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Hesperidin increases intestinal β,β-carotene 15-15′ mono-oxygenase 1 (BCMO1) activity in Mongolian gerbils (*Meriones unguiculatus*) fed with β-carotene-free diet

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ABSTRACT

β,β-Carotene 15-15' mono-oxygenase 1 (BCMO1) is a key enzyme in vitamin A (VitA) metabolism in mammals. Dietary compounds, such as carotenoids and polyphenols, were reported to influence BCMO1 activity. The aim of this study was to evaluate the effect of hesperidin (Hes), on the VitA bioefficacy of β-carotene (Bc) from orange-fleshed sweet potato, using Mongolian gerbils, focussing on BCMO1 activity. Gerbils ($n = 50$) depleted in VitA were divided into five groups fed with basal diet containing 3% white- or orange-fleshed sweet potatoes supplemented or not with Hes. Liver BCMO1 activity was low, with no significant differences between groups. Interestingly, intestinal mucosal BCMO1 activity was significantly higher in the gerbils fed without Bc or VitA than those fed with a VitA/Bc-supplemented diet. Finally, our results show that, under a low VitA status, Hes dramatically stimulated intestinal BCMO1 activity, an effect that could possibly be related to its action as an agonist of PPARγ.

1. Introduction

Provitamin A activity is the primary role of β-carotene (Bc) known in humans. Oxidative central cleavage of β-carotene (Bc), by β,β-carotene 15-15′ mono-oxygenase 1 (BCMO1), yields two molecules of all-*trans*-retinal which is a direct precursor of retinoic acid, the active form of vitamin A (VitA) or retinol. The two major sites of Bc bioconversion in humans are the intestine and the liver. VitA status affects the absorption of Bc and is one of the most important factors that influence the BCMO1 activity (Lietz, Lange, & Rimbach, 2010). To investigate VitA metabolism and BCMO1 activity, two kinds of animal models are commonly used: rats and mice to focus on BCMO1 transcriptional regulation and gerbils to study bioefficacy of carotenoids from foods, since these latter animals are an appropriate model for evaluation of the Bc conversion into VitA (Lee et al., 1999).

Recently, by using BCMO1 knockout mice, Lobo, Amengual et al. (2010) revealed that retinoic acid induced expression of the intestinal transcription factor ISX to represse BCMO1 expression. The same study demonstrated that the intestinal lipid/carotenoid transporter scavenger receptor B class 1 (SR-B1) is also controlled by retinoid signalling. Therefore the existence of a diet-responsive regulatory network, that controls Bc absorption and VitA production by negative feedback regulation, is highly possible (Lobo, Hessel et al., 2010). Moreover, dietary fat (During, Nagao, & Terao, 1998) and an adequate intake of protein (Hosotani & Kitagawa, 2005; Parvin & Sivakumar, 2000) were found to increase BCMO1 activity in rat intestine. Conversely, Bc conversion was decreased by the addition of other carotenoids (Ershov, Dmitrovskii, & Bykhovskii, 1993; Grolier, Duszka, Borel, Alexandre-Gouabau, & Azais-Braesco, 1997; vanVliet, vanVlissingen, vanSchaik, & vanden-Berg, 1996). On the other hand, other nutritional studies were conducted with gerbils or ferrets to better assess dietary provitamin A bioefficacy from foodstuffs (Mills et al., 2009). These authors showed the influence of dietary fat, but not of soluble fibres, on the VitA bioefficacy of Bc from sweet potato, using gerbils. Other

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dietary phytochemicals, such as flavonoids have proved to influence BCMO1 activity. Some *in vitro* studies showed that several flavonoids were able to decrease the intestinal BCMO1 activity to different degrees (Nagao, Maeda, Lim, Kobayashi, & Terao, 2000). These *in vitro* observations suggested that some antioxidants derived from food sources might modulate conversion of Bc to VitA in intestinal cells. To the best of our knowledge, only one *in vivo* study investigated the effect of two flavonoids (quercetin and rutin) on both carotenoid absorption and conversion in mice (Bando, Muraki, Murota, Terao, & Yamanishi, 2010). Nevertheless, no data are available in gerbils, which are purported to be the best adapted model to study carotenoid metabolism *in vivo* (Lee et al., 1999). No study to date has tested the effect of flavonoid on Bc absorption, using the gerbil model to examine the bioefficacy of Bc to VitA after a depletion phase. Therefore, the purpose of the present study was to evaluate the effect of a specific citrus flavonoid, hesperidin (Hes), on the VitA bioefficacy of Bc in Mongolian gerbils. Hesperidin (hesperetin-7-*O*-rutinoside) is a functional compound with many physiological and pharmacological effects and was reported to be a lipid lowering, an anti-inflammatory, and a cardiovascular protection agent. (Assini, Mulvihill, & Huff, 2013; Chanet, Milenkovic, Manach, Mazur, & Morand, 2012). Moreover, this flavanone is among the flavonoid compound displaying the highest bioavalability. The powerful biological properties of this flavonoid and its involvement in lipid metabolism both justify our interaction study.

