Eur J Nutr 41 : 47–53 (2002)

ORIGINAL CONTRIBUTION

F. Granado
B. Olmedilla
I. Blanco

Serum depletion and bioavailability of lutein in Type I diabetic patients

Summary *Background* Lutein, a non-provitamin A carotenoid, is frequently consumed in the human diet. It is distributed preferentially in certain human tissues (i. e., retina) and shows a high antioxidant activity. Type 1 diabetic patients have been considered to be at risk of increased oxidative stress that may contribute to accelerated atherogenesis and to the microangiopathic complications of the disease. *Aim of the study* To assess the influence of type 1 diabetes mellitus on the serum depletion rate and bioavailability of lutein. *Subjects and Methods* Ten type 1 diabetics and eight controls consumed a low carotenoid diet for 21 days and the bioavailability study was performed in 7 diabetics and 5 controls on day 15 with the administration of a capsule of lutein esters from marigold extract. Samples were collected at baseline and on days 1, 2, 3, 6, 11, 15 (eight times during 9 h), 16, 17 and 21. Lutein and other carotenoids were determined by HPLC in triglyceride-rich lipoprotein (TRL) fractions and plasma or serum. *Results* Serum depletion curve, area of concentrations under time curve (AUC) and final concentration percentages were similar in diabetics and controls. In the bioavailability study, all-trans-lutein increased in both groups and AUC, maxima concentrations in TRL and serum and time required for maxima concentration in serum were similar in diabetics and controls. *Conclusions* These data suggest that in the group of patients assessed, type 1 diabetes mellitus does not apparently influence the absorption and depletion rate of lutein in serum.

Key words Lutein – serum depletion rate – bioavailability – carotenoids – type 1 diabetes mellitus

Received: 24 July 2001
Accepted: 18 December 2001

F. Granado · B. Olmedilla · I. Blanco
Unidad de Vitaminas
Sección de Nutrición
Clínica Puerta de Hierro
28035 Madrid, Spain
Fax: +34-91/373 76 67
E-Mail: bolmedilla@hpth.insalud.es

Introduction

Lutein, a non-provitamin A xanthophyll, is, along with β-carotene, one of the most widely distributed carotenoids in fruits and vegetables frequently consumed by different populations. Lutein shows a marked antioxidant activity *in vitro* [1, 2], and several putative *in vivo* oxidative metabolites, referred to as ketocarotenoids, have been described in humans [3, 4].

In human blood, lutein is predominantly transported by HDL and preferentially deposited in certain tissues (i. e., retina and lenses), where lutein, zeaxanthin (referred to as macular pigments) and several oxidation products, including ketocarotenoids, have been identified as the major carotenoids [5–7]. In this regard, several epidemiological studies have shown that serum and dietary lutein and zeaxanthin are specifically associated with a lower risk of eye disease in the elderly [8] and, additionally, dietary interventions in humans have been found to effectively increase macular pigment density [9, 10] and visual function in patients with cataracts [11]. Although the role of the macular pigment is still uncertain, a reduction of the photo-oxidative damage of blue light and protection against photochemical reactions, because of their antioxidant properties, have been suggested [12].

Type 1 diabetic patients have long been considered to be at risk for marginal vitamin status [13, 14] and increased oxidative stress because of higher levels of markers of oxidative stress and/or lower concentrations of antioxidants [15–19]. However, similar or even higher

EJN 351

circulating levels of α-tocopherol (and α-tocopherol/cholesterol ratio) and several carotenoids in serum have been also reported in these subjects [20–22] and, specifically, serum levels of lutein and zeaxanthin in type 1 diabetics and their first-degree relatives were not different after adjusting for other potential confounding factors [22].

Hyperglycemia, a key clinical manifestation of diabetes mellitus, not only generates reactive oxygen species (ROS), but also attenuates anti-oxidative mechanisms by scavenging enzymes and antioxidant substances [17]. A greater oxidative damage in insulin-dependent diabetic (IDDM) patients has been described and these changes may contribute to accelerated aging and atherogenesis in diabetes and to the microangiopathic complications of the disease [16, 17]. Within diabetic microvascular complications, visual loss due to diabetic retinopathy has become the most frequent cause of new cases of blindness among adults aged 20–74 years, and the association between poor glucose control, glucose-induced changes in refraction of ocular media (macular region) and retinopathy has been consistently documented in observational studies [23].

