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Review

Interaction of divalent minerals with liposoluble nutrients and phytochemicals during digestion and influences on their bioavailability – a review

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ABSTRACT

Several divalent minerals, including the macroelements calcium and magnesium, are essential nutrients for humans. However, their intake, especially via high-dose supplements, has been suspected to reduce the availability of lipophilic dietary constituents, including lipids, liposoluble vitamins, and several phytochemicals such as carotenoids. These constituents require emulsification in order to be bioavailable, and high divalent mineral concentrations may perturb this process, due to precipitations of free fatty acids or bile salt complexation, both pivotal for mixed micelle formation. Though in part based on *in vitro* or indirect evidence, it appears likely that high-dose supplements of divalent minerals around or even below their recommended dietary allowance perturb the availability of certain liposoluble miroconstituents, in addition to reducing absorption of dietary lipids/cholesterol. In this review, we investigate possible negative influences of divalent minerals, including trace elements (iron, zinc), on the digestion and intestinal uptake of lipophilic dietary constituents, with a focus on carotenoids.

1. Introduction

For the past two decades, there has been an increasing interest in phytochemicals and recognition of their potential benefits in human nutrition and health. Phytochemicals cover a wide range of secondary plant compounds that are considered not to be essential for humans, i.e. their absence in the diet does not cause any deficiency symptoms. However, the intake of several dietary phytochemicals has been associated with the prevention of certain chronic conditions, such as cancer (Giovannucci, 2002; Surh, 2003), type 2 diabetes and insulin resistance (Coyne et al., 2005), and cardiovascular diseases (Voutilainen, Nurmi, Mursu, & Rissanen, 2006). Some of these phytochemicals and other food microconstituents are highly lipophilic molecules, i.e. with high octanol-water partition coefficients (log P_{oct} > 8), and tend to readily dissolve in oil. These include, among other, carotenoids, mono- and diterpenes, and triterpenes/triterpenoids such as phytosterols, as well as fat soluble vitamins (A, E, D, and K) (Fig. 1).

Given their highly lipophilic nature, these food microconstituents tend to share similar crucial steps during their digestion, including the transfer from the food matrix into a lipid phase, emulsification, inclusion into mixed micelles, diffusion through the mucus to the unstirred water layer, binding to the enterocyte, and cellular uptake (Gropper & Smith, 2013). The transfer of lipophilic food microconstituents from the food matrix via lipid droplets into mixed micelles is especially important and a limiting step in their bioavailability, i.e. the fraction of a nutrient/non-nutrient that is absorbed and available for use and/or storage in body organs. In recent years, dietary aspects influencing the bioavailability of highly lipophilic food microconstituents, especially carotenoids, have been reviewed comprehensively (Borel, 2003), highlighting the importance of sufficient lipids (Unlu, Bohn, Clinton, & Schwartz, 2005) and a limited amount of dietary fibre (Palafox-Carlos, yala-Zavala, & Gonzalez-Aguilar, 2011) as crucial factors influencing their bioavailability.

One dietary factor that has recently been proposed to affect *in vitro* carotenoid bioaccessibility and cellular uptake is the presence of divalent minerals, especially at concentrations that are common for dietary supplements, usually at half to full the recommended dietary allowance (RDA). For example, calcium at concentrations between 12.5 and 25 mM reduced the bioaccessibility of beta-carotene from a spinach based meal by approximately 80% (Biehler, Hoffmann, Krause, & Bohn, 2011; Corte-Real, Bertucci et al., 2017 Table 1). Very recently, a human trial corroborated the potential negative effects of divalent minerals on carotenoid absorption, showing that a dietary supplement containing 500 mg of calcium reduced lycopene absorption from tomato paste by

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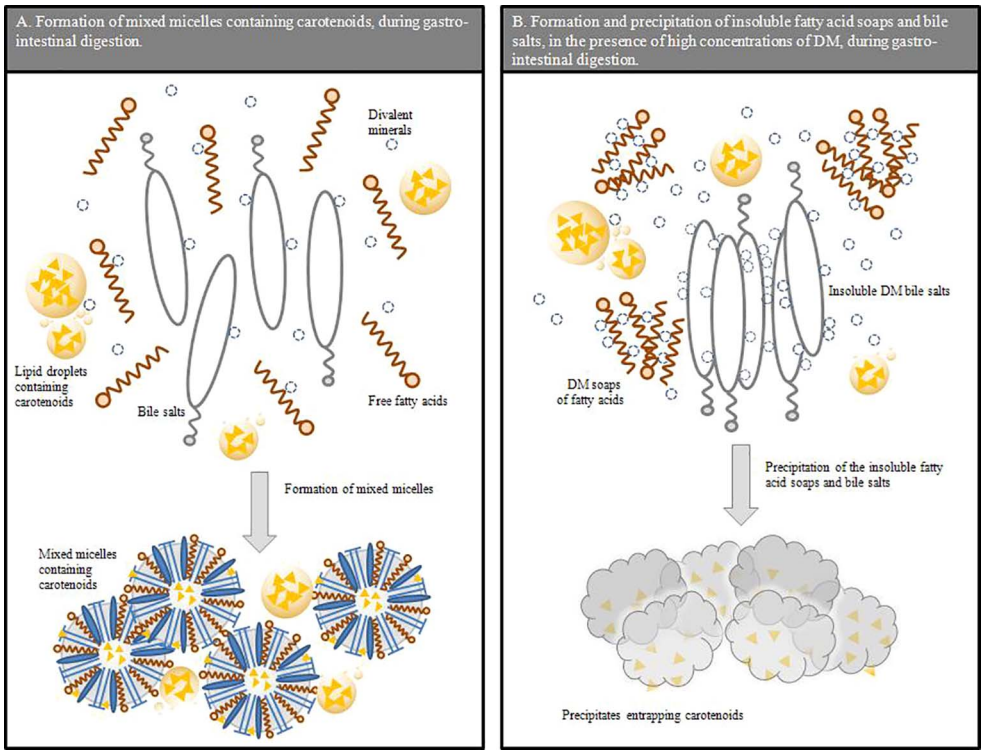

