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Influence of simulated upper intestinal parameters on the efficiency of beta carotene micellarisation using an *in vitro* model of digestion

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Abstract

This study was undertaken to better understand how variations in the parameters used to simulate the upper intestinal environment influence the transfer of a lipophilic molecule, beta carotene (BC), from the oily to the aqueous phase of digestate using a static model of digestion. Bile, pancreatin and pH were important and inter-related factors in determining the efficiency of BC transfer ($P < 0.05$). Less than 4% and 8% of the BC was transferred to the aqueous phase in the absence of bile and pancreatin, respectively. Generally, the proportion of BC transferred increased with bile and pancreatin concentrations and with pH. Under conditions which simulated the fed versus fasted states of digestion, significantly more BC was incorporated in the aqueous fraction (46.5% versus 18.8%). These results underscore the need to carefully consider and define the experimental parameters used for *in vitro* assays to study the digestion of carotenoids and other lipophilic molecules.

Keywords: Beta carotene; *In vitro* digestion; Intestinal; Micellarisation; Fasted; Fed

1. Introduction

The carotenoids are a group of lipophilic pigment molecules with important consequences for human health because of their pro-vitamin A activity and nutraceutical potential. Despite their prevalence in fruits and vegetables, however, the bioavailability of carotenoids from foods, and even some supplements, may be low and highly variable (Faulks & Southon, 2001). In fact, food matrix structure and interactions with dietary constituents, including between carotenoids, critically influence carotenoid digestion and absorption (Borel, 2003; Tyssandier et al., 2003).

During gastric digestion, carotenoids are released from foods and dissolve in the oily phase (Rich, Fillery-Travis, & Parker, 1998), which gets emulsified due to the shearing forces present (Furr & Clark, 1997; Parker, 1996). Digestive enzymes then hydrolyse the emulsified lipids into free fatty acids (FFA) and partial acylglycerols (Charman, Porter, Mithani, & Dressman, 1997). Pancreatic lipase activity, specifically, accounts for most of the lipid hydrolysis during digestion (Pafumi et al., 2002). The products of lipid digestion (i.e., primarily 2-monoacylglycerols and free fatty acids) play an important role in helping to solubilise lipophilic molecules into the bile salt micelles which form in the small intestine. These species intercalate into the bile salt micelles, causing them to swell and increasing their solubilisation capacity (Porter & Charman, 2001) for lipophilic molecules like carotenoids. Due to their lipophilic nature, carotenoids need to be incorporated into the mixed micelles in order to pass through the unstirred water layer and to approach the enterocyte membrane for absorption (Charman et al., 1997). Therefore, the efficiency of micellarisation and solubilisation of carotenoids within the micelles that form during digestion are important determinants of carotenoid bioavailability (Borel, 2003; Tyssandier, Lyan, & Borel, 2001; Tyssandier et al., 2003). Despite this, the processes by which lipophilic molecules are transferred

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from the food matrix to the gastric emulsion and ultimately to the aqueous, micellar phase are not well understood.

In vitro assays have been adapted in order to study the processes of carotenoid digestion and absorption (Rich, Bailey, Faulks, Parker, Wickham, & Fillery-Travis, 2003a; Rich, Faulks, Wickham, & Travis, 2003b). This often involves quantifying how much of a carotenoid is transferred from a food matrix to the aqueous, micellar phase of the digestate after mimicking the conditions of the gastrointestinal tract. It is assumed that those carotenoids which are associated with the aqueous phase would be the most readily absorbable by the body (Porter & Charman, 2001).