Due to the absence of markers for biological activity (sensitive, reliable and predictive) that respond specifically to carotenoids intake in humans, the assessment of their bioavailability has largely been taken to mean "assimilation efficiency" or the ability to accumulate them in some defined body pools [24]. In the evaluation of bioavailability, chylomicron carotenoids represent newly-absorbed material and then, the so-called triacylglycerol-rich plasma fraction is potentially very useful in assessing bioavailability of carotenoids (absorption and conversion)[24].

Due to the potential role of lutein in ameliorating the photo-induced oxidative damage in the macula region and its impact on visual function, our aim was to study the bioavailability and serum depletion rate of lutein in type 1 diabetic patients as part of the metabolism of this biologically relevant carotenoid in humans.

Subjects and methods

Subjects

A total of 21 clinically diagnosed type 1 diabetics were screened for participation. Ten patients accepted to take part in the depletion study, whereas seven were involved in the bioavailability test. Exclusion criteria included the absence of clinical signs of long-term diabetic complications, the use of vitamin/carotenoid supplements and absence of any other disorder that could affect absorption and/or metabolism of carotenoids. All diabetics received subcutaneous insulin, 8 out of 10 being under intensive therapy (≥3 injections/day). Eight sex- and age-matched controls (recruited among first-degree relatives of patients and friends) with hematological and biochemical profile within the normal values participated in the depletion study of whom five agreed to take part in the bioavailability test. Baseline characteristics of the subjects are shown in Table 1.

Table 1 Baseline characteristics of the subjects (mean ± SD, range)

Contacted	21 Type 1 diabetic patients	
Included: "Depletion study"	10 type 1 DM	8 controls
"Bioavailability"	7 type 1 DM	5 controls
	Type 1 DM (n=10)	Control (n=8)
Age (y)	24±6	27±3
Sex (men/women)	7/2	6/1
BMI (kg/m^2)*	22.6±1.6	25.7±1.3
Disease evolution (years median)	6	
(range)	(1–23)	
Cholesterol (mmol/L)	4.34±1.14	4.60±0.52
cHDL (mmol/L)	1.50±0.34	1.74±0.47
Triglycerides (mmol/L)	1.06±0.55	0.96±0.54
Fasting glycemia (mmol/L)*	12.6±5.8	5.3±0.3
HbA_{1c} (%)**	7.7±2	4.8±0.5
Fructosamine (mmol/L)*	1.9±0.5	1.3±0.12
Lutein (μmol/L)	0.32±0.12	0.23±0.09
(range)	(0.11–0.60)	(0.09–0.37)

Type 1 DM type 1 diabetic patients; *HbA_{1c}* glycated hemoglobin
* $p < 0.01$; ** $p < 0.005$.

The study was performed in accordance with the ethical standards of the Ethical Committee of the Clínica Puerta de Hierro and all subjects gave written informed consent.

Experimental protocol

A scheme of the experimental protocol is shown in Fig. 1. To study the depletion rate of lutein in serum, a low-carotenoid diet was maintained for 21 days during which food intake was recorded by all volunteers. Participants received a list with the major carotenoid-containing fruits and vegetables consumed in Spain [25] to avoid and were advised about other foods (i. e., mixed dishes, dairy products treated with food coloring) that should be consumed as little as possible. The objective of this low-carotenoid diet was to consume ≤10% of the season-adjusted reference Spanish intake of each carotenoid [26] and/or less than 0.5 mg/person/day of total carotenoids. Carotenoid intake was assessed using carotenoid data on Spanish fruits and vegetables generated by HPLC [25].

To study the bioavailability (absorption), a single-dose test consisting of the administration of a capsule containing lutein (mixed esters from the marigold flower) was carried out on day 15 of the depletion diet. Capsules were supplied by Quest International