Fig. 1. Possible interactions of divalent minerals and liposoluble dietary constituents during digestion, exemplified for carotenoids.

up to 83%, as measured by plasma appearance (Borel et al., 2016). The ability of divalent minerals to bind free fatty acids and bile acids, leading to the formation of poorly soluble soaps (Govers & Van der Meet, 1993) and salts (Hofmann & Mysels, 1992), respectively, may in fact inhibit the transfer of lipophilic food microconstituents from the food matrix into mixed-micelles, limiting eventually their bioavailability. The non-absorbed fraction would then pass on to the colon, where they will likely not become available either, or are in part degraded by the microbiota (Goni, Serrano, & Saura-Calixto, 2006), and are finally excreted via the faeces.

Dietary divalent minerals include several essential macrominerals, such as calcium and magnesium, and microminerals, also termed trace elements, such as iron and zinc, as the most predominant ones. Due to their participation in many essential functions in the human body, a sufficient intake of these minerals is paramount. While calcium is especially of relevance for bone metabolism, magnesium plays a crucial part in many energy-related metabolic functions, acting as a cofactor for many enzymes. Iron is part of haemoglobin and required for the formation of functional erythrocytes, and zinc is likewise required for energy metabolism and constitutes a cofactor for many enzymes (Gropper & Smith, 2013).

The RDA for calcium, magnesium, iron and zinc are 1000, 400, 8 and 11 mg/d, respectively, for adult men (age category 19–40). Yet, a significant percentage of the population fails to meet 100% of the RDA for mineral and trace element intake, as well as of certain vitamins. For example, it is estimated that in the United States, more than half of the population aged ≥2, does not meet the RDAs for vitamin B-6, vitamin A, magnesium, calcium, and zinc (Cordain et al., 2005). As a solution, due to their dietary importance and convenience, supplements are widely offered on the market and are frequently consumed. For calcium, typical dietary supplements contain between 250 and 1200 mg per unit (mostly, a tablet), for magnesium these are 100–600 mg, and for iron and zinc up to 65 and 50 mg, respectively.

Especially in developed Westernized countries, up to 66% of the population consumes regularly dietary multimineral/multivitamin supplements (Skeie et al., 2009), at least in several, though not all of these countries. Given their frequent use in today's society, and the potential inhibitory effects of divalent minerals on the bioavailability of dietary lipophilic microconstituents, we aimed at providing an overview of literature, shedding evidence on the interaction between dietary minerals/trace elements and lipophilic food microconstituents during intestinal digestion and cellular uptake. This would also be of interest for subjects with digestive malfunctions, such as those with pancreatitis, or for subjects with already poor absorption of certain micronutrients, such as those with inflammatory bowel disease. Often, these conditions are associated with deficient bile and pancreatic enzyme secretion, causing steatorrhea and absorption deficiencies of certain nutrients, such as of vitamin E, most likely due to the impeded ability to form mixed-micelles during the small intestinal passage (Stahl et al., 2002). It could be hypothesised that intake of divalent minerals as supplements would worsen the effects of these conditions. For this purpose, literature (Pubmed, Medline databases) was searched for English reports, from all years until today, employing the search terms: ("minerals" or "calcium" or "magnesium" or "iron" or "zinc") and ("digestion" or "bioavailability" or "bioaccessibility" or "intestine") and ("carotenoid" or "vitamin" or "lipid" or "fat").

