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Original Article

Contribution of violaxanthin, neoxanthin, phytoene and phytofluene to total carotenoid intake: Assessment in Luxembourg

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ABSTRACT

Dietary carotenoid intake has been associated with a low incidence of several chronic diseases, including cardiovascular complications, cancer, and macular degeneration. While food composition and intake of some carotenoids such as β-carotene and lycopene is frequently available, information on the contribution of less studied epoxycarotenoids such as neoxanthin, violaxanthin, and phytoene/phytofluene, is scant. The present study describes the assessment of ten individual carotenoids in frequently consumed food items and estimates their contribution to total carotenoid intake in Luxembourg. For this purpose, 50 frequently consumed food items were collected from local groceries, and combined with food consumption data obtained from the first epidemiological Luxembourgish cardio-vascular risk factor study (ORISCAV-LUX). Highest epoxycarotenoid content was found in bell peppers (4.5 mg/100 g), highest amount of phytoene/phytofluene in apricot (9.6 mg/100 g) and tomato ketchup (4.5 mg/100 g). National daily per capita intake was assessed as 7.6 mg α- and β-carotene, 2.0 mg phytoene, 1.8 mg lycopene, 1.5 mg lutein, 1.4 mg β-cryptoxanthin, 1.2 mg violaxanthin, 0.7 mg phytoene, 0.5 mg neoxanthin, and 0.3 mg zeaxanthin, with 10% of total daily carotenoid intake from epoxycarotenoids and 16% from phytoene/phytofluene. While intake in Luxembourg appears to be comparable to other European data, this study highlights the importance of taking less frequently analysed carotenoids into account for determining total intake.

1. Introduction

Carotenoids are lipophilic pigments widespread in nature, with more than 700 species known today (Britton et al., 2004), although only about 60 are estimated to be of importance for the human diet (Bohn, 2008). They can be synthesized by all plants, many bacteria and some fungi, with animals including humans solely relying on dietary uptake. Some carotenoids are precursors of vitamin A, the latter exerting manifold functions during embryonic development (Morris-Kay and Ward, 1999), immune response (Hughes, 1999) and in the vision cycle (Simpson and Chichester, 1981; Bendich and Olson, 1989). There is further growing evidence for the preventive role of non-provitamin A carotenoids in a number of chronic diseases, partly but not exclusively due to their antioxidant properties. For example, lycopene has been proposed to prevent the onset and proliferation of prostate cancer (Kucuk et al., 2001; Giovannucci et al., 2002), increase gap-junction intercellular communication (Stahl et al., 2000; Livny et al., 2003), while lutein and zeaxanthin appeared of importance for the protection of the retina (Landrum et al., 1999; Bernstein et al., 2001). It has been suggested that carotenoids, especially when consumed in the form of fruits and vegetables, could prevent cardiovascular disease (Voutilainen et al., 2006; Hozawa et al., 2009) and may play a beneficial role for bone health and density (Wattanapenpaiboon et al., 2003; Sahni et al., 2009).

Dietary carotenoid sources are, to a large extent, coloured fruits and vegetables. In addition, there exist secondary carotenoid sources, including animal products, such as some fish, seafood, eggs and further processed foods, such as beverages, soups, margarine or butter (Souci et al., 2000; O'Neill et al., 2001), some of which are fortified with carotenoids, especially β-carotene.

Given the accumulating evidence for beneficial health effects, consumption of several carotenoid species, such as lycopene, lutein and zeaxanthin and the vitamin A precursors α- and β-

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carotene, has been studied in nutritional surveys in several European countries (Pelz et al., 1998; O'Neill et al., 2001; Lucarni et al., 2006) and the US (USDA, 2010). The occurrence of carotenoids in food items, especially of the more frequently consumed β-carotene and lycopene, has been addressed in several carotenoid databases in Europe and the US (Mangels et al., 1993; O'Neill et al., 2001). However, these databases do not take into account additional carotenoids that are also frequently found in the diet, including the epoxycarotenoids violaxanthin and neoxanthin, or the carotenoid precursors phytoene and phytofluene (Fig. 1). Although the precursors, compared to, e.g. lycopene, contain a shorter deconjugated π-electron system, which is essential for singlet oxygen quenching, they still exert a certain antioxidant activity (Shaish et al., 2008). Further health beneficial properties, such as protection of the skin from excess UV exposure (Aust et al., 2005; Engelmann et al., 2011), were also reported. Similarly, digestion-derived furanoid-carotenoids were shown to inhibit tumor cell proliferation (Asai et al., 2004).

This study aimed at investigating the contribution of the less studied carotenoids neoxanthin, violaxanthin, phytoene and phytofluene toward total dietary carotenoid intake. In addition, we estimated, for the first time, carotenoid food composition patterns and consumption in Luxembourg, based on the measured composition of frequently consumed fruits and vegetables and food consumption data collected within the framework of the ORISCAV-LUX cardio-vascular risk factor study (Alkerwi et al., 2010b). Creating new data for Luxembourg seem warranted due to its multi-ethnical composition and cuisines compared to neighbouring countries, from where results are usually simply transferred. These data are, in sight of the potential beneficial health effects of carotenoids, of substantial interest for national and international health-care stakeholders.

2. Materials and methods

2.1. Food items and preparations

Food items that were estimated to be rich in carotenoids (Souci et al., 2000; O'Neill et al., 2001) and frequently consumed (Alkerwi et al., 2010b) were included in this study, given that their measured carotenoid content was above 50–100 μg/100 g per edible portion (Table 1). Food products were purchased and analysed at mature conditions and procured predominantly from the main Luxembourgish supermarket chain (CACTUS S.A., Esch/Alzette, Luxembourg). Solid food items were aliquoted on the day of purchase, analysed on the same day or stored at −25 °C for a maximum of 4 weeks until processing, while juices, eggs and oils were stored in the original package in a cold chamber at 4 °C in the dark and analysed within 2 weeks.

Details of the sampling and amount and parts taken for further analysis are outlined in Table 1. For fruits and vegetables, materials were prepared according to typical kitchen preparation procedures, via removing the non-edible parts such as peel, kernels, while the skin (e.g. potatoes, apples, and pears) was usually kept, unless otherwise stated (Table 1). The material was then cut into small pieces of approx. 3–4 cm^3 or 3–4 cm^2 (for leafy materials) with a sharp knife, and smaller aliquots were then further cut into ca. 1 cm^3 or 1 cm^2 size, and approx. 20–30 g aliquots were then rapidly frozen for further analyses by adding liquid nitrogen, or assayed directly. Material kept for storage was put into 50 mL