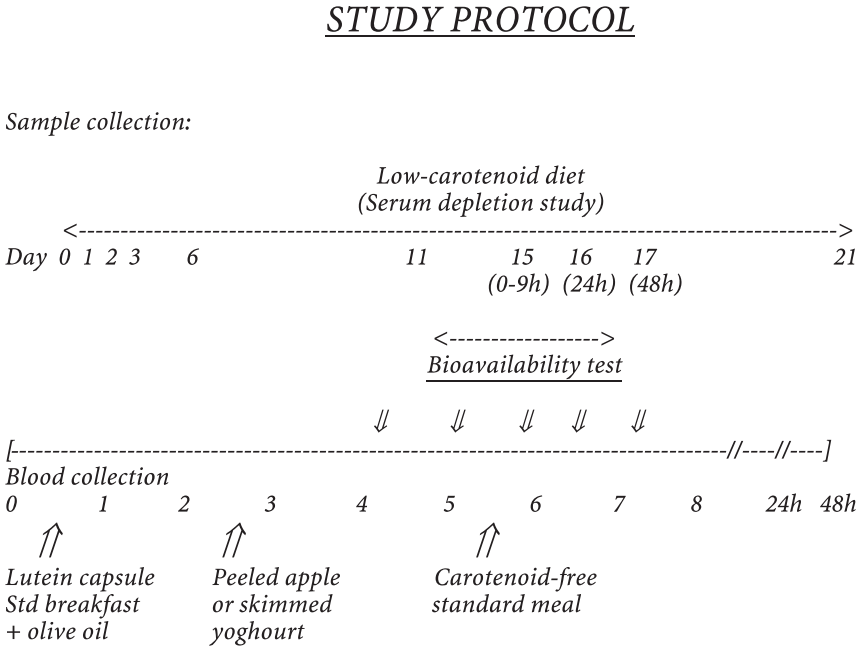

Fig. 1 Time diagram during the depletion and bioavailability study.

(Unilever, Vlaardingen, The Netherlands), and HPLC analysis of saponified extracts gave a content per capsule of 12 mg of all-trans-lutein, 3 mg of 13/15-cis-lutein and 3.3 mg of α-tocopherol, whereas zeaxanthin was not detected.

The study was performed in the hospital and diabetes-experienced nurses and physicians controlled the course of the study. Because the aim of the study was to assess the influence of type 1 diabetes under usual conditions, patients maintained their ordinary insulin injections schedule throughout the experimental day. All volunteers received a capsule together with a standard breakfast consisting of two slices of bread with 40 g of olive oil (68 % oleic acid) and 200 ml of skimmed milk. Breakfast supplied about 670 Kcal (56 % from fats, 35 % from carbohydrates and 9 % from proteins). All the participants ate a peeled apple or skimmed yogurt 2.5 h after the capsule and a standard meal (carotenoid-free) 3 h later. Patients adjusted the number of insulin units according to their respective glycemic control and dietary intake throughout the experimental day.

Blood collection and sample preparation

For the depletion rate study, blood samples were taken at baseline (day 0) and on days 1, 2, 3, 6, 11 and 15. In the bioavailability test, blood samples were collected from an antecubital vein before breakfast (baseline) and during 9 hours (eight samples) after the capsule administration and at 24 h (day 16), 48 h (day 17) and six days later (day 21).

Determination of lutein, zeaxanthin and major carotenoids in serum and triglyceride-rich lipoprotein fractions (TRL), including ketocarotenoids and lutein esters, was carried out by a quality-controlled HPLC method [21] (QA Fat-soluble Vitamins and Carotenoids Assurance Programme, NIST, USA). Briefly, 0.5 ml of serum (depletion study) or plasma (bioavailability test) were mixed with 0.5 ml of ethanol containing internal standard (retinyl acetate), vortexed and extracted twice with 2 ml of methylene chloride/hexane (1:5). Organic phases were pooled, evaporated to dryness and reconstituted to be injected onto the HPLC. Samples were analyzed using a Spheri-5-ODS column (Applied Biosystems) and a mobile phase of acetonitrile:methanol (85/15) for 3 minutes and linear gradient elution to acetonitrile:methylene chloride:methanol (70/20/10) at 5 min and up to 20 minutes. Flow rate 1.8 ml/min with detection at 450 nm.

The "triglyceride-rich lipoprotein" fraction (TRL) were prepared from plasma (EDTA, 5 %) according to the protocol described by Griffiths et al. [27]. Plasma obtained within 20 min of blood collection was stored at –20 °C until analysis (< 20 days). After slow thawing at 4 °C, duplicate 0.5 ml of plasma at each time-point were transferred to Eppendorf tubes overlayered with saline solution (density 1.006 kg/l) and centrifuged at 12,600 × g for 2 hours. Upper layers (TRL fraction) were aspirated carefully, washing the tube walls, and duplicates were pooled to be extracted as described above. The protocol used for the preparation of TRL fractions yielded intra- and between-day variation coefficients for lutein of 2 % and 4 %, respectively.