2. Effect of divalent minerals on digestion

Dietary mineral release typically starts in the stomach, where digestive enzymes (pepsin, gastric lipase) and peristaltic movements work towards the food matrix breakdown, and the rather acidic gastric pH (fasting 1-5-2.0; with food 3–5) (Alminger et al., 2014) aids in releasing the minerals from their bound forms, such as in organic complexes including phytic acid or oxalic acid. Solubilized minerals, present in their free ionised form, can be taken up in the small intestine, both via passive transport and facilitated uptake, via e.g. calcitriol-regulated transporters such as for calcium (Gropper & Smith, 2013), though a smaller fraction may also reach the colon (Gopalsamy et al., 2015). Trace elements (e.g. iron and zinc) are principally absorbed in the upper small intestine (duodenum and jejunum), while the macrominerals calcium and magnesium appear to be absorbed throughout the small intestine, either by active or passive transport, but especially in the latter sections of the small intestine, the ileum (Gropper & Smith,

Table 1
Overview of studies demonstrating negative interactions of divalent minerals and trace elements with lipophilic dietary constituents on aspects of their bioavailability. *: all salts in *in vitro* trials were in chloride form.

Type of study	Interaction studied	Divalent mineral/trace element	Study design	Concentration ranges of divalent minerals	Observed effects	Literature
*In vitro**	Cholesterol	Calcium	Simulated digestion	Up to 11 mM Ca (440 mg/L)	Significant reduction of cholesterol bioaccessibility	Vinarova et al. (2016)
	Isolated pure carotenoids: Lutein Neoxanthin Beta-carotene Lycopene	Calcium Magnesium Zinc	Simulated digestion	250 < Ca^{2+} < 1000 mg/L 100 < Mg^{2+} < 300 mg/L 50 < Zn^{2+} < 200 mg/L	Presence of: Ca^{2+} significantly decreased bioaccessibility of all investigated carotenoids, starting from 250 mg/L Mg^{2+} significantly decreased bioaccessility of beta-carotene and lycopene at concentrations > 100 mg/L, and of lutein and neoxanthin at concentrations > 200 mg/L Zn^{2+} significantly increased the bioaccessibility of lutein and neoxanthin at lower concentrations (50 mg/L), while it decreased the bioaccessibility of all carotenoids at 200 mg/L.	Corte-Real et al. (2016)
	Dietary carotenoids: Lutein Neoxanthin Beta-carotene Lycopene Phytoene Phytofluene	Calcium Magnesium Zinc	Simulated digestion	250 < Ca^{2+} < 1000 mg/L 100 < Mg^{2+} < 300 mg/L 12.5 < Zn^{2+} < 200 mg/L	Presence of: Mg^{2+} significantly decreased bioaccessibility of all investigated carotenoids Ca^{2+} significantly increased the bioaccessibility of beta-carotene from vegetable and fruit juices at 250 mg/L, and decreased bioaccessibility of all carotenoids at concentrations > 500 mg/L Zn^{2+} significantly increased carotenoid bioaccessibility in green leafy matrices	Corte-Real et al. (2017)
	Carotenoids from spinach: Beta-carotene Lutein	Calcium Magnesium Iron (Fe^{2+}) Zinc	Simulated digestion	12.5 < Ca^{2+}, Mg^{2+} < 25 mM 3.8 < Zn^{2+}, Fe^{2+} < 12.5 mM	All divalent minerals and trace elements significantly decreased the bioaccessibility of the investigated carotenoids, especially at the higher end of the range of tested concentrations	Biehler Hoffmann et al. (2011)
Animal	Calcium and magnesium absorption	Rats receiving either fat-free, 5%, or 25% of either a triolein, tripalmitin or tristearin diet together with calcium and magnesium	Animal experimental feeding trial, 1 week	560 mg/week calcium, 250 mg/week magnesium	Rats receiving 25% tripalmitin or tristearin reduced calcium and magnesium digestibility (from 62 to 25% and 55 to 30%, respectively) and absorption, (chemical balance method), no effect was found for triolein	Tadayyon and Lutwak (1969)
	Calcium absorption	Calcium absorption from ^{47}Ca labelled soaps of fatty acids	Rat trial, postprandial absorption	100 mg of each soap per rat	Calcium absorption was inversely correlated with the chain lengths and level of saturation of the fatty acids, and was < 5% for C14:0, C16:0 and C18:0 compared to $CaCl_2$	Gacs and Barltrop (1977)
	Lipid digestion	High vs. low calcium diets,	Feeding trial (23 d) on calves, studying fat digestion	12.4 vs. 5.2 g/kg calcium diet	5.6% lower fat digestibility and 90% increased bile acid excretion in the faeces with high compared to a low calcium control diet	Xu et al. (1998)
Human observational and meta-analyses	Serum carotenoids	Calcium	6 months intervention trial	2 g calcium (as calcium carbonate)/d	Calcium supplementation significantly reduced total serum carotenoids by 14%	Chai et al. (2013)
	Meta-analysis, serum cholesterol	Calcium supplements	2 wk-5 y intervention trials	Up to 1200 mg/d calcium	Calcium supplementation significantly reduced LDL-cholesterol, on average by 0.12 mM	Chen et al. (2017)
	Meta-analysis, serum triglycerides and LDL-cholesterol	Calcium supplements	4 d to over 1 year intervention trials	Calcium doses from 400 to 2660 mg/d	Majority of trials reported significant reductions of the participants' serum triglycerides (up to 25%) and also LDL-C levels (up to 11%)	Reid (2004)
Human intervention	Lycopene from tomato paste	Calcium	Postprandial trial	Calcium carbonate supplement, containing 500 mg of Ca^{2+}	Calcium supplementation significantly decreased plasma lycopene appearance by 83%	Borel et al. (2016)
	Beta-carotene and lutein from a spinach meal	Calcium	Postprandial trial	Calcium carbonate supplement, containing 500 mg or 1000 mg of Ca^{2+}	Calcium supplementation did not significantly affect the concentration of neither carotenoid in the plasma triacylglycerol-lipoprotein fraction	Corte-Real et al. (2017)
	Serum lipids	Calcium	Postprandial trial	350 or 800 mg calcium from dairy products	Calcium reduced triglyceride chylomicron response postprandially by up to 19%	Lorenzen et al. (2007)