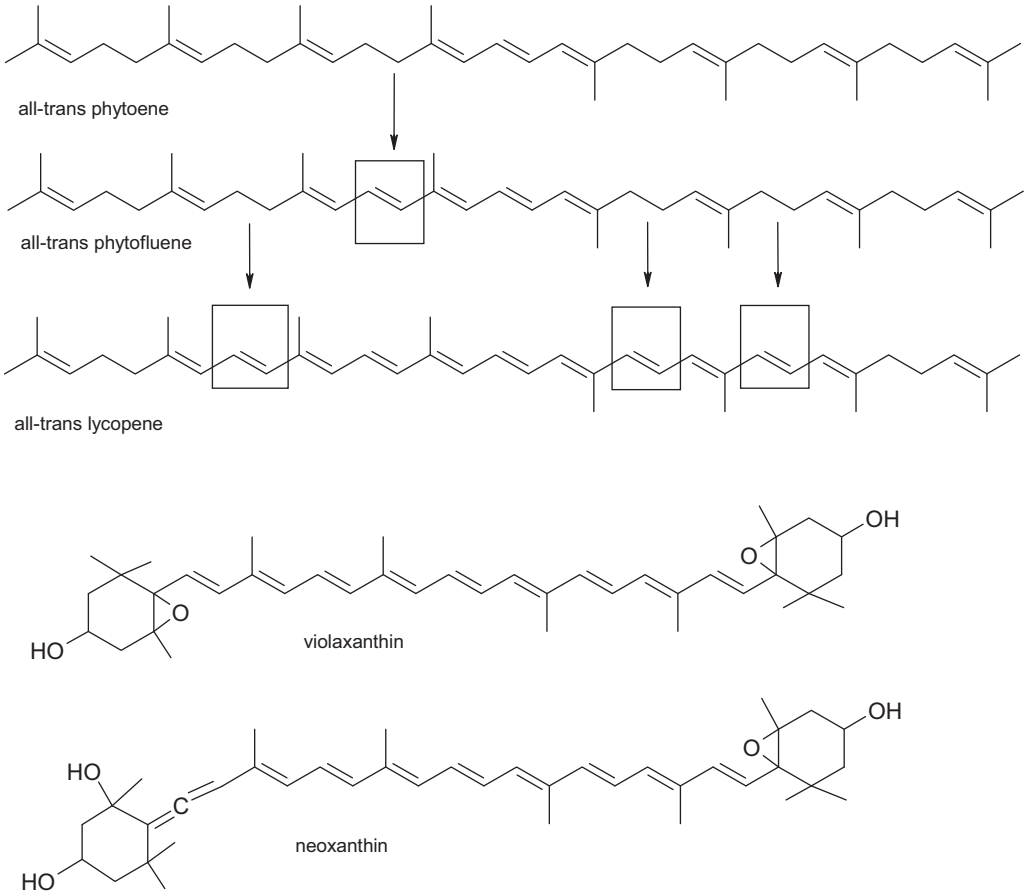

Fig. 1. Chemical structures of less well studied carotenoids with respect to carotenoid intake.

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Table 1
Detailed sampling plan for the food items investigated. All items were purchased from the main Luxembourgish supermarket chain (Cactus S.A., Windhof, Luxembourg).

Food items	Time of purchase	Origin	Number/amount further processed	Sampling, preparation and processing
Apple (red)	Autumn 2010	Italy	2 from a box of 6 chosen	Cubes from edible parts including skin, removal of inner parts
Apricot	Summer 2008	Israel	10 fruits	Removal of kernels, cubes cut and mixed
Arugula	Summer 2008	France	1 box	Slices and cubes, removal of bottom end
Aubergine	Summer 2010	France	1 of 2 chosen	Cubes from edible parts including skin, removal of top and bottom end
Banana	Autumn 2009	[a]	2 from a bundle chosen	Cubes from peeled fruits, cubes mixed
Butter	Summer 2010	Luxembourg	1 pack of 250 g	Thin slices were scraped from the surface with a kitchen knife
Bean (green)	Summer 2010	Belgium	30 beans chosen from a box	Removal of non-edible parts at both ends, cubes from appr. 30 beans
Blackberry	Summer 2008	[a]	50 berries chosen from a box	Cubes from blackberries, approx. 8 cubes / fruit
Broccoli	Summer 2008	France	1 broccoli	Removal of non-edible parts (stem), cubes cut and mixed
Cabbage (green)	Summer 2008	France	1 cabbage	Removal of bottom end, slices and cubes, mixed
Carrot	Summer 2008	Belgium	3 chosen from a bundle	Upper and lower ends were removed, cubes from un-peeled carrots
Carrot (juice)	Autumn 2010	Germany	1 L, glass bottle	Juice was transferred to a beaker, aliquots weighed in centrifuge tubes
Cheese (25% fat)	Autumn 2010	Luxembourg	1 box, 200 g	Cottage cheese was stirred in a mortar and transferred to centrifuge tubes
Cheese (32% fat)	Autumn 2010	Luxembourg	1 pack, 250 g	Dry cheese was sliced with a kitchen knife, then cubes were cut
Cherry	Summer 2008	Luxembourg	50 chosen from a box	Removal of kernels, cutting and mixing of cubes
Corn, sweet	Summer 2008	[a]	2 from a box of 4 chosen	Sweet corn seeds were scraped from the trunk with a kitchen knife
Courgette	Summer 2008	Belgium	2 chosen from a bag of 6	Top and bottom end were removed, cubes cut and mixed
Eggs (total)	Autumn 2010	Netherlands	3 from a box of 6 chosen	Liquid inner parts of eggs transferred in a mortar, mixed and homogenized
Endive (curly)	Summer 2008	Belgium	1 endive	Removal of bottom end, slices and cubes from leaves (1 endive head)
Grapefruit (pink)	Autumn 2010	[a]	3 chosen from a bag of 7	Peel and other non-edible fibrous parts removed, flesh cut into cubes
Grapefruit (juice)	Autumn 2010	Germany	1 L, glass bottle	Juice was transferred to a beaker, aliquots weighed in centrifuge tubes
Grapes (green)	Autumn 2009	Italy	30 chosen from 500 g	Seedless grapes; stems removed, all other parts used and cut in cubes
Lamb's lettuce	Summer 2008	France	20 chosen from 250 g	Roots were removed, leaves sliced and cut
Leek	Summer 2008	France	2 chosen from a box of 4	Roots were removed, sliced in small rings and rings cut into cubes
Lentil, green, dry	Autumn 2010	France	100 g from a box of 1 kg	Lentils were directly ground in a mortar
Lentil, white, dry	Autumn 2010	France	100 g from a box of 1 kg	Lentils were directly ground in a mortar
Lettuce	Summer 2008	Belgium	1 lettuce head	Cubes from one salad, removal of bottom end
Margarine (normal)	Autumn 2010	Luxembourg	1 pack of 500 g	Thin slices from the surface were scraped with a kitchen knife
Margarine (light)	Autumn 2010	Luxembourg	1 pack of 500 g	Thin slices from the surface were scraped with a kitchen knife
Melon (cantaloupe)	Summer 2008	[a]	1 melon, ca. 1.5 kg	Peel was removed, flesh cut into cubes with a kitchen knife
Orange	Autumn 2009	[a]	3 chosen from a bag of 8	Peel and other non-edible fibrous parts removed, flesh cut into cubes)
Orange (juice)	Autumn 2009	Germany	1 L, glass bottle	Juice was transferred to a beaker, aliquots weighed in centrifuge tubes
Orange (mandarin)	Autumn 2009	[a]	3 chosen from a net of 10	Peel and other non-edible fibrous parts removed, flesh cut into cubes
Peach	Summer 2010	[a]	2 chosen from a box of 10	Removal of kernels, cubes were cut and mixed
Pear	Autumn 2010	France	2 chosen from a box of 3	Removal of stem and inner parts, cubes from edible parts
Peas (garden)	Summer 2010	[a]	100 g from a box of 250 g	Peas were not further cut, stored as they were
Pepper (green)	Summer 2008	Spain	2 peppers purchased loose	Removal of upper and inner parts, cubes from edible parts
Pepper (orange)	Summer 2009	Spain	2 peppers purchased loose	Removal of upper and inner parts, cubes from edible parts
Pepper (red)	Summer 2008	Spain	2 peppers purchased loose	Removal of upper and inner parts, cubes from edible parts
Pepper (yellow)	Summer 2008	Spain	2 peppers purchased loose	Removal of upper and inner parts, cubes from edible parts
Plum	Summer 2010	[a]	10 chosen from a box of 500 g	Removal of kernels, cubes were cut and mixed
Potato (parisienne)	Autumn 2010	Luxembourg	3 chosen from a net of 1.5 kg	Potatoes were cut into cubes, skin was kept, cubes were mixed
Potato (victoria)	Autumn 2010	France	3 chosen from a net of 1.5 kg	Potatoes were cut into cubes, skin was kept, cubes were mixed
Sour cream (30% fat)	Autumn 2010	Luxembourg	1 box of 250 g	Cream was stirred in a mortar and transferred to centrifuge tubes
Spinach	Summer 2008	France	5 from as box of 250 g	Removal of lower part of, leaves sliced and cut into cubes
Spinach (creamed)	Summer 2008	Germany	100 g from a box of 500 g	Frozen spinach was slightly thawed, homogenized in a kitchen mixer
Tomato	Summer 2008	Spain	3 from a net of 1 kg	Removal of stem and upper greenish parts, cut into cubes and mixed
Tomato (juice)	Autumn 2010	Germany	1 L, glass bottle	Juice was transferred to a beaker, aliquots weighed in centrifuge tubes
Tomato (ketchup)	Summer 2010	[a]	100 g from a bottle of 300 mL	Liquid was poured into a beaker, stirred, and aliquoted in centrifuge tubes
Watermelon	Summer 2008	[a]	1 melon (1.5 kg)	Removal of skin and kernels, cubes were cut from flesh and mixed