Plasma lipid profile (total cholesterol, cHDL and triglycerides) was determined at each time point

throughout the entire study, glycated hemoglobin (HbA1c) and fructosamine levels were evaluated at the start of the study (day 0). These analyses were carried out at the General Biochemistry Laboratory of the hospital according to routine quality-controlled standard methods.

Statistical analysis

Concentration curves versus time (AUC) were calculated by the trapezoidal method after correction for baseline concentrations. Student's t test and the Mann-Whitney U test were used to assess statistical differences ($p < 0.05$) between groups in terms of their lutein intake during depletion diet, serum depletion of lutein and bioavailability study. To assess relationships among different parameters measured, Pearson correlation coefficients were calculated. All analyses were performed using SPSS (version 8.0).

Results

Low-carotenoid diet and depletion rate in serum

Lutein intake (median) during the consumption of the low-carotenoid diet was 74 µg/person/day (range: 46–103) and 51 µg/person/day (range: 33–101) in diabetics and controls (not significant), respectively. This lutein intake/day accounts for 13% (diabetics) and 9% (controls) of the season-adjusted reference intake in the Spanish population [26]. The mean total carotenoid intake/day during this period was 135 µg/person/day in diabetics and 91 µg/person/day in controls, representing about 3–4% of the reference intake, respectively.

Depletion of lutein in serum is shown in Fig. 2. Areas of concentration versus time curve, final percentages (62 versus 65% in controls and diabetics) and estimated half-life values for lutein in serum (> 15 days, as extrapolated from regression-adjusted curves), showed no significant differences between diabetics and controls. Regression-adjusted curves (Fig. 2) showed that curves for lutein depletion in serum for diabetics and controls overlap and display two stages, with initial rapid clearance and then a much slower clearance. No significant changes were observed in the lipid profile during this period in either of the groups.

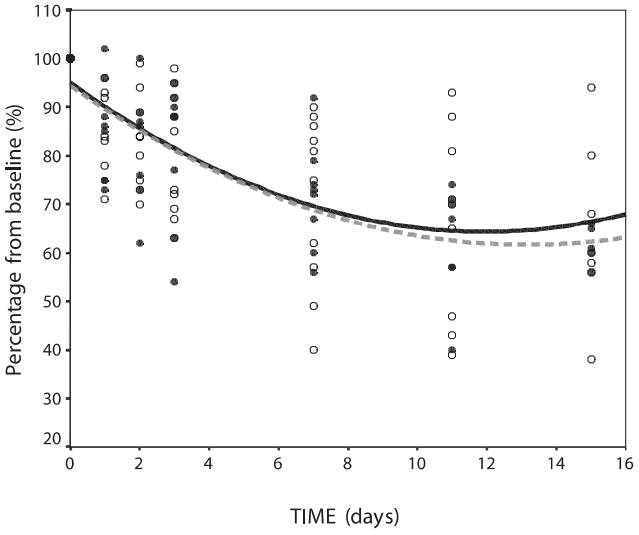

Fig. 2 Lutein depletion (percentages from baseline concentrations) in serum upon low-carotenoid diets. Lines represent quadratic-regression adjusted curves for individual values. (Dotted line: control group; solid line: diabetic group.)

Bioavailability study

Lutein concentrations, after baseline correction, are shown in Table 2 and Figs. 3a, b. After lutein administration, an increase in all-trans-lutein and the all-trans-lutein/ ketocarotenoids ratio was observed during the 9-h post-prandial period, both in TRL fraction and plasma (Figs. 3a, b). No significant changes in 13/15cis-lutein, zeaxanthin or 2'3'-anhydrolutein concentrations could be detected in TRL or in plasma.