From a nutritional point of view, this experimentation was embedded in the general approach of a fight against VitA-deficiency. Indeed, it was realised that orange fleshed sweet potato (OFSP) was a promoted tuber Bc-rich staple food and therefore a source of VitA in developing countries (Hagenimana et al., 2001; Huang, Tanudjaja, & Lum, 1999; Liu, Lin, & Yang, 2009). *Citrus* Hes was chosen because: (1) *citrus* fruits are commonly consumed in Africa and used in household preparations (Agbola, Maitra, & McLaren, 2003), (2) Hes belongs to the flavanone class and several flavanones have biological activities in the digestive tract (Uesawa et al., 2011) and (3) flavanones have already been shown to interact *in vivo* with lipid metabolism (Gorinstein et al., 2007; Miwa et al., 2004; Monforte et al., 1995) and *in vitro* on carotenoid micellarisation (Poulaert, Borel, Caporiccio, Gunata, & Dhuique-Mayer, 2012). Through this bioefficacy study one of the main objectives was to evaluate the impact of Hes on both intestinal and liver BCMO1 activities. Because both SR-BI and BCMO1 are under the same negative feedback control, we also investigated SR-BI protein expression in the intestinal mucosa.

2. Materials and methods

2.1. Chemicals and standards

Hes, β-apo-8′-carotenal, canthaxanthin, retinol, retinal, retinyl acetate, retinyl palmitate and dithiothreitol were purchased from Sigma–Aldrich (St Quentin-Fallavier France). Bc was obtained from Extrasynthese (Lyon, France) and other retinyl esters were a generous gift from Catharine Ross (Pensylvania State University, United States).

2.2. Animal and diet

Male Mongolian gerbils ($n = 50$), 40 d old, were obtained from Janvier (S^t Berthevin, France). Animals were housed at 20 ± 1 °C, subjected to a 12 h light/dark cycle and had free access to food and water. Animals and food were weighed daily. Animals were handled in compliance with European Union rules and according to the guidelines of the national institute of Health (National Institutes of Health, 1985) and the Committee for Animal Care at the University of Montpellier (France). During the acclimatisation period (3 d), gerbils were fed with commercial diet A04 (Specific rodent diet) (Table 1) (SAFE: Scientific Animal Food and Engineering; Augy, France). For the experimentation, six different diets were used: the classical A04 diet without any carotenoid or VitA (VitA-deficient), the A04 diet supplemented with VitA (VitA-supplemented diet), as well as the experimental diet constituted of the A04 VitA-deficient diet supplemented with either white-fleshed sweet potatoes (“WFSP”) or orange-fleshed sweet potatoes (“OFSP”) and supplemented or not with Hes. Boiled sweet potatoes were lyophilised (Usifroid SMH 15) during 72 h and ground with a grinder (Prolabo Dangoumill 300). The incorporation of lyophilised sweet potato and/or Hes in extruded diet was done by society SAFE. Bc and Hes contents in the diet were 10 mg/kg (Mills et al., 2009) and 4.6 g/kg (based on human daily consumption of 1 g/d), respectively, and the VitA-supplemented diet contained 1980 μg of VitA/kg. The concentration of each added constituent was checked prior to the experiment. Diets were vacuum-packed and stocked at 4 °C in a dark room. Bc and Hes were also quantified

Table 1
Composition of basal (A04) and experimental diets with added sweet potato powder and/or Hes fed to Mongolian gerbil.[a]

	Dietary component	Control−	WFSP	WFSP + Hes	OFSP	OFSP + Hes	Control+
A04	Protein (g/100 g)	16.1	16.1	16.1	16.1	16.1	16.1
	Lipids (g/100 g)	3.1	3.1	3.1	3.1	3.1	3.1
	Glucid (g/100 g)	60	60	60	60	60	60
	Starch (g/100 g)	45.8	45.8	45.8	45.8	45.8	45.8
	Total sugar (g/100 g)	2	2	2	2	2	2
	Cellulose (g/100 g)	3.9	3.9	3.9	3.9	3.9	3.9
	Mineral mix (g/100 g)[b]	5.1	5.1	5.1	5.1	5.1	5.1
	VitD3 (μg/kg)	22.5	22.5	22.5	22.5	22.5	22.5
	VitE (mg/kg)	30	30	30	30	30	30
	VitA (μg/kg)	/	/	/	/	/	1980
	Bc (mg/kg)[c]	/	/	/	10.9	9.5	/
	Hes (g/kg)[d]	/	/	3.7	/	4.1	/

Control−, VitA/Bc-deficient diet; Control+, VitA-supplemented diet; OFSP, orange-fleshed sweet potato; WFSP, white-fleshed sweet potato; Hes, hesperidin. VitD3, vitamin D3. VitE, vitamin E; VitA, vitamin A; Bc, β-carotene.

[a] Basal diet (A04) provided by SAFE-diets. Instead of 1% cereal flour in basal diet, 3% OFSP and 3% WFSP were used. Hesperidin was incorporated into the basal medium at 0.46%.

[b] Mineral Mix provided the following (mg/kg): Ca, 8400; P, 5700; Na, 2500; K, 6400; Mn, 70; Cu. 17.

[c] Bc was provided by OFSP. The theoretical Bc content was 10 mg/kg as mentioned by Mills et al. (2009).

[d] Hes was added as crystalline powder. The theoretical Hes content was 4.6 mg/kg based on human physiological data of a daily consumption of 1 g/day.