[a] Origin not known.

sealable centrifuge Falcon tubes (Fisher Scientific, Schwerte, Germany) leaving as little free air space as possible. For further analyses, the 10 g frozen aliquots were taken, ground by mortar and pestle under liquid nitrogen with the addition of weighted amounts of quartz sand to facilitate homogenization, and extracted for carotenoids as described below. For liquid or semi-liquid items, including fruit and vegetable juices, ketchup, sour cream and oils, aliquots stored at 4 °C were used directly after shaking or stirring. For butter and margarine, small blocks of ca. 1 cm^3 were taken from various parts of the package, and several aliquots of approx. 10 g were stored at 4 °C and further processed within several days. From these aliquots, subsamples were removed with a kitchen knife and further processed as described under carotenoid extraction. As for eggs, 3 eggs without shell were mixed in a mortar, and subsamples were immediately further processed as described below.

2.2. Carotenoid extraction

Unless otherwise stated, all chemicals used were of analytical grade or superior, and purchased from Sigma–Aldrich (Bornem, Belgium). The methodology of carotenoid extraction for the majority of food items, including fruits, vegetables, liquid and semi-liquid items and eggs was described elsewhere (Biehler et al., 2010). Approximately 1–3 g of material was used for further processing, and all analyses were carried out in independent replicas of 3 or larger. Matrices containing high concentrations of lipids (>10%) were enzymatically treated in order to remove lipids prior to extraction. To this end, 0.2–0.5 g of homogenised food matrix was weighed into a 50 mL centrifuge tube and 200 mg of monoolein, 10 mg of oleic acid, 120 mg of lecithin and 0.5 mL of canola oil of negligible carotenoid content (Colzol, Clemency, Luxembourg) were added to achieve emulsification of lipids

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including carotenoids. The mixture was brought to a total volume of 50 mL with physiological saline containing sodium taurocholate (1.1 mg/mL) and sonicated for 20 min. An aliquot of 15 mL was taken and 9 mL of a bile salt (commercial porcine bile mixture; 24 mg/mL) and porcine pancreatin (commercial mixture of pancreatic digestive enzymes; 4 mg/mL, both diluted in 0.1 M $NaHCO_3$) solution were added, filled up to 50 mL with physiological saline (final pH approx. 7) and incubated at 37 °C for 4 h. An aliquot of 4 mL was extracted with an equal volume of hexane:acetone (1:1, v/v) twice, 15 mL of saturated aqueous sodium chloride solution was then added, and vigorously shaken to foster transition of the remaining carotenoids from the aqueous phase into the organic phase. The carotenoid-containing organic fractions were combined, evaporated to dryness under a stream of nitrogen, and analysed directly or covered with a blanket of argon and stored under −80 °C for a maximum of 2 weeks. Residues were reconstituted in 150 μL MTBE:methanol (3:7, v/v) and 25 μL were injected for high pressure liquid chromatography (HPLC) analysis.

2.3. Carotenoid quantification

For carotenoid quantification, including the less frequently studied carotenoids neoxanthin, violaxanthin, phytoene and phytofluene, earlier protocols were applied (Biehler et al., 2010). Briefly, a YMC C30 column (Waters Inc., Zellik, Belgium, 150× 4.6 mm, 3 μm particle size, set at 28 °C) was used in combination with a Dionex HPLC instrument together with a UVD340S photodiode array detector (Dionex Benelux B.V., Amsterdam, The Netherlands). The detector was simultaneously set at 285 nm (detection of phytoene), 350 nm (phytofluene), 450 nm (β-carotene, lutein, β-cryptoxanthin and zeaxanthin) and 472 nm (neoxanthin, violaxanthin and lycopene). Employing this configuration, violaxanthin and neoxanthin concentrations were approx. 5% and 16% underestimated, respectively, as optimal sensitive absorption wavelengths could not be chosen simultaneously for all compounds. Quantification was based on external calibration and was carried out in triplicate. Retention times were used to differentiate the major abundant carotenoids, including the main *cis*-isomers such as 9-*cis* beta-carotene. Individual *cis/trans* isomers were, if detected, expressed as concentrations of the respective *trans*-isomer. The retention times and the spectra were compared to data from the literature (e.g. Britton et al., 2004) and to standards. Carotenoid standards and their extinction coefficients were obtained from CaroteNature (Lupsingen, Switzerland: neoxanthin (115,000 L mol^{-1} cm^{-1}), violaxanthin (145,000 L mol^{-1} cm^{-1}), phytoene (40,000 L mol^{-1} cm^{-1}), and phytofluene (120,000 L mol^{-1} cm^{-1})), Extrasynthèse (Lyon, France: β-cryptoxanthin (135,000 L mol^{-1} cm^{-1}), lycopene (180,000 L mol^{-1} cm^{-1}), zeaxanthin (140,000 L mol^{-1} cm^{-1})), and Sigma–Aldrich (β-carotene (140,000 L mol^{-1} cm^{-1}), lutein (150,000 L mol^{-1} cm^{-1})). Extinction coefficients for Sigma–Aldrich products were taken from literature (Budavari, 1989). Purity for all standards was above 95% (as certified by the suppliers), except for zeaxanthin, which was of 90% purity.

