
1. INTRODUCTION

Green leafy vegetables are principal sources of the two carotenoids lutein (Lut) and β -carotene (β -Car) [1], which have been associated with positive effects on Age-related Macular Degeneration (AMD) [2,3], cataract [4] and vitamin-A related disorders [5]. Carotenoids are not synthesized in primates and we therefore rely on intake, accessibility and bioavailability from food or supplements for their actions [6].

Bioavailability of carotenoids is low and depends on a complex set of factors. Bioavailability is defined as the fraction of ingested nutrients that is available for utilisation in normal physical functions or storage and is thus a key concept for nutritional effectiveness [7]. Moreover, it includes bio-accessibility, which is the fraction of ingested nutrients released from the food matrix in the intestinal tract and thereby made available for intestinal absorption. Accessibility strongly depends on carotenoid species, food matrix and food processing, whereas influencing factors for bioavailability include micelle formation, uptake into intestinal mucosa cells, packing into chylomicrons and secretion into the lymphatic system [8]. Bioavailability and bio-accessibility are both complicated to assess *in vivo*. A simpler and faster approach, however, is offered by *in vitro* methods, which may be useful for screening the availability potential as a function of numerous different factors i.e. physiological variations (age, pH, transit time etc.), matrices, meals and food pre-processing. Several different *in vitro* digestion methods are found in laboratories around the world and through the literature [9-11], however, conflicting information and results have been reported [8], making comparison of results challenging. A new European consensus *in vitro* digestion protocol has been elaborated within the COST action FA 1005 Infogest collaboration [12,13]. In order to test the suitability of the protocol as a useful tool to in estimating bio-accessibility and possibly bioavailability, the new system needs to be validated with concomitant *in vivo* data.

Validation of results obtained from *in vitro* digestion systems with human data is a key point, as stated by several authors [8,14], however, correlating *in vitro-in vivo* data is still, to a large degree lacking. A few authors have compared results obtained from different *in vitro* digestion protocols with results reported in other studies [11,15,16], after long intervention periods [16] and in serum/plasma [8,16], however this is far from a direct comparison. Direct or semi-direct comparisons between data obtained in *in vitro* digestion models and correlating *in vivo* studies have, to our knowledge, only been reported in a few cases [17-19].

Absorption and metabolism are highly dependent on a functional gastro-intestinal tract [20]. Studies investigating carotenoid absorption from patients with a reduced absorption area following gastro-intestinal surgery i.e. gastric-by-pass, short bowel and ileostomy patients, who could potentially be at risk of malabsorption of these fat-soluble phytochemicals, are limited.

With this PhD thesis I intended to shed more light on the usefulness of the consensus *in vitro* digestion method as a model for evaluations of the *in vitro* accessibility potential of the foremost nutritionally relevant carotenoids specifically present in green leafy vegetables. Furthermore, it was my objective to validate these outcomes through results obtained *in vivo*. The results will be discussed according to the current evidence.