In the TRL fraction, the partial AUC for lutein and peak concentration reached during the 9-h post-prandial period were greater, although not significant, in

Table 2 Area under the time-response curve (AUC) (mean, SD and range) in the bioavailability study*

	CONTROL (n=5)		Type 1 DM (n=7)		p
	AUC nmol/L h	Cmax (t) nmol/L	AUC nmol/L h	Cmax (t) nmol/L	
Lutein in "TRL"	8.62±3.52 (5.28–14.43)	22.3 (8 h)	5.98±3.87 (1.40–12.14)	16.36 (8 h)	NS
Lutein in plasma	20.76±13.72	59.64 (9 h)	21.29±14.43	91.66 (9 h)	NS
C.max serum (0–6 days)	–	153.76 (24 h)	–	158.34 (24 h)	NS

* Baseline corrected values.
NS not significant (Mann-Whitney U test, $p < 0.05$)

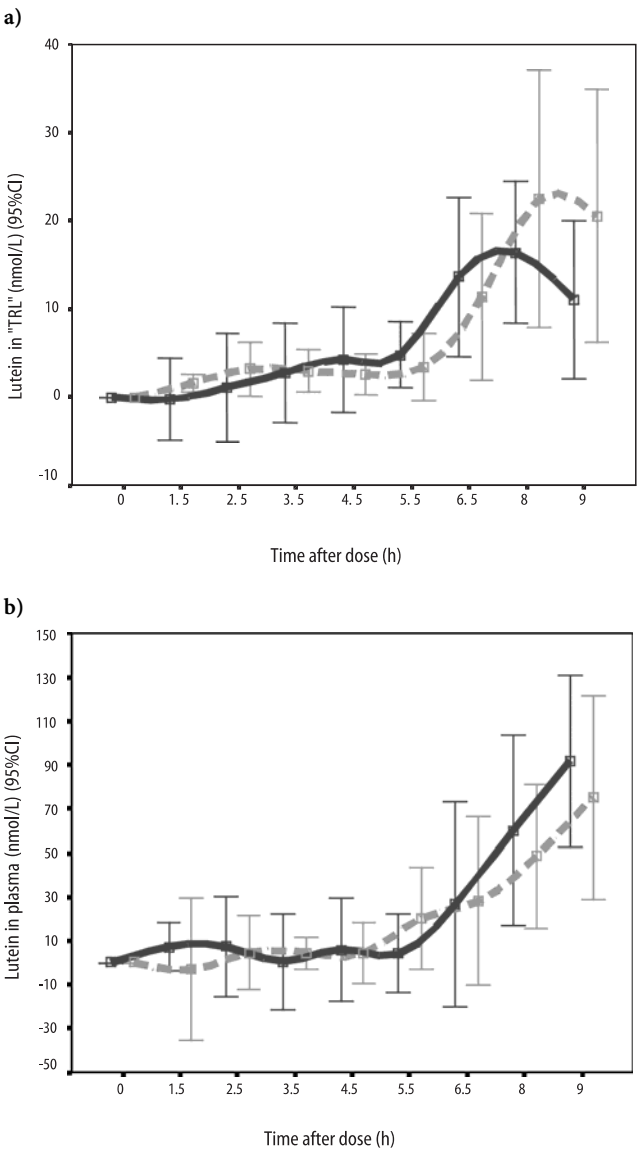

Fig. 3 Time course of lutein (mean and confidence interval 95 %) in (**a**) "TRL" and (**b**) plasma of controls and type 1 diabetic patients. (Dotted lines: control group; solid lines: diabetic group.)

controls than in diabetics. In plasma, partial AUC, peak concentration, time required to reach peak maximum and absorption curves during the 9 h period were not significantly different in diabetics and controls (Table 2; Fig. 3). Maximum concentration of lutein in serum was reached at 24 h (Table 2) in both groups and, on average, baseline levels were not recovered six days after the capsule administration and maintaining the low-carotenoid diet.

Mean plasma triglyceride AUC was similar in both groups. In diabetic subjects, lutein response was highly correlated with fasting triglycerides concentrations ($r > 0.9$; $p > 0.01$) but not with clinical parameters of metabolic control (HbA1c, fructosamine). Percentages of absorption during the period of study (9 h) were calculated based on the AUC in TRL fractions, correcting for plasma volume (assuming 4 % body weight), and in both groups it was < 2 % of the dose supplied.