2.4. Food consumption and carotenoid intake

Data regarding the population carotenoid intake, in terms of frequency and food content, were obtained from the ORISCAV-LUX study (Alkerwi et al., 2010b); the first nationwide, cross-sectional, population-based study ever carried out in Luxembourg, between November 2007 and January 2009. It was based on a stratified systematic random sample selection from the general residents in Luxembourg, aged 18–69 years ($n = 1432$). The ORISCAV-LUX study collected a wide range of nutritional data. The dietary habits during the preceding 3 months were assessed using a self-administered Food Frequency Questionnaire, which consisted of 9 alimentary groups (starchy foods, fruits, cooked and raw vegetables, meat-poultry-fish-egg, prepared dishes, dairy products, fats, miscellaneous and drinks), subdivided into 134 items. Estimates of daily intake of energy, food, beverages and selected nutrients were calculated according to the French Table of Composition, which has been developed for the French SU.VI.MAX study (Hercberg et al., 2004).

In a first step, 8 out of 9 food categories, excluding prepared dishes, were further investigated for food items potentially rich in carotenoids (>50–100 μg/100 g). In a second step, subcategories rich in food items containing high amounts of carotenoids, 20 out of 107, were selected (Table 3). Then, the global mean values for carotenoid intake, for both genders, were calculated.

As some of the selected subcategories were pooling various individual food items of different carotenoid content and patterns, without detailing the per capita consumption for each individual food item, the relative (percentage) contribution of the individual food items had to be estimated for each subgroup by additional means, i.e. through integrating Luxembourgish food disappearance data, generously provided by CACTUS S.A., Windhof, Luxembourg, into the analyses. This percentage contribution of each individual food item within a subgroup was then multiplied by the total consumption of food in this subgroup according to the ORISCAV-LUX data, yielding the total amount of each consumed food item in the respective subgroup. An example is given for a hypothetical group:

$$m(\text{Food}_1) = m\left(\sum \text{Foods}\right) \times \%\text{Food}_1 \quad (1)$$

with $m(\text{Food}_1)$ being the amount of a food item consumed per capita in this subgroup, $m(\sum \text{Foods})$ the total amount of all consumed food items combined in this subgroup per capita, obtained from the ORISCAV-LUX study, and $\%\text{Food}_1$ the percentage contribution of the food item in relation to the total amount of consumed foods in this subgroup, based on Luxembourgish food disappearance data if not available from the ORISCAV-LUX study.

In addition, in case of non availability of local data, calculation of percentage of contribution was further validated by consumption data obtained from the 5th Swiss Report of Nutrition (Gremaud et al., 2005). These data were chosen, on the one hand, owing to the detailed nature of this report and on the other hand, because the culinary culture of Luxembourg is to some extent similar to Swiss cuisine, both of which are strongly influenced by German and French gastronomy.

In a final step, carotenoid intake was obtained by multiplying the carotenoid content of locally grown, produced, and/or consumed foods determined via our HPLC analyses by multiplication with the per capita consumption of this food item derived as explained above.

3. Results

3.1. Carotenoid content in frequently consumed fruits and vegetables

In total, of the 50 chosen food items, carrot (20.0 mg/100 g), fresh spinach (13.2 mg/100 g) and apricot (12.3 mg/100 g) were food items rich in total carotenoids (Table 2) whereas sour cream (0.03 mg/100 g) contained very little carotenoids. Regarding individual carotenoid species, carrots presented a rich source of α- and β-carotene (17.9 mg/100 g), as was carrot juice (4.9 mg/100 g) and cream-spinach (4.2 mg/100 g). β-Cryptoxanthin (also a vitamin A precursor) concentration in mandarin was determined at 2.6 mg/100 g, followed by oranges (1.3 mg/100 g) and orange juice (0.4 mg/100 g). Concerning the non-provitamin A carotenes, lycopene in tomato ketchup was determined at 8.8 mg/100 g, in

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Table 2
Carotenoid content in food items sampled in Luxembourg as determined by high pressure liquid chromatography. Values are presented in mg/100 g edible portion.