2013).

However, solubilisation of divalent minerals does not necessarily ensure absorption in the small intestine. Within neutral to slightly alkaline pH range, as found in the small intestine, divalent minerals can complex with bile acids and dietary constituents such as organic acids (Etcheverry, Grusak, & Fleige, 2012), polysaccharides (Debon & Tester, 2001), and fatty acids (Cheng, Morehouse, & Deuel, 1949). For example, it is well comprehended that iron absorption can be negatively influenced by phytic acid (Petry, Boy, Wirth, & Hurrell, 2015) present in many legumes and oilseeds, and a similar behaviour has been shown for other minerals, e.g. magnesium (Bohn, Davidsson, Walczyk, & Hurrell, 2004b). Other organic acids, such as oxalic acid, present in many leafy vegetables may also form complexes with minerals, reducing their availability (Bohn, Davidsson, Walczyk, & Hurrell, 2004a). In general, the strength of complexation has been reported to follow the following ranking: Ca = Zn = Fe > Mg (Bohn, 2008).

Perhaps the most relevant complexes formed by the binding of divalent minerals, concerning aspects of liposoluble food constituent bioaccessibility, are those formed between cations and bile acids or free fatty acids, given their importance regarding the formation of lipid-bile mixed micelles. Fatty acids, when released from the diet during digestion of triglycerides and/or phospholipids such as lecithin, have been reported to form insoluble soaps with divalent minerals (Vaskonen, 2003). Similar reports have been published demonstrating that divalent minerals may react with bile acids, forming poorly soluble complexes (Capurso et al., 1999). In addition, the presence of divalent minerals may enhance the binding of bile salts to fatty acids (Pandolf & Clydesdale, 1992), likewise resulting in poorly soluble complexes. In the following, these two main potential pathways of inhibiting mixed micelle formation, i.e. binding with fatty acids and/or bile salts, and their impact on the bioaccessibility of liposoluble constituents, will be evaluated, with a certain focus on carotenoids.

2.1. Interaction of divalent minerals with bile salts during digestion

Bile acids, which consist of a steroid backbone, include primary, secondary, and tertiary bile acids. Primary bile acids are produced in the hepatocytes of the liver from cholesterol; the most prominent ones are cholic acid and chenodeoxycholic acid (Wilde & Chu, 2011). They are then further metabolized by colon bacteria to secondary bile acids such as deoxycholic and lithocholic acid. Finally, they are then conjugated with taurine and glycine in the liver to form glycoconjugates and tauroconjugates, respectively, and are then secreted via the bile into the duodenum, i.e. the uppermost part of the small intestine, to act as emulsifiers during intestinal digestion. These tertiary bile acids include e.g. glycocholic acid and taurocholic acid. The amount secreted daily is approx. 250–1000 mL, of which the bile concentration is around 4–45 mmol/L (Boyer, 2013). The polarity of bile acids has been reported to decrease in the following order: glycine-conjugated < taurine-conjugated < lithocholic acid < deoxycholic acid < chenodeoxycholic acid < cholic acid < ursodeoxycholic acid. Due to their amphiphatic properties they can act as surfactants, aiding in the micellization of liposoluble constituents. If missing, severe malabsorption of lipophilic constituents may occur (Venkat et al., 2014). In subjects without functional bile salt secretion, very low circulating levels of lipophilic vitamins have been reported. For example, Setchell et al. (2013) reported that in children genetically lacking the possibility for bile salt conjugation, low serum concentrations of especially vitamin D and E were encountered, and in part also low vitamin K and A levels, emphasizing the importance of sufficiently high conjugated bile salt concentrations in the gut for optimal micellisation and absorption of lipophilic constituents. The requirement of bile salts for vitamin E absorption, such as in children with obstructive jaundice, occurring when the essential flow of bile to the intestine is blocked, has been highlighted earlier, and lack of bile salts reduced circulating vitamin E from 0.8 to 0.1 mg/100 mL in serum (Muller, Harries, & Lloyd, 1974).

