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Relation between in vitro lipid digestion and β-carotene bioaccessibility in β-carotene-enriched emulsions with different concentrations of L-α-phosphatidylcholine

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ABSTRACT

In view of consumer health, it is desirable to promote the bioaccessibility of lipid-soluble compounds like carotenoids, while limiting the lipid intake. The objective of this work was to examine the relation between in vitro lipid digestion and β-carotene bioaccessibility of carrot-based model food emulsions containing water, 5% olive oil enriched with β-carotene (from carrots) and different concentrations (1–2–3–4%) of L-α-phosphatidylcholine (PHC), as an emulsifier. The lipid digestion (hydrolysis of triacylglycerols (TAGS) and incorporation of free fatty acids (FFAs) and monoacylglycerols (MAGs) in the micelles) and the β-carotene bioaccessibility (incorporation of β-carotene in the micelles) were studied after an in vitro digestion procedure wherein the stomach phase was mimicked for 2.0 h (37 °C) and the small intestinal phase was mimicked for 1.0 h, 1.5 h and 2.0 h (37 °C) (both end-over-end rotations). As a consequence, not only the influence of the emulsifier concentration, but also the influence of the duration in the small intestinal phase was investigated in this study. The oil droplet size distributions of the emulsions at different stages of digestion were shown to be dependent on the phosphatidylcholine concentration, but independent on the duration in the small intestinal phase (1.0 h–2.0 h). Furthermore, all TAGs were already hydrolysed into FFAs and MAGs after 1.0 h small intestinal phase and the incorporation of FFAs and MAGs into micelles seemed to reach a maximum for all emulsions (approximately 26.5%), independent on the phoshpatidylcholine concentration and thus on the particle size distributions. Finally, the β-carotene bioaccessibility increased with increasing phosphatidylcholine concentration, ranging from 33.2% to 79.8% for a 1% and 4% PHC emulsion respectively. No significant differences in β-carotene bioaccessibility were however noticed for the different durations in the small intestinal phase tested. In conclusion, a higher phosphatidylcholine concentration in emulsions leads to higher β-carotene bioaccessibility while the incorporation of lipids into micelles did not increase.

1. Introduction

Consumers are becoming more and more aware of the importance of a balanced and healthy diet. This balanced and healthy diet includes besides fruit and vegetables, containing carotenoids, also a low amount of fat or oil. Carotenoids (e.g. β-carotene) are a group of lipid-soluble bioactive compounds which seem to have a positive impact on human health, because of their antioxidant capacity or immune system enhancing properties. In addition, carotenoids such as β-carotene act as provitamin A (Dutta, Chaudhuri, & Chakraborty, 2005; Fernández-Garcia et al., 2012). Mammals however need to obtain carotenoids from their diet, hence a good level of absorption or bioavailability from the food is desirable. The bioavailability is the fraction of the ingested (micro)nutrient that is available for utilization in physiological functions and for storage in the body. It is partially determined by its bioaccessibility, which is in case of a lipid soluble (micro)nutrient, the fraction of the ingested (micro)nutrient that is incorporated into micelles and thus becomes available for absorption in the body (Hedren, Diaz, & Svanberg, 2002; Palafox-Carlos, Ayala-Zavala, & Gonzalez-Aguilar, 2011). Bioavailability is dependent on several factors, grouped in the word "SLAMENGHI". The letters of "SLAMENGHI" stand for Species of carotenoids, molecular Linkage, Amount of carotenoids consumed in a meal, Matrix in which the carotenoid is incorporated, Effectors of absorption and bioconversion, Nutrient status of the host, Genetic factors, Host-related factors and Interactions (Castenmiller & West, 1998). Because carotenoids are lipophilic, the presence of lipids constitutes an effector of absorption (E) (Castenmiller & West, 1998; Hedren et al., 2002). In this context, it has already been demonstrated that the absorption of carotenoids increases by adding lipids to the diet, hence a minimum amount of lipids is needed for the carotenoid absorption (Castenmiller & West, 1998). Over-consumption of lipids on the other hand can lead to obesity and increasing risks of cardiovascular diseases (McClements & Decker, 2009; Simopoulos, 1999).