Support for the use of *in vitro* digestion assays to study the pre-absorptive events which affect carotenoid digestion, is provided by the correlations which have been observed between *in vitro* and *in vivo* studies (Failla & Chitchumroonchokchai, 2005; Reboul et al., 2006). Still, there is much to learn about carotenoid digestion and about the relationships between the complex and interconnected factors which govern carotenoid digestion and absorption *in vivo* as well as during *in vitro* digestion assays. For example, carotenoid bioavailability is influenced by the presence and nature of food in the gastrointestinal tract (Borel, 2003). Despite this, most *in vitro* studies are conducted using conditions intended to simulate the fasted state of digestion. For example, pH values in the range of 6.5–7.5 are often used to simulate the intestinal environment (Garrett, Failla, & Sarama, 1999; Hedren, Diaz, & Svanberg, 2002; Liu, Glahn, & Liu, 2004; Veda, Kamath, Platel, Begum, & Srinivasan, 2006). While this may be representative of the duodenal fasted environment, the intestinal pH is influenced by the presence of food and is significantly lower in the fed state (Kalantzi, Goumas, Kalioras, Abrahamsson, Dressman, & Reppas, 2006). Depending on where measurements are made, fed pH values as low as 2 have been observed (Charman et al., 1997). Since carotenoids are often consumed as part of a mixed meal, there is strong rationale for studying the influence of the fed state on the efficiency of carotenoid transfer and micellarisation. The objective of this study was to determine the influence of simulated upper intestinal parameters on the efficiency of BC transfer from the oily to the aqueous fraction of digestate. Specifically, the influence of bile extract and pancreatin concentrations as well as pH are reported, in addition to the influence of conditions which are broadly representative of the fasted and fed states of digestion.

2. Materials and methods

2.1. Materials

All chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO). This included all-*trans*-beta carotene (BC, Type 1 synthetic, >95% purity, C9750), porcine bile extract (B8631) and pancreatin (from porcine pancreas, P1750, 4 × USP). High purity nitrogen was supplied by BOC Gases (Guelph, ON).

2.2. Sample preparation

Refined, bleached and deodorised canola oil (CO) was generously provided by Bunge Canada (Toronto, Canada). The oil was re-bleached to ensure the removal of coloured impurities by the addition of 5 wt% bleaching clay (Engelhard F105, also provided by Bunge Canada) to the oil which was then heated to 90 °C, under vacuum in a rotary evaporator for 20 min prior to vacuum filtration.

A stock solution of BC in bleached canola oil (i.e., BC–CO) was initially prepared at a concentration of 0.1 mg BC per 100 mg CO. This concentration was selected based on reports that BC solubility in bulk triacylglycerols ranges from 0.11–0.14 wt% (Borel et al., 1996). To remove any potentially crystalline BC, the BC–CO was heated to room temperature and filtered (0.22 μm MAGNA, nylon, Fisher Scientific Inc.). The BC–CO was placed in an amber glass jar, flushed with nitrogen and stored at -20 °C until use within 2 weeks. To minimise BC degradation, all experiments were conducted under reduced lighting and at temperatures not exceeding 37 °C.

For each digestion experiment, the BC–CO was heated to 37 °C for 30 min and the desired amount was weighed into amber glass jars (typically). The concentration of the stock BC–CO was determined for each experiment (as described below) and found to be within the range of 0.035 mg BC/100 mg CO. Therefore, roughly 4.1×10^{-5} mg BC was present in a typical digestion sample. Samples were blanketed with nitrogen and stored at 4 °C overnight.

2.3. In vitro digestion assay

An *in vitro* digestion procedure which mimicked the upper intestinal stage of digestion was adapted from those described in the literature (Garrett et al., 1999; Hedren et al., 2002). During the gastric phase of digestion, carotenoids are transferred to the oily phase (Rich et al., 1998). In this study, samples consisted of BC already dissolved in the oily phase, and therefore only the intestinal phase of digestion was considered. In previous experiments, elimination of the gastric step did not significantly change the proportion of BC transferred to the aqueous phase (Garrett et al., 1999), while eliminating the intestinal phase of digestion resulted in a 90% reduction in transfer (Hedren et al., 2002).

At the start of each digestion, the desired amounts of bile and pancreatin were dissolved in 40 ml of saline (0.9 wt% NaCl) and 10 ml of 100 mM sodium bicarbonate solution, respectively, by vigorous mixing. Twenty millilitres of the bile solution and 10 ml of the pancreatin solution was then added to an amber glass jar, containing BC–CO and 1.3 mg butylated hydroxytoluene as an antiox-