Discussion

Serum carotenoids reflect short-term dietary intake [28] and display two different depletion rates in serum depending on their chemical structure [28, 29]. In the present study, diabetics and controls consuming a very low-lutein diet showed equal depletion curves of lutein in serum. In regression-adjusted curves, the rate of disappearance of lutein in serum and the estimated half-life (> 15 days) was similar to that previously described [28, 30], and showed a two-slope curve suggesting the existence of two body storage pools [28]. These results support previous data in the sense that lutein in serum should be considered a marker of recent (days) dietary intake and also suggest that the depletion/disposal rate (and half-life) of lutein in serum is unaffected by the presence of type 1 diabetes mellitus. If, as proposed [31], depletion of serum antioxidants may be indicative of oxidative stress, then the present results would indicate both that a higher oxidative stress was not present in these type 1 diabetic patients and/or that concentrations and depletion rate of lutein in serum are unreliable markers of oxidative stress. In this regard, we did not observe any significant association between serum depletion rate (AUC, half-life or final percent concentration) and clinical parameters of metabolic control (HbA1c, fructosamine), coinciding with that reported by other authors using different markers of oxidative stress [16, 18].

Bioavailability studies with carotenoids are difficult to compare because of the distinct protocols used [32]. The so-called triacylglycerol-rich lipoprotein fraction is considered to be potentially very useful in assessing bioavailability of carotenoids (absorption and conversion) [24]; however since these carotenoids already exist in plasma at appreciable concentrations, "relative bioavailability" is more conveniently assessed when different treatments/groups are compared.

Our aim was to study whether the presence of type 1 diabetes mellitus influences lutein absorption and thus, the results provide useful information in relative terms and for comparative purposes, although some special features of diabetes should be considered. A slower clearance of chylomicron remnants by the liver has been reported in subjects with type 1 diabetes mellitus [33, 34] which may lead to their accumulation in plasma during the post-prandial state and thus to a higher recovery during isolation of the TRL fraction. The relative influence of different clearance mechanisms would have an impact on the clearance kinetics for normal and diabetic

individuals. In the present study, triglycerides in TRL were not measured, but AUC for triglycerides in plasma were not different between groups, suggesting that if different clearance mechanisms are involved then it should be at the chylomicron remnants uptake by the liver coinciding with that reported by others [33, 34]. This difference in clearance kinetics seems to be independent of the metabolic control of the disease and would be related to the route of insulin administration (subcutaneous), leading to a higher peripheric insulinemia and lower stimulation of LDL or LRP receptors of the liver which provokes a longer permanence of chylomicrons remnants in plasma (33). An impaired (delayed) chylomicron clearance in diabetics might explain the lower peak maxima in TRL but higher in plasma of diabetic patients when compared to controls. Alternatively, the higher lutein content in the diabetic plasma at 8 hours may also be related to the presence of longer half-life lipoproteins in plasma (i. e., VLDL and LDL) to which the lutein has been transferred.

The extent to which the chylomicron post-prandial model may be applied to study xanthophylls bioavailability has been questioned because of the potential transference to other lipoprotein fractions during the post-prandial state [24, 29] and thus, the chylomicron post-prandial model may under-represent the true extent of lutein absorption [24]. In this study, the amount of lutein entering in plasma during the 9 hours of study, calculated from AUC in TRL fractions was < 2 % (in both groups) and < 4.5 % using plasma AUC values, both estimates being lower than previous reports [30, 32, 35]. Contrary to those studies, in order to not to alter diet/insulin regimen in the patients, we used a daily meal consumption pattern and olive oil as an absorption enhancer. This approach may have contributed to the delayed post-prandial response observed and the time required to reach maximum concentrations [36, 37] and possibly determined the lack of baseline recovery during the time monitored.

Post-prandially, no lutein esters (supplied in the capsule) were detected in TRL or plasma at any time point. Although we can not rule out the possibility of the presence of ester forms in amounts below our detection limit, this observation coincides with the absence of xanthophyll esters in chylomicrons described by Wingerath et al. [38], who suggest that cleavage of ester forms is a general step in the metabolism of xanthophylls before release into the circulation. Also, the presence of diketo- and monoketo-monohydroxy-carotenoids in human sera [3] and their increase upon supplementation with lutein [3, 4] have led to the suggestion that these substances are possible in vivo oxidative metabolites of lutein/zeaxanthin [3]. Interestingly, we detected no increase in ketocarotenoids in TRL or in plasma at any time point, and the all-trans-lutein/keto-carotenoid ratio increased in parallel to that of all-trans-lutein which suggests that these metabolites are formed post-absorptively in vivo. Finally, plasma concentrations 24–48 h after dose administration have been considered to reflect different aspects of carotenoid metabolism (hepatic turnover, absorption, clearance, tissue uptake, etc). In this study, maximum concentrations in serum were reached at 24 h and showed a similar decline during the following 6 days upon low-carotenoid-diet both in controls and diabetic patients.