Food item[a]	Latin term	Mean concentration in mg/100 g edible portion									
		Carotenes[b]	β-Crypto	Neoxanthin	Lutein	Lycopene	Phytofluene	Phytoene	Violaxanthin	Zeaxanthin	Total ± SD
Apple (red)	*Pyrus malus*	0.041 ± 0.008	–[c]	0.150 ± 0.033	–[c]	–[c]	–[c]	–[c]	0.105 ± 0.033	–[c]	0.30 ± 0.03
Apricot	*Prunus armenica*	2.409 ± 0.432	–	–	–	0.321 ± 0.042	2.382 ± 0.294	7.187 ± 0.963	–[c]	–	12.23 ± 1.82
Arugula	*Eruca sativa*	3.425 ± 0.714	–	0.966 ± 0.017	3.563 ± 0.859	–	–	–	2.409 ± 0.319	–	10.36 ± 1.89
Aubergine	*Solanum melongena*	0.040 ± 0.012	–	–	0.135 ± 0.051	–	–	–	0.044 ± 0.014	–	0.22 ± 0.07
Banana	*Musa paradisia*	0.242 ± 0.195	–	–	0.082 ± 0.048	–	–	0.032 ± 0.022	–	–	0.36 ± 0.19
Butter	–	0.318 ± 0.006	–	–	–	–	–	–	–	–	0.32 ± 0.01
Bean (green)	*Phaseolus vulgaris*	0.800 ± 0.023	0.012 ± 0.002	0.072 ± 0.018	0.672 ± 0.030	–	–	–	0.628 ± 0.014	–	2.18 ± 0.06
Blackberry	*Rubus ulmifolius*	0.123 ± 0.012	–	–	0.154 ± 0.016	–	–	–	–	–	0.28 ± 0.03
Broccoli	*Brassica oleracea*	0.180 ± 0.011	–	–	–	–	–	–	–	–	0.18 ± 0.01
Cabbage (green)	*Brassica oleracea*	0.653 ± 0.091	–	0.211 ± 0.039	0.701 ± 0.103	–	–	–	0.432 ± 0.060	–	2.00 ± 0.29
Carrot	*Daucus carota*	17.932 ± 2.058	–	–	0.081 ± 0.012	–	0.567 ± 0.051	1.399 ± 0.155	–	–	19.98 ± 2.25
Carrot (juice)	*Daucus carota*	4.885 ± 0.088	–	–	0.197 ± 0.004	–	0.551 ± 0.007	1.287 ± 0.021	–	–	6.92 ± 0.11
Cheese (25% fat)	–	0.083 ± 0.019	–	–	–	–	–	–	–	–	0.08 ± 0.01
Cheese (32% fat)	–	0.075 ± 0.013	–	–	–	–	–	–	–	–	0.08 ± 0.01
Cherry	*Prunus avium*	0.032 ± 0.006	–	–	–	–	–	0.039 ± 0.009	0.303 ± 0.032	–	0.37 ± 0.05
Corn, sweet	*Zea mays*	–	–	–	0.618 ± 0.087	–	–	–	–	0.439 ± 0.065	1.06 ± 0.16
Courgette	*Curcubita pepo*	0.082 ± 0.023	–	0.101 ± 0.019	0.864 ± 0.226	–	–	–	0.326 ± 0.040	–	1.37 ± 0.31
Eggs (total)		–	–	–	0.634 ± 0.106	–	–	–	–	0.051 ± 0.006	0.69 ± 0.10
Endive (curly)	*Cichorium endivia*	0.707 ± 0.064	0.290 ± 0.040	0.609 ± 0.158	0.221 ± 0.041	–	–	–	0.430 ± 0.093	0.023 ± 0.009	2.28 ± 0.36
Grapefruit (pink)	*Citrus paradise*	1.716 ± 0.145	0.400 ± 0.041	–	0.077 ± 0.012	2.553 ± 0.315	0.208 ± 0.034	0.617 ± 0.060	–	0.186 ± 0.036	5.76 ± 0.47
Grapefruit (juice)	*Citrus paradise*	0.632 ± 0.466	–	–	0.028 ± 0.006	0.941 ± 0.134	0.077	0.212 ± 0.013	–	0.069 ± 0.016	1.96 ± 0.22
Grapes (green)	*Vitis vinifera*	0.050 ± 0.007	–	–	–	–	–	–	–	–	0.05 ± 0.01
Lamb's lettuce	*Valerianella locusta*	2.486 ± 0.338	–	0.863 ± 0.061	2.941 ± 0.170	–	–	–	1.407 ± 0.085	–	7.70 ± 0.60
Leek	*Allium ampeloprasum*	0.179 ± 0.027	–	0.988 ± 0.236	0.358 ± 0.057	–	–	–	0.698 ± 0.130	–	2.22 ± 0.45
Lentil, green, dry	*Lens culinaris*	0.020 ± 0.004	–	0.042 ± 0.006	1.196 ± 0.160	–	–	–	0.051 ± 0.017	0.319 ± 0.050	1.63 ± 0.22
Lentil, white, dry	*Lens culinaris*	0.028 ± 0.001	–	–	1.061 ± 0.062	–	–	–	–	0.290 ± 0.028	1.39 ± 0.09
Lettuce	*Lactuca sativa*	0.725 ± 0.163	–	0.078 ± 0.044	0.657 ± 0.183	–	–	–	0.362 ± 0.124	–	1.82 ± 0.63
Margarine (normal)	–	0.598 ± 0.035	–	–	0.053 ± 0.010	–	–	–	–	0.063 ± 0.017	0.71 ± 0.05
Margarine (light)	–	0.512 ± 0.015	–	–	0.037 ± 0.024	–	–	–	–	0.031 ± 0.025	0.58 ± 0.04
Melon (orange)	*Cucumis melo*	3.214 ± 0.590	–	–	–	–	–	0.247 ± 0.027	–	–	3.46 ± 0.62
Orange	*Citrus reticulate*	–	1.275 ± 0.073	–	–	–	0.185 ± 0.034	0.554 ± 0.047	–	–	2.02 ± 0.11
Orange (juice)	*Citrus reticulate*	–	0.412 ± 0.026	–	–	–	–	–	–	–	0.41 ± 0.03
Orange (mandarin)	*Citrus sinensis*	0.175 ± 0.017	2.568 ± 0.231	–	–	–	0.102 ± 0.005	0.200 ± 0.021	–	–	3.04 ± 0.28
Peach	*Prunus persica*	0.086 ± 0.028	0.081 ± 0.001	–	–	–	0.070 ± 0.003	0.163 ± 0.007	1.157 ± 0.059	–	1.56 ± 0.05
Pear	*Pyrus communis*	–	–	–	0.048 ± 0.016	–	–	–	–	–	0.05 ± 0.02
Peas (garden)	*Pisum sativum*	0.912 ± 0.041	0.021 ± 0.003	0.520 ± 0.079	1.766 ± 0.062	–	–	–	0.365 ± 0.047	0.028 ± 0.007	3.61 ± 0.11
Pepper (green)	*Capsicum annuum*	0.457 ± 0.011	0.017 ± 0.006	0.503 ± 0.055	0.373 ± 0.020	–	–	–	0.457 ± 0.009	0.361 ± 0.019	2.17 ± 0.06
Pepper (orange)	*Capsicum annuum*	0.784 ± 0.103	–	–	3.517 ± 0.363	–	0.317 ± 0.389	1.005 ± 0.112	0.252 ± 0.013	4.206 ± 0.725	10.08 ± 1.68
Pepper (red)	*Capsicum annuum*	3.647 ± 0.782	–	–	0.510 ± 0.590	–	0.511 ± 0.014	1.698 ± 0.520	0.577 ± 0.185	1.516 ± 0.040	8.46 ± 1.26
Pepper (yellow)	*Capsicum annuum*	–	–	–	2.221 ± 0.623	–	0.221 ± 0.553	0.418 ± 0.106	4.165 ± 0.902	0.027 ± 0.007	7.05 ± 1.82
Plum	*Prunus domestica*	0.031 ± 0.004	–	0.115 ± 0.019	0.033 ± 0.009	–	–	–	0.054 ± 0.010	–	0.23 ± 0.04
Potato (parisienne)	*Solanum tuberosum*	–	–	0.244 ± 0.007	0.134 ± 0.001	–	–	–	0.293 ± 0.025	–	0.67 ± 0.01
Potato (victoria)	*Solanum tuberosum*	–	–	0.173 ± 0.017	0.101 ± 0.007	–	–	–	0.240 ± 0.014	–	0.52 ± 0.03
Sour cream (30% fat)	–	0.032 ± 0.000	–	–	–	–	–	–	–	–	0.03 ± 0.00
Spinach	*Spinacia oleracea*	4.215 ± 0.656	0.062 ± 0.019	0.841 ± 0.143	4.866 ± 0.387	–	–	–	2.765 ± 0.242	0.445 ± 0.021	13.20 ± 1.37
Spinach (creamed)	–	4.259 ± 0.198	0.025 ± 0.008	0.603 ± 0.047	3.442 ± 0.200	–	–	–	2.447 ± 0.052	0.330 ± 0.018	11.11 ± 0.50
Tomato	*Solanum lycopersicum*	0.522 ± 0.114	–	–	–	2.719 ± 0.357	0.401 ± 0.054	1.388 ± 0.156	–	–	5.03 ± 0.26
Tomato (juice)	*Solanum lycopersicum*	0.131 ± 0.003	–	–	–	3.040 ± 0.223	0.441 ± 0.014	1.642 ± 0.025	–	–	5.26 ± 0.59
Tomato (ketchup)	–	0.169 ± 0.021	–	–	–	8.774 ± 0.438	1.034 ± 0.097	3.494 ± 0.279	–	–	13.47 ± 0.83
Watermelon	*Citrullus lanatus*	0.878 ± 0.055	–	–	–	0.309 ± 0.020	0.443 ± 0.026	1.172 ± 0.077	–	–	2.80 ± 0.15

[a] For more details on food items including number of investigated items, see Table 1.
[b] Carotenes: α- and β-carotene; β-crypto: β-cryptoxanthin.
[c] Not detected (LOD for β-carotene and β-cryptoxanthin: 12 μg/100 g; lycopene 16 μg/100 g; phytofluene 17 μg/100 g; zeaxanthin 22 μg/100 g; lutein 28 μg/100 g; violaxanthin 44 μg/100 g, neoxanthin 30 μg/100 g; phytoene 38 μg/100 g) as determined by Zorn et al. (1997).

tomato juice at 3.0 mg/100 g and in tomatoes at 2.7 mg/100 g. Apricot yielded the highest measured content of phytofluene (2.4 mg/100 g), ensued by tomato ketchup (1.0 mg/100 g) and carrot/carrot juice/red bell pepper (0.5 mg/100 g). Apricot was also the richest source of phytoene (7.2 mg/100 g), followed by tomato ketchup (3.5 mg/100 g) and red bell pepper (1.7 mg/100 g).