Previous *in vitro* trials have shown the dependency of carotenoid bioaccessibility on the concentration of bile during gastro-intestinal digestion (Tyssandier, Lyan, & Borel, 2001), and that low concentration of bile salts compromised bioaccessibility, i.e. the proportion of a compound available for absorption, of carotenoids from spinach (Biehler, Kaulmann, Hoffmann, Krause, & Bohn, 2011), highlighting the importance of micellization for especially the more apolar compounds. Similar results were found by Garrett, Failla, Sarama, and Craft (1999) regarding carotenoid bioaccessibility from a mixed salad (1999). Likewise, in a study by Gireesh and Sudhakaran (2009), uptake of beta-carotene into human exfoliated colonic cells increased with increasing bile salt concentration. In a study with gerbils, adding taurocholate at concentrations of 0, 0.5, or 1% to the diet significantly increased hepatic and plasma beta-carotene concentrations with increasing bile acid concentration (Sundaresan et al., 2005). In an *in vitro* trial employing spinach and bile salts, glycodeoxycholate was more effective in micellarizing carotenoids than taurocholate, possibly due to higher polarity and acting as a less efficient emulsifier of the latter (Rich, Faulks, Wickham, & Fillery-Travis, 2003).

As bile acids have a pKa of 4.2–7.3 (Cabral, Hamilton, & Small, 1986) and are weak acids, they are in part present in the gut in form of a salt. Sodium bile salts have generally a high solubility (Jones, Hofmann, Mysels, & Roda, 1986), and also a lower critical micelle concentration (CMC) (Jones et al., 1986), i.e. the concentration threshold above which spontaneous formation of bile salt mixed micelle formation occurs, than their free form. In contrast, binding of calcium ions to bile acids, tends to increase the CMC and lower the solubility product of the respective bile salt (Jones et al., 1986), which could lead to bile salt precipitation. However, it is likely that the presence of Na^+ also lowers the potential negative effect of divalent minerals by competition, as bile salts and micelles bind both calcium ions and counterions. Such an effect may explain results in an *in vitro* study by Biehler et al. (2011), where sodium concentrations of 900 mM in the digesta increased bioaccessibility of spinach originating beta-carotene by 60%, though not remarkably influencing xanthophylls.

Addition of divalent minerals to *in vitro* digestion models has also negatively affected the surface charge of digested particles, and potentially the stability, of the digesta. This effect was particularly visible with increasing concentrations of calcium, through the drastic decrease of the zeta potential of the micellar fraction of the digesta (Corte-Real, Bertucci et al., 2017). The presence of bile confers a negative charge to the system, as a result of repulsive forces created by the surfactant effect of bile salts, which stabilizes the system and prevents aggregation. The authors suggested that the decreased zeta potential might have been due to the removal of bile salts from the solution and surface of particles via precipitation. Another study reported a similar effect of calcium ion concentration on the zeta potential of a phospholipid stabilized emulsion (Wickham, Garrood, Leney, Wilson, & Fillery-Travis, 1998). In their study, the reduction of the zeta potential was however attributed to the shielding of the surface charge of the emulsion droplets by calcium ions, which could constitute an additional negative influence, destabilizing particles. It also has to be considered that factors related to cellular uptake, such as better adhesion to the epithelium, have in general been related to more negatively charged particles (Xu, Ling, & Zhang, 2013).

The increased excretion of bile acids by high calcium doses has been shown earlier in rat trials (Lapre, De Vries, & van der Meer, 1991), and proposed earlier as a protective effect against colon cancer development (Kleibeuker, Cats, van der Meer, Lapre, & de Vries, 1994). In addition to removing bile acids via precipitation, high calcium doses have also been shown to alter bile acid profile in humans towards the more polar deoxycholic acid compared to lithocholic acid, which may have implications for the absorption of liposoluble microconstituents (Lupton et al., 1996).

Although calcium is by far the most investigated divalent mineral both *in vitro* and *in vivo*, other minerals and trace elements also bind bile

acids, namely iron, copper and zinc (Feroci, Fini, Fazio, & Zuman, 1996). However, *in vivo*, the latter are normally present at micromolar concentrations, and are hence not likely to significantly affect bile salt solubility.

2.2. Interaction of divalent minerals with lipids during digestion

Earlier animal trials had described negative interactions between lipids and divalent minerals during gastro-intestinal digestion, resulting in both the unavailability of the mineral and the lipids for absorption. In a study by Tadayyon and Lutwak (1969) on rats receiving either fat-free, 5%, or 25% of either a triolein, tripalmitin or tristearin diet together with calcium and magnesium, it was found that diets with 25% tripalmitin or tristearin reduced calcium and magnesium absorption, while no effect was found for triolein. In another study (Gacs & Barltrop, 1977), the authors investigated whether calcium from ^{47}Ca labelled soaps of fatty acids would be bioavailable in rats. It was found that calcium absorption was inversely correlated with the chain lengths and level of saturation of the fatty acids, presumably related to the lower solubility of long-chain and of saturated fatty acid soaps. However, no significant soap formation or reduction in calcium absorption was observed when fats were given as triglycerides, unlike the earlier trial by Tadayyon and Lutwak (1969). It should be pointed out that levels of dietary lipids were quite high in the latter study, and that it is likely that the slow and stepwise release in the small intestine, coupled with parallel ongoing calcium absorption in the small intestine, reduced the negative interactions of calcium with triglycerides, compared to the free fatty acids in the study of Gacs and Barltrop (1977).