In summary, absorption and depletion/disposal rate of lutein in serum do not seem to be significantly influenced by the presence of type 1 diabetes mellitus in the participating patients. However, because of the small number of subjects involved and the differential post-prandial lipid metabolism in IDDM, these results need further confirmation in larger groups.

Acknowledgments This work was funded by the Fondo de Investigaciones Sanitarias, Spain (FIS 97/0966). The authors are indebted to Dr. A. Simal and Dr. F. Botella for clinical advice, to Teresa Motilla and Pilar Martínez for blood collection and to the participating patients, relatives and friends.

References

1. Chopra M, Thurnham DI (1993) In vitro antioxidant activity of lutein. In: Waldron KW, Johnson IT, Fenwick GR (eds) Food and Cancer Prevention: Chemical and Biological Aspects. Royal Society of Chemistry, Bath (UK), pp 125–129
2. Packer L (1993) Antioxidant action of carotenoids in vitro and in vivo and protection against oxidation of human low-density lipoproteins. Ann N Y Acad Sci 691: 48–60
3. Khachick F, Beecher G, Smith JC (1995) Lutein, lycopene and their oxidative metabolites in chemoprevention of cancer. J Cel Biochem 22 (suppl): 236–246
4. Olmedilla B, Granado F, Gil-Martínez E, Blanco I, Rojas-Hidalgo E (1997) Supplementation with lutein (4 months) and α-tocopherol (2 months), in separate or combined oral doses, in control men. Cancer Letters 114: 179–181
5. Parker R (1996) Absorption, metabolism and transport of carotenoids. FASEB J 10: 542–551
6. Bone RA, Landrum JT (1988) Preliminary identification of the human macular pigment. Vis Res 25: 1531–1535
7. Khachick F, Berstein PS, Garland DL (1997) Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. Invest. Ophthalmol. Vis Sci 38: 1802–1811
8. Bunce GE (1994) Nutrition and eye disease of the elderly. J Nutr Biochem 5: 66–77
9. Landrum JT, Bone RA, Joa H, Kilburn MD, Moore L, Sprague K (1997) A one year study of the macular pigment: The effect of 140 days of a lutein supplement. Exp Eye Res 65: 57–62
10. Hammond BR, Johnson EJ, Russell RM, Krinsky NI, Yeum KJ, Edwards RB, Snodderly DM (1997) Dietary modification of human macular pigment density. Invest Ophthalmol Vis Sci 38: 1795–1801