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To understand and control the absorption of lipophilic micronutrients, knowledge about lipid digestion is needed. Lipid digestion can be divided into two steps, (i) the hydrolysis and (ii) micellization of the dietary lipids. Specifically, triacylglycerols (TAGs) (like triolein, mostly present in olive oil) are hydrolyzed into diacylglycerols (DAGs), monoacylglycerols (MAGs) and free fatty acids (FFAs), after which the MAGs and FFAs can be incorporated into micelles. Micelle formation is essential for the absorption in the enterocytes. Micelles contain, besides MAGs and FFAs, bile salts, phospholipids and lipid-soluble compounds, like β-carotene (Hofmann & Borgstrom, 1962; Yonekura & Nagao, 2007). Hence the ingestion and hydrolysis of lipids (resulting in MAGs and FFAs) along with carotenoids is thought to be essential for the absorption of carotenoids (van het Hof, West, Weststrate, & Hautvast, 2000). The rate of hydrolysis by lipase can depend on different factors. The oil droplet size for instance influences the area of lipid surface available to lipase. Moreover, the composition of the droplet surfaces and the rheological properties of the surrounding medium are important as well. Very small oil droplets covered with surfactants which are strongly bound to the oil droplets in a viscous medium will be hydrolysed slower by lipase than larger oil droplets covered with less bound surfactants in a low viscous medium for example (McClements & Decker, 2009). Finally, the rate of micelle formation can be affected by the surfactant type and concentration, the viscosity of the surrounding medium and the amount of hydrolysed products among others (McClements & Decker, 2009).

In view of consumer health, it is desirable to promote the bioaccessibility of lipid-soluble compounds like carotenoids, while on the other hand the lipid intake should be reduced. As a consequence, knowledge on the link between lipid digestion and β-carotene bioaccessibility is important and can be used to design food systems in which these properties are controlled. Specifically to understand this link, simple model emulsions with a low amount of lipids and different properties, like different oil droplet size (distributions) can be used as study object. The oil droplet size (distribution) seems in turn to influence the activity of lipase (Armand et al., 1996; Mun, Decker, & McClements, 2007) and is expected to influence the lipid digestion and β-carotene bioaccessibility therefore. To prepare emulsions with different oil droplet size distributions, different concentrations of emulsifiers can be utilized because the emulsifier concentration is expected to influence the oil droplet size distribution (Hur, Decker, & McClements, 2009; Mun et al., 2007). As emulsifier, a natural, harmless and useful surfactant, phosphatidylcholine, was chosen. Phosphatidylcholine is the major membrane phospholipid in mammalian cells, critical for the membrane structure and function (Gennis, 1989; Venturoli, Sperotto, Kranenburg, & Smit, 2006). Besides this, phosphatidylcholine is an important source of choline, an essential nutrient, in the body, which is needed for vital functions like the synthesis of cell signaling molecules and neurotransmitters involved in muscle control and memory (Sanders & Zeisel, 2007; Zeisel, 2006).

In the present work, the influence of the L-α-phosphatidylcholine concentration on the in vitro lipid digestion and β-carotene bioaccessibility in low fat emulsion systems was investigated. To investigate lipid digestion, lipid extraction and quantification was optimized and implemented, based on a method of Helbig, Silletti, Timmerman, Hamer, and Gruppen (2012). Furthermore, the particle size distributions of the digests at different stages of digestion as well as the influence of the duration in the small intestinal phase on lipid digestion and β-carotene bioaccessibility were investigated. In this way, more information about lipase activity, micelle formation and β-carotene incorporation could be obtained. Finally, the link between the micellar lipid incorporation and the β-carotene bioaccessibility was evaluated.