Similar studies have also been performed in humans. In an older study (Steggerda & Mitchell, 1951), a diet rich in butter fat did not decrease calcium balance in humans, perhaps due to the limited content of butter fat (up to 32% fat content of diet), or due to the higher solubility of short chain fatty acids partly present in butter. In another human study, targeting negative effects on lipids, calcium intake from dairy products (800 mg and 350 mg), but not from calcium carbonate (850 mg) reduced postprandial lipid response in plasma and chylomicrons, employed as an indicator of postprandial fat absorption, also suggesting negative interactions with lipid availability and calcium intake. It is noteworthy that there was no effect of the calcium carbonate supplement, perhaps due to the differences in the chemical form of calcium and kinetics of dissolution (calcium carbonate has to solubilize in the acid milieu of the stomach first) during digestion (Lorenzen et al., 2007), which may have slowed down potential negative interactions.

In addition to negative effects on the mineral side, also negative effects regarding lipid digestion and availability were reported. In a previous study employing high concentrations of calcium (12 g/kg fodder) during a 23 d feeding trial, fat digestion in calves was inhibited, resulting in 5.6% lower fat digestibility and 90% increased bile acid excretion in the faeces, compared to a low calcium control diet (Xu, Wensing, & Beynen, 1998). In fact, calcium supplementation has been used for a few decades as a possible therapeutic means to lower serum circulating lipids. In a number of randomized (n = 6) and non-randomized (n = 6) human trials, reviewed by Reid (2004), lasting a minimum of 4 days to over one year, with calcium doses ranging from 400 to 2660 mg/d, the majority of these trials reported significant reductions of the participants' serum triglycerides and also LDL-C levels. While calcium gluconate and citrate were found to be effective, one study employing calcium carbonate did not result in any benefits, which may or may not have been due to the lower solubility of the carbonate form (Goss, Lemons, Kerstetter, & Bogner, 2007). Some of the studies reviewed by Reid also reported an increase in faecal lipid excretion, supporting the hypothesis that minerals and lipids interact in the gut and limit one another in their absorption.

Another recent meta-analysis found significant reduction in LDL-cholesterol by 0.12 mM (Chen et al., 2017) with calcium supplementation of up to 1200 mg/d, though lower amounts interestingly appeared to be more effective. Though rather hormonal effects on adipocytes were discussed, effects at the level of dietary absorption of cholesterol could also have played a role. In line with this, a recent *in vitro* study found that calcium, dose dependently with concentrations employed up to 11 mM (440 mg/L), reduced cholesterol micellization in simulated digestion experiments (Vinarova et al., 2016), which at least may contribute to the effect of calcium on serum cholesterol.

However, the formation of fatty acids soaps with divalent minerals and the faecal fat excretion seems to depend on the solubility of the formed soap, which is again related to the digestibility of triglycerides (Cheng et al., 1949) and fatty acid composition. Addition of calcium and magnesium appears to have a particularly negative effect on the solubility of natural fats with high melting points (i.e. > 50 °C) and of long-chain saturated fatty acids (Tadayyon & Lutwak, 1969).

Divalent minerals may also effect the extent of lipolysis. An increase in lipase activity in the presence of millimolar concentrations of calcium has been reported (Hu, Li, Decker, & McClements, 2010). Interestingly, it also has to be stated that lipolysis could be enhanced by higher concentrations of calcium (Wickham et al., 1998), perhaps also due to precipitation of lipolysis products, shifting the equilibrium of the reaction to the product side. Whichever the underlying mechanism, the formed products would no longer be available for emulsification and would thus be expected to hamper micellization of liposoluble micro-constituents.

3. Divalent minerals and lipophilic food microconstituents

Lipophilic food microconstituents, which include liposoluble vitamins (A, E, D and K), carotenoids, other triterpenes such as ursolic acid, phytosterols, and some liposoluble phenols (e.g. curcumin) are assumed to share similar steps during gastro-intestinal digestion. Due to their low water solubility, these compounds have to be emulsified in order to diffuse through the unstirred water layer and to the apical surface of the enterocytes for absorption. For this purpose, they are transferred from the food matrix via lipid droplets into mixed micelles, emulsified particles containing amphiphilic constituents such as bile salts, mono- and diglycerides, free fatty acids on the outside, and more apolar compounds (cholesterol esters, carotenoids) in their interior (Sy et al., 2012). It could be assumed that any dietary factor that potentially interferes with the ability to form mixed-micelles in the gut, including the presence of divalent minerals, would also affect food constituents that depend on mixed micelle incorporation in order to be absorbed.