11. Olmedilla B, Granado F, Blanco I, Vaquero M, Cajigal C (2001) Lutein in patients with cataracts and age-related macular degeneration: a long-term supplementation study. J Sci Food Agric 81(9): 904–910
12. Beatty S, Boulton M, Henson D, Koh HH, Murray IJ (1999) Macular pigment and age-related macular degeneration. Br J Ophthalmol 83: 867–877
13. Mooradian AD, Morley JE (1987) Micronutrient status in diabetes mellitus. Am J Clin Nutr 45: 877–895
14. Mooradian AD, Failla M, Hoowerf B (1994) Selected vitamins and minerals in diabetes. Diabetes Care 17 (5): 464–479
15. Wolff SP (1993) Diabetes and free radicals. Br Med Bull 49: 642–652
16. Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, Nicotera T (1996) Oxidative damage to DNA in diabetes mellitus. Lancet 347: 444–445
17. Giugliano D, Ceriello A, Paolisso G (1996) Oxidative stress and diabetic vascular complications. Diabetes Care 19 (3): 257–267
18. Jain SK, Krueger KS, McVie R, Jaramillo JJ, Palmer M, Smith T (1998) Relationship of blood thromboxane-B2 (TxB2) with lipid peroxides and effect of vitamin E and placebo supplementation on TxB2 and lipid peroxide levels in type 1 diabetic patients. Diabetes Care 21: 1511–1516
19. Tsai EC, Hirsch IB, Brunzell JD (1994) Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. Diabetes 43: 1010–1014
20. Bates JH, Young IS, Galway L, Traub AI, Hadden DR (1997) Antioxidant status and lipid peroxidation in diabetic pregnancy. Br J Nutr 78: 523–532
21. Olmedilla B, Granado F, Gil-Martínez E, Blanco I, Rojas-Hidalgo E (1997) Reference levels of retinol, α-tocopherol and main carotenoids in serum of control and insulin-dependent diabetic Spanish subjects. Clin Chem 43 (6): 1066–1071
22. Granado F, Olmedilla B, Gil-Martínez E, Blanco I, Millán I, Rojas-Hidalgo E (1998) Carotenoids, retinol and tocopherols in insulin-dependent diabetics and their immediate relatives. Clin Sci 94 (2): 189–195
23. American Diabetes Association (ADA) (2000) Diabetic retinopathy. Diabetes Care 23 (Suppl. 1): S73–S76
24. Parker RS, Swanson JE, You ChS, Edwards AJ, Huang T (1999) Bioavailability of carotenoids in human subjects. Proc Nutr Soc 58: 155–162
25. Olmedilla B, Granado F, Blanco I, Gil-Martínez E, Rojas-Hidalgo E (1996) Contenido de carotenoides en verduras y frutas de mayor consumo en España. Instituto Nacional de la Salud (INSALUD). Secretaría General, Madrid 1996
26. Granado F, Olmedilla B, Blanco I, Rojas-Hidalgo E (1996) Major fruit and vegetable contributors to the main serum carotenoids in the Spanish diet. Eur J Clin Nutr 50 (4): 246–250
27. Griffiths AJ, Humphreys SM, Clark ML, Frayn K (1994) Immediate metabolic availability of dietary fat in combination with carbohydrates. Am J Clin Nutr 59: 1025–1032
28. Rock CL, Swenseid ME (1992) Plasma carotenoid levels in human subjects fed a low-carotenoid diet. J Nutr 122: 96–100
29. Pateau I, Chen H, Goh NMY (1997) Interactions in the postprandial appearance of β-carotene and canthaxanthin in plasma triacylglycerol-rich lipoproteins in humans. Am J Clin Nutr 66: 1133–1143
30. Kostic D, White WS, Olson JA (1995) Intestinal absorption, serum clearance and interactions between lutein and β-carotene when administered to human adults in separate or combined oral doses. Am J Clin Nutr 62: 604–610
31. Nourooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tristchler H, Rosen P, Halliwell B, Betteridge DJ (1997) Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. Diabetologia 40: 647–653
32. Van Vliet T, Schreurs WHP, van den Berg H (1995) Intestinal β-carotene absorption and cleavage in men: response of β-carotene and retinyl esters in triglyceride rich lipoprotein fraction after a single oral dose of β-carotene. Am J Clin Nutr 62: 110–116
33. Georgopoulos A (1994) Are chylomicrons remnants involved in the atherogenesis of insulin-dependent diabetes mellitus? J Lab Clin Med 123: 640–646
34. Georgopoulos A, Saudek CD (1994) Intraperitoneal insulin delivery decreases the levels of chylomicron remnants in patients with IDDM. Diabetes Care 17 (11): 1295–1299
35. O'Neill ME, Thurnham DI (1998) Intestinal absorption of β-carotene, lycopene and lutein in men and women following a standard meal: response curves in the triacylglycerol rich lipoproteins fraction. Br J Nutr 79: 149–159
36. Fielding B, Callow J, Owen RM, Samra J, Mathews DR, Frayn KN (1996) Postpandrial lipemia: The origin of an early peak studied by specific dietary fatty acid intake during sequential meals. Am J Clin Nutr 63: 36–41
37. Higashi K, Ishikawa T, Shige H, Tomiyasu K, Yoshida H, Ito T, Nakajima K, Yonemura A, Sawasa S, Nakamura H (1997) Olive oil increases the magnitude of postprandial chylomicron remnants compared to milk fat and safflower oil. J Am Coll Nutr 16(5): 429–434
38. Wingerath T, Stahl W, Sies H (1995) Bcryptoxanthin selectively increases in human chylomicrons upon ingestion of tangerine concentrate rich in β-cryptoxanthin esters. Arch Biochem Biophys 324 (2): 385–390