2. Materials and methods

2.1. Materials

Fresh carrots (*Daucus carota* cv. Nerac) were purchased in a local shop in Belgium and stored at 4 °C. Olive oil (extra virgin) was kindly donated by Vandemoortele (Ghent, Belgium). All chemicals and reagents were from Sigma Aldrich, except for NaCl, HCl, urea, anhydrous sodium sulfate and ethanol (from VWR); $CaCl_2 \cdot 2H_20$, NH_4Cl and $MgCl_2$ (from Merck); hexane, sulphuric acid and acetone (from Chem Lab); glucose and $NaHCO_3$ (from Fisher Scientific); heptane (from Fluka); KCl (from MP Biomedicals) and diethylether (from Riedel-De Haën). All chemicals and reagents were of analytical grade. The Phosphatidylcholine Assay Kit is from Cayman Chemical Company.

2.2. Preparation of β-carotene enriched oil-in-water emulsions

Olive oil enriched with β-carotene (from carrots) was prepared according to the procedure described by Verrijssen et al. (2014). Emulsions were prepared by blending (10 min, Ultra-Turrax (Waring Commercial, Torrington, CT, USA)) 5% (w/w) of this enriched oil with demineralized water, in which 1, 2, 3 or 4% (w/w) L-α-phosphatidylcholine (PHC) was dissolved. After blending, the emulsions were homogenized (Stansted Fluid Power, Pressure cell homogenizer, U.K.) at 100 MPa using a single cycle. The pH was adjusted to 6.0 using a (1 M) sodium hydroxide solution. The different emulsions are further indicated as "1% PHC emulsion", "2% PHC emulsion", "3% PHC emulsion" and "4% PHC emulsion". Emulsions were prepared in duplicate. Each emulsion was independently submitted to the in vitro digestion procedure.

2.3. In vitro digestion of β-carotene enriched emulsions

2.3.1. Digestion model

Digests were passed through a two-step static in vitro digestion model based on the method described by Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005). The first step in the digestion method is the simulation of the stomach phase by addition of 12 ml stomach juice (pH 1.3), which contains mainly electrolytes, bovine serum albumin, pepsin and mucin (Versantvoort et al., 2005) to 6 g emulsion and rotating this mixture 2.0 h end-over-end (40 rpm) at 37 °C. After the stomach phase, the intestinal phase is simulated by mixing (end-over-end, 40 rpm) 12 ml duodenal juice, 6 ml bile juice and 2 ml 1 M bicarbonate with the emulsion and stomach juice (37 °C). The duration of the small intestinal phase was 1.0 h, 1.5 h and 2.0 h (further indicated as "SIP 1.0 h", "SIP 1.5 h" and "SIP 2.0 h" respectively). To minimize the influence of light and oxygen, the samples were kept in the dark during the whole digestion procedure and the headspace of the tubes was flushed with nitrogen prior to each incubation step.

2.3.2. Particle size distribution during digestion

The particle size and particle size distribution of the samples were measured by laser diffraction (Beckman Coulter Inc., LS 13 320, Miami, Florida) during digestion, i.e. before digestion, after the stomach phase (2.0 h) and after the small intestinal phase (SIP 1.0 h, SIP 1.5 h and SIP 2.0 h). The sample was poured into a stirred tank, filled with deionized water, and pumped into the measurement cell wherein the laser light (wavelength main illumination source: 750 nm; wavelengths halogen light for Polarization Intensity Differential Scattering (PIDS): 450 nm, 600 nm, 900 nm) is scattered by particles in the sample. All analyses were carried out in duplicate.

Besides laser diffraction, microscopy pictures were taken to visualize the microstructure of the samples. This was done using a light microscope (Olympus BX-41) equipped with an Olympus XC-50 digital camera (Olympus, Optical Co., Ltd., Tokyo, Japan).

2.3.3. Lipid digestion

The amount of lipids was measured before digestion (in the initial emulsions), after the small intestinal phase (SIP 1.0 h, SIP 1.5 h and SIP 2.0 h) and in the micelle fraction (collected after the small intestinal phase). As a consequence, not only the release of FFAs and MAG from