Concerning the xanthophylls, lutein in spinach was 4.9 mg/100 g, ensued by arugula (3.6 mg/100 g) and orange peppers (3.5 mg/100 g). Orange bell pepper was a rich source of zeaxanthin (4.2 mg/100 g), as were red bell peppers (1.5 ± 0.1 mg/100 g) and spinach/sweet corn (both 0.4 mg/100 g). Regarding the epoxycarotenoid xanthophylls, leek (1.0 mg/100 g) and arugula (1.0 mg/100 g) constituted rich sources of neoxanthin, as was lamb's lettuce (0.9 mg/100 g). Yellow bell peppers were especially rich in violaxanthin (4.4 mg/100 g), as was spinach (2.8 mg/100 g) and creamed spinach (2.5 mg/100 g). Further details are presented in Table 2.

3.2. Carotenoid intake including epoxycarotenoids and lycopene precursors

Combining food per capita consumption data [g/day] with the respective measured carotenoid content, the average carotenoid intake of the Luxembourgish population was estimated. Notwithstanding phytoene and phytofluene, combining α- and β-carotene, β-cryptoxanthin, neoxanthin, lutein, lycopene, violaxanthin and zeaxanthin, total carotenoid intake per person and per day was calculated as 14.3 mg. Including carotenoid precursors, this was estimated as 17.0 mg/day. Main contributors to daily intake were carrots (5.5 mg total carotenoids/day), green leafy vegetables (1.9 mg/day) and citrus fruits (1.9 mg/day, Table 3).

The contribution of the epoxycarotenoids to total carotenoid intake was estimated as 10% (1.7 mg), while the contribution of the precursors phytoene and phytofluene to total carotenoid intake was assessed as 16% (2.7 mg), i.e. a total of 26% of carotenoids were consumed in the form of these less frequently investigated species. This high contribution was mostly due to the consumption of green-leafy vegetables and tomato products for epoxycarotenoids and precursors, respectively, contributing to >33% and 20% of epoxycarotenoid and carotenoid precursor intake, respectively. Considering individual carotenoid species, α- and β-carotene constituted 44.8% (7.6 mg) of daily intake followed by phytoene (12.0% or 2.0 mg) and lycopene (10.6% or 1.8 mg).

3.3. Carotenoid intake compared with other countries

Compared to other European countries (Pelz et al., 1998; O'Neill et al., 2001; Lucarni et al., 2006), estimated comparable total carotenoid intake (α- and β-carotene, β-cryptoxanthin, lutein, lycopene and zeaxanthin) in Luxembourg appeared to be slightly lower, especially when compared to Italy. However, people in Germany and in Madrid (Spain) seemed to consume less carotenoids (Fig. 2). With respect to non-European-countries, carotenoid intake in Israel exceeded that of Luxembourg by more than 60% (Chaiter et al., 2007) and that of Australia by approximately 20% (Manzi et al., 2002).

While the intake of α- and β-carotene appeared above that of other European countries, especially compared to Italy, Luxembourgish lycopene intake was lower in comparison to all other countries, except for Spain and Germany. Notably, α- and β-carotene consumption in Israel alone surpassed total carotenoid

Table 3
Estimated carotenoid intake in Luxembourg. Food intake (g/day) from Luxembourgish food subgroups was combined with average carotenoid concentrations in the consumed food subgroups to yield carotenoid intake (mg/day).

Rank	Food group	Mean (g/day)[a]	Carotenoid intake (mg/day)[a]									
			Carot[b]	Lyco[b]	Lutein	Zeaxa[b]	β-Crypto[b]	Viola[b]	Neo[b]	Phyto[b]	Phytofl[b]	Total ± SEM
1	Carrots (fresh and processed)	27.5	4.932	–[c]	0.022	–	–	–	–	0.385	0.156	5.496 ± 3.45
2	Green-leafy vegetables (spinach, endive, etc.)	25.6	0.603	–	0.616	0.057	0.035	0.391	0.184	–	–	1.885 ± 1.90
3	Citrus fruits	82.4	0.135	0.143	0.004	–	1.149	–	–	0.323	0.113	1.867 ± 2.25
4	Tomatoes (raw, processed, and sauce)	36.6	0.191	0.994	–	–		–	–	0.508	0.147	1.834 ± 0.86
5	Bell peppers	18.3	0.223	–	0.161	0.186	0.001	0.223	0.021	0.190	0.061	1.067 ± 1.54
6	Fruit juices (canned, bottled, tetra-packed)	114.1	0.240	0.358	0.011	0.026	0.157	–	–	0.086	0.031	0.909 ± 0.76
7	Pear, apple, pineapple, melons	90.3	0.485	0.014	0.021	0.004	0.014	0.056	0.079	0.095	0.031	0.800 ± 0.64
8	Salads (lettuce, lambs lettuce, ice-berg salad, etc.)	21.1	0.194	–	0.182	–	–	0.104	0.032	–	–	0.512 ± 0.64
9	Plum, grape, nectarine, cherry, peach, apricot	22.0	0.093	0.012	0.001	–	–	0.042	–	0.269	0.090	0.507 ± 0.32
10	Vegetable juice (canned, tetra-packed, bottles)	6.9	0.172	0.104	0.007	–	–	–	–	0.101	0.034	0.418 ± 1.01
11	Potatoes (all kinds of preparations)	65.8	–	–	0.077	0.001	–	0.175	0.137	–	–	0.391 ± 0.87
12	Other vegetables: aubergine, cucumber, courgette	39.9	0.074	–	0.162	0.002	0.015	0.134	–	–	–	0.386 ± 0.23
13	Ketchup	1.9	0.033	0.170	–	–	–	–	–	0.068	0.020	0.290 ± 0.45
14	Broad beans, lentils, french beans, peas (chick)	15.0	0.038	–	0.101	0.014	0.001	0.024	0.014	–	–	0.192 ± 0.20
15	Banana	37.6	0.091	–	0.031	0.006	–	–	–	0.012	0.001	0.141 ± 0.14
16	Eggs (all kinds and preparations)	13.4	–	–	0.085	0.007	–	–	–	–	–	0.115 ± 0.09
17	Cabbage, average	18.9	0.037	–	0.025	0.001	0.008	0.023	0.022	–	–	0.092 ± 0.09
18	Butter, margarine, sour cream	14.1	0.054	–	0.005	0.003	–	–	–	–	–	0.061 ± 0.09
19	Olive oil	11.9	0.016	–	0.019	–	–	–	–	–	–	0.035 ± 0.02
20	Cheese total	21.5	0.017	–	0.002	–	–	–	–	–	–	0.019 ± 0.02
	Σ:	684.6	7.6	1.8	1.5	0.3	1.4	1.2	0.5	2.0	0.7	17.0 ± 10.5

[a] Food consumption data from $n = 1432$ subjects were collected within the ORISCLAV-LUX study (Alkerwi et al., 2010b); mean: mean intake; SEM: standard error of the mean.
[b] Carot: α- and β-carotene; [+]lyco: lycopene; zeaxa: zeaxanthin; β-crypto: β-cryptoxanthin, viola: violaxanthin, neo: neoxanthin, phyto: phytoene, phytofl: phytofluene.
[c] Carotenoid content of the food group below limit of detection (below ca. 0.0005 mg/day).