However, data on the potential effects of divalent minerals/trace elements on the bioavailability of lipophilic food microconstituents, particularly the liposoluble vitamins, is scarce and in need of better elucidation. However, some *in vitro* and *in vivo* studies have looked especially into the interactions between divalent minerals and carotenoids, as well as vitamin A.

3.1. Divalent minerals and liposoluble vitamins

Perhaps one of the most well studied interactions of divalent minerals and liposoluble food microconstituents is the one of calcium and vitamin D, where 1,25-dihydroxy vitamin D3 regulates active calcium absorption in the colon and small intestine.

One pilot study, looking into the effect of supplementation of calcium alone (2 g/d as calcium carbonate), or in combination with vitamin D, on blood pressure and serum lipids of colorectal adenoma patients, has also looked into plasma carotenoid levels (Chai, Cooney, Franke, & Bostick, 2013). The authors found that daily calcium supplementation, either alone or in combination with vitamin D3, for a period of 6 months, was able to reduce, though not significantly, serum triglycerides by approximately 30 and 32% ($p = 0.1$), respectively, and plasma carotenoid levels by 14% ($p = 0.07$) and 9% ($p = 0.1$), respectively. It was hypothesised that these effects were likely due to the formation of calcium-lipid complexes in the gut, and that, despite the

absence of statistical significance, calcium supplementation could indeed lower blood triglycerides and consequently carotenoids on the long term. However, no effect on vitamin D status has been reported.

No data is available regarding the influence of calcium or other mineral supplements and other vitamins. In an early animal trial on weaning male rats receiving a hypervitamin A rich diet, addition of calcium (as carbonate) did not reduce fractures caused by vitamin A, not proposing any significant interaction with vitamin A absorption (Moore & Sharman, 1979).

3.2. Carotenoids and divalent minerals

3.2.1. In vitro studies with carotenoids

A few *in vitro* studies have meanwhile investigated interactive effects of various divalent minerals, as well as sodium, and carotenoids during simulated gastro-intestinal digestion. In these studies, solutions of divalent minerals and sodium (all in chloride form) were added either to various test meals (green leafy vegetables or vegetable/fruit juices), or to individual carotenoids, dissolved in an artificial emulsification system consisting of oleic acid, lecithin, and monoolein (Corte-Real et al., 2016). The first report mentioning a negative interaction between minerals and carotenoid bioaccessibility emphasized negative effects of calcium on beta-carotene at concentrations of 5 mM (Biehler, Kaulmann, Hoffmann, Krause, & Bohn, 2011), while sodium enhanced beta-carotene bioaccessibility by up to 60% (at 0.9 M vs. controls, i.e. 0.15 M). In a first systematic study (Biehler, Hoffmann, Krause, & Bohn, 2011), increasing concentrations of iron, zinc, magnesium and calcium, added at concentrations of 3.8 (212 mg/L), 3.8 (250 mg/L), 12.5 (300 mg/L) and 7.5 mM (300 mg/L), respectively, resulted in a dose-dependent decrease of individual and total carotenoid bioaccessibility (lutein, beta-carotene), as well as Caco-2 cellular uptake of carotenoids from spinach. IC50 (half-inhibitory concentration) values for bioaccessibility were reported to be approximately 7 (390 mg/L), 9 (590 mg/L) and 15 mM (600 mg/L) for iron, zinc, and calcium, respectively, when compared against physiological saline (0.15 M). The reduction of beta-carotene was significantly more pronounced than that of lutein, indicative of a stronger effect of minerals on the more apolar carotenoids, presumably related to their lower aqueous solubility and their higher sensitivity toward factors disturbing micellization.

In fact, it has been observed in several *in vitro* studies that lower concentrations of calcium, but also of magnesium and zinc, resulted in higher solublilization of carotenoids compared to the absence of added divalent minerals. For example, the presence of approx. 250 mg/L calcium improved the bioaccessibility of beta-carotene from various juices/nectars by up to 100% (Corte-Real, Bertucci et al., 2017). Again, also the addition of sodium has been reported to enhance bioaccessibility of carotenoids by up to 300% at 1500 mg/L (Corte-Real, Bertucci et al., 2017), interestingly much lesser so from matrix-free digestion (Corte-Real et al., 2016) compared to various juices and leafy vegetables (Corte-Real, Bertucci et al., 2017), perhaps due to the suppression of rather negative effects the food matrix may pose on the formation of mixed micelles, suggesting that indeed this effect could be seen in a complex food matrix.