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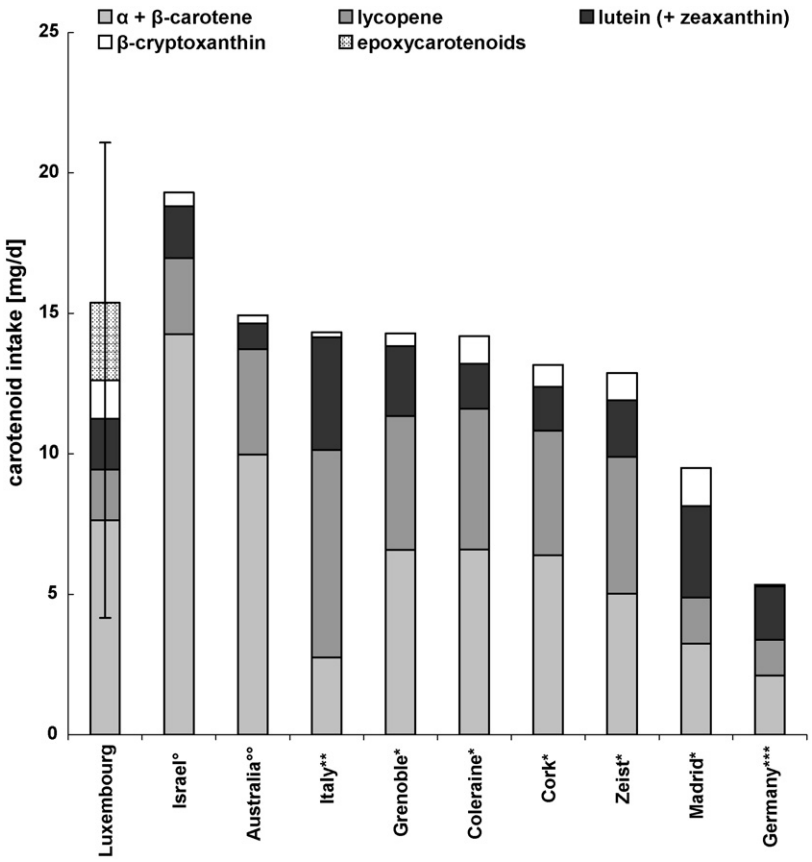

Fig. 2. Comparison of carotenoid intake in Luxembourg to other European countries (* Grenoble/France; * Coleraine/Northern Ireland; *Cork/Republic of Ireland; *Zeist/The Netherlands; *Madrid/Spain (O'Neill et al., 2001); ** Italy (Lucarni et al., 2006), and *** Germany (Pelz et al., 1998)) and to °Israel (Chaiter et al., 2007) and°°Australia (Manzi et al., 2002). Intake from own study includes additionally epoxycarotenoids, data represents mean ± SEM.

intake in Luxembourg and other European countries (Fig. 2); however, the third highest intake was detected in Luxembourg. Although lutein intake in Luxembourg was low compared to Italy and Madrid, it was relatively high when compared to all other countries.

4. Discussion

4.1. Epoxycarotenoids and lycopene precursor significance

Food databases including information on selected carotenoids, such as β-carotene, lutein/zeaxanthin and lycopene, have existed for many years, mostly in European countries and the US (Mangels et al., 1993; Souci et al., 2000; O'Neill et al., 2001; BAG, 2004; USDA, 2010), but also in others, such as Israel (Chaiter et al., 2007). While often listing a large number of food items, none of these databases have aimed to take into account other carotenoid species, including epoxycarotenoids and precursors such as neoxanthin, violaxanthin, and phytoene and phytofluene, respectively. In addition, the intake of other carotenoids also remains poorly addressed, including astaxanthin from some fish varieties (Rufer et al., 2008), and algae carotenoids such as fucoxanthin (Asai et al., 2008), albeit their consumption is possibly limited. In the present first carotenoid assessment study in Luxembourg, we have estimated that the combined contribution of neoxanthin, violaxanthin, phytoene and phytofluene to carotenoid intake is greater than 25%. To our knowledge, there exist no other attempts to estimate their contribution to total carotenoid intake.

Although neither epoxycarotenoids nor carotenoid precursors phytoene and phytofluene are assumed to exhibit any pro-vitamin A activity, a number of beneficial health effects have been reported. For example, similar anti-cancer propagation effects were found for phytoene and phytofluene compared to lycopene (Nishino, 1998; Hirsch et al., 2007), and comparable effects were reported for epoxycarotenoids and their digestion products (Asai et al., 2004). Furthermore, phytoene and phytofluene could protect the skin from harmful UV light effects due to excess sun exposure (Aust et al., 2005; Engelmann et al., 2011), and inhibited LDL oxidation, at least in vitro (Shaish et al., 2008).

A prerequisite for carotenoid activity *in vivo* is a sufficiently high bioavailability. While absorption via appearance in the blood stream for phytoene and phytofluene has been demonstrated and is at least comparable to other carotenoids (Paetau et al., 1998; Aust et al., 2005; Bohn, 2008), it is known that epoxycarotenoids such as neoxanthin and violaxanthin *per se* are not or only to a minor extend absorbable (Asai et al., 2008). Epoxycarotenoids have been shown to undergo rapid expoxide-furanoid transition in the acid milieu of the stomach, resulting, e.g. in the formation of neochrome from neoxanthin and auroxanthin and luteoxanthin from violaxanthin (Asai et al., 2004; Biehler et al., 2011). Even though the further fate of the resulting carotenoids remains to be elucidated, their uptake into human intestinal epithelial cells has recently been demonstrated (Biehler et al., 2011), and some products such as neochrome were detected in the plasma of mice in earlier studies (Asai et al., 2004). Thus, as epoxycarotenoids and their degradation products do not appear to be well absorbable, it is possible that they are

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further metabolized prior to their secretion into the lymphatic system.

4.2. Carotenoids in food items

On average, total carotenoid content in Luxembourgish food items (Table 2) were similar to those reported in other studies in the US and European countries, i.e. France, Germany, Ireland, The Netherlands, Spain and the UK (Bauernfeind, 1972; Mangels et al., 1993; Müller, 1997; O'Neill et al., 2001). Considerable differences compared to published data, however, were detected for individual food items, up to a factor of 3, e.g. as found for watermelon compared to O'Neill et al. (2001).

Carotenoid content in plant food depends on a multitude of factors, such as differences in climate, the availability of water and nutrients in the soil (Chenard et al., 2005), varying cultivars, and time of harvest and ripening state (reviewed by Britton and Khachik, 2009). Ripening state alone was shown to impact carotenoid content by approximately 25%, as shown in carrots (Fraser and Bramley, 2004). For tomatoes, substantial differences in lycopene content were detected (up to a factor 3) between varieties matured in green house vs. grown on fields (Müller, 1997). Similar as for total carotenoids, considerable differences for individual carotenoids were also detected compared to literature. For example, carotene in carrots, a major source of carotenoids, was found as 17.9 mg/100 g in the present study, identical to the Israeli database, but differing from a US (28.9 mg/100 g, USDA, 2010) and a European database (10.2 mg/100 g, O'Neill et al., 2001). Similarly, for lutein, over 4.5-fold differences in concentration were found for some items such as for lettuce (Müller, 1997).

Lycopene, a potent antioxidant (Bohn, 2008), was also found to differ in concentrations from other reports. Concentrations in watermelons were only 15% of values reported by Souci et al. (2000), and even lower concentrations down to 0.3 mg/100 g (Chaiter et al., 2007) were reported. Besides the conditions listed above, a highly aqueous matrix with varying water content as in watermelon, may pose additional analytical challenges during homogenization, weighing and extraction (Chaiter et al., 2007).

Concentrations of epoxycarotenoids and carotenoid precursors in foods are generally not described in food databases. When comparing Luxembourgish data to earlier results from a study on selected German food items (Müller, 1997), violaxanthin and neoxanthin concentrations were, on average, found to be similar, while here the presented concentrations of carotenoid precursors phytoene and phytofluene were higher. Although generally of lower abundance, for some food items, such as yellow bell pepper, epoxycarotenoids constituted up to 68% of total carotenoids, while phytoene/phytofluene contribution in apricot was as high as 78% (Table 2).

While plant foods generally show a high variability, carotenoid contents in animal products, such as in eggs, appeared more uniform in their concentration, perhaps due to strict regulations by the European Union concerning additives in foods and feeds (reviewed by Breithaupt, 2008). Lutein concentrations in Luxembourgish eggs were almost identical compared to other reports (O'Neill et al., 2001). Differences in dairy products, such as butter, margarine and cheese were also low, ca. 10–30% (Souci et al., 2000; O'Neill et al., 2001).

4.3. Carotenoid intake

Although differences in total carotenoid intake existed when comparing Luxembourg to other countries (Fig. 2), main contributing items (carrots and green leafy vegetables) were similar across all countries compared, except for Italy where tomatoes played a more predominant role. Compared to other European countries, total carotenoid intake in Luxembourg was between 1% (The Netherlands) and 13% (Italy and Grenoble (France), Fig. 2) lower and between 25% (Madrid/Spain) and 58% (Germany) higher. Concerning individual carotenoids, Luxembourgish β-cryptoxanthin intake (1.4 mg) was estimated to be very high, α- and β-carotene intake high (7.6 mg), lutein/zeaxanthin intake rather low (1.5 mg) and lycopene intake very low (1.8 mg, Fig. 1). Furthermore, the heterogeneous study designs and databases made the inter-countries comparison an extremely difficult task. Studies that also investigated carotenoid intake differed in the population examined, e.g. global population (Pelz et al., 1998) vs. subjects in larger cities (O'Neill et al., 2001), including various age populations ranging from all ages (Lucarni et al., 2006) to a cohort averaging 70 years (Chaiter et al., 2007), influencing data structure. For example, data presentation as either median and range (O'Neill et al., 2001; Chaiter et al., 2007) vs. mean and standard deviation (SD) (Lucarni et al., 2006) suggests differing intake distribution patterns (Gaussian vs. non-Gaussian).

With respect to the pro-vitamin A carotenoids, existing knowledge appears insufficient to establish an 'adequate intake' or a 'recommended dietary allowance (RDA)', partly due to the unknown and controversially discussed conversion rates to vitamin A (Institute of Medicine 2000). To cover the RDA of 800 μg vitamin A (Institute of Medicine 2000), approximately 9.6 mg of β-carotene would be required. Considering the already high intake of vitamin A in industrialised countries (approx. 2200 μg), such as the US (Chasan-Taber et al., 1999), it appears that the intake of provitamin A carotenoids in Luxembourg is more than sufficient.

It is difficult to compare intake data of epoxycarotenoids and the precursors phytoene and phytofluene across countries, due to the limited studies available. As phytoene and phytofluene are mostly consumed within tomato products, it is possible to estimate their intake if lycopene intake is known; however, the ratio of lycopene to its precursors in fruits and vegetables is somewhat variable, around 1:2–1:4 due to own and published data (Khachik et al., 2002; Aust et al., 2005). Although epoxycarotenoids appear more ubiquitously distributed, a ratio of approximately 1:1 compared to β-carotene, at least in green leafy vegetables, can be roughly estimated based on published (Müller, 1997) and own data.

Differences in the intake of other individual carotenoids were observed. For example, lycopene intake in one Italian study (Lucarni et al., 2006) exceeded that of Luxembourg by a factor of 4 (Fig. 2). In addition to varying local dietary patterns, these differences might be to some extent due to our incomplete local carotenoid database with respect to complete dishes such as fast- and convenience foods, which are often rich in processed tomato products, such as tomato sauce and ketchup, and hence in lycopene (Khachik et al., 1986; O'Neill et al., 2001; Khachik et al., 2002). These food items were not included in our study, as detailed analyses of all potential carotenoid containing dishes would have burst the scope of the present investigation. However, when Luxembourgish consumption data of pizza, pasta and vegetable soup were multiplied with carotenoid contents from the O'Neill data base (2001) and taken into account, a similar lycopene intake compared to other European cities was obtained (4.7 mg/day vs. 1.8 mg/day without these dishes).

It is important to note some limitations of the present investigations. First of all, we only investigated 50 individual food items, sampled during two years, and only from 1 to 3 independent samples. Secondly, although the ORISCAV-LUX sample representativeness has been validated, we cannot exclude the possibility that the non-responders had different eating patterns compared to the responders in the present study (Alkerwi et al., 2010a). It appeared that non-responders were especially workers, younger

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subjects and Portuguese, with potentially different dietary behaviour. Finally, longer dietary history assessments have been criticized for being less precise in assessing dietary intake, as subjects could forget many items over time, such as small snacks, resulting in under-reporting of food intake (Kortzinger et al., 1997). In addition, the ORISCAV-LUX study was originally not foreseen for estimating carotenoid intake, and many relevant items with high carotenoid content were not individually noted, but merged into consumption groups such as green and leafy vegetables. Therefore, alternative sources, such as food disappearance data, were needed to assess the consumption frequency of lacking individual items. However, disappearance data were only employed to estimate relative contributions of individual food species inside the food groups and not to calculate total food intake.

On the other hand, considering the small country size and low number of approximately 500,000 residents (STATEC, 2010), the ORISCAV-LUX study was done on a sufficiently large representative sample of the population. Other studies, such as the carotenoid uptake study in Italy (Lucarni et al., 2006) were conducted with similar numbers of participants, although the entire populations were much (>120 times) larger, or with a low number of subjects (ca. 75/city) (O'Neill et al., 2001).

5. Conclusion

In summary, the present study reports, for the first time, an estimate for the significant contribution of the less frequently studied epoxycarotenoids violaxanthin and neoxanthin and the precursors phytoene and phytofluene, toward total carotenoid intake, and further supplied the first set of data of carotenoid consumption in Luxembourg.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jfca.2011.07.005.

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