Thus, negative effects were observed starting from doses equivalent to an intake of approx. 210 mg, 250 mg, 300 mg, and 300 mg for iron, zinc, calcium and magnesium, respectively, given their dissolution in approx. 1 L gastro-intestinal fluid, i.e. if taken within one meal or supplement. The doses are therefore physiological only for calcium and magnesium, with RDAs for 19–30 y old men of 1000 and 400 mg/d for adults, respectively, though doses are difficult to achieve even for the latter 2 via dietary intake without supplements. Dietary supplements for magnesium range approx. between 50 and 250, and for calcium between 250 and 1000 mg, while only up to approximately 65/50 mg for iron/zinc. It was however unclear whether the observed effects were related to either decreased bile salt or fatty acid concentration, due to precipitation or complexation or both, or due to other unknown mechanisms such as micelle destabilization. Furthermore, the influence was tested merely on one test meal (spinach).

These limitations were avoided in later studies (Corte-Real, Bertucci et al., 2017; Corte-Real, Richling, Hoffmann, & Bohn, 2014; Corte-Real et al., 2016). In a first approach, individual carotenoids (beta-carotene, lutein, lycopene and neoxanthin) were emulsified without a further food matrix in order to produce micelles based on lecithin, oleic acid and monoolein, and the effect of mineral concentrations of sodium (0–1500 mg/L, as a control), zinc (0–200 mg/L), calcium (0–1000 mg/L) and magnesium (0–300 mg/L) were studied. All minerals except sodium inhibited the bioaccessibility of all observed carotenoids, with an average IC50 of 270 ± 18, 253 ± 75 and 420 ± 322 mg/L for calcium, magnesium and zinc, respectively. These values were somewhat lower than those reported by Biehler et al. (590 mg/L for zinc and 600 mg/L for calcium), perhaps indicative that in more complex test meals the IC50 values tend to be higher, due to higher viscosity, or binding of divalent minerals by organic acids (oxalic acid, phytic acid) or other complexes, such as dietary phosphates, all of which have been reported to chelate divalent minerals. In addition, it was found that the addition of minerals decreased viscosity and enhanced surface tension, which is in line with massive precipitations observed during the intestinal phase of digestion, likely including fatty acids and also bile salts, though this was not clearly proven.

In a follow-up study by Corte-Real et al. (2017), the influence of adding divalent minerals (calcium, magnesium, zinc) to real food matrixes (spinach, field salad, tomato juice, apricot nectar, carrot juice) on the bioaccessibility of individual carotenoids was studied, and also physicochemical properties of the digesta (macroviscosity and surface tension, in addition to zeta potential of mixed micelles) were studied, at similar concentration ranges than in the previous articles. In general, the negative effects of increasing mineral concentrations were observed for all matrices and at higher mineral concentrations (at 500 mg/L or higher for calcium and at 300 mg/L or higher for magnesium). A decrease in the absolute values of the zeta potential indicated decreased mixed micelle stability with higher mineral concentrations, also in line with the observed precipitations. However, as *in vivo* conditions could be much more varying and dynamic than during simulated *in vitro* digestion in static models (Alminger et al., 2014), a broader scope of gastro-intestinal digestion conditions and their influence on mineral-carotenoid bioaccessibility was studied recently (Corte-Real, Desmarchelier et al., 2017). More specifically, combinations of either high and low bile extract (1 and 8 mM) and high and low pancreatin (100 and 720 mg/L) were investigated regarding the influence of magnesium on spinach carotenoids digested with either canola oil or coffee creamer (containing a similar amount of lipids). In general, the results demonstrated that the negative effect of magnesium addition was observed for all bile/pancreatin combinations, suggesting that such interactions are indeed likely to have effects *in vivo*, given a sufficiently high concentration of divalent minerals. The effect was also seen for both types of lipid sources.

Conversely, some positive effects of carotenoids on trace element absorption at more physiological doses have also been reported. One study reported that adding pure beta-carotene (200 µg or 400 µg, constituting non-physiological doses) to an *in vitro* digestion model significantly improved the bioaccessibility of zinc (16.5–118.0%) and iron (19.6–102.0%) from food grains – rice (*Oryza sativa*), sorghum (*Sorghum vulgare*), green gram (*Phaseolus aureus*) (whole) and chickpea (*Cicer arietinum*) (whole) (Gautam, Platel, & Srinivasan, 2010). Authors also reported that the addition of beta-carotene rich vegetables, carrot and amaranth, also enhanced iron, but not zinc bioaccessibility. Although the authors attributed these results to the presence of beta-carotene, it is actually difficult to discern whether other food constituents played a role as well, such as vitamin C, which may have positively affected iron absorption.

Some of the interactions between carotenoids and divalent minerals may be related to factors other than the digestive process. Work from

García-Casal and colleagues, for example, has shown that carotenoids, but not vitamin A, were able to improve iron uptake, in the form of NaFe-EDTA and ferrous fumarate, in a Caco-2 cell model (Garcia-Casal, Leets, & Layrisse, 2000; García-Casal & Leets, 2014). Increases in iron absorption were statistically significant ($p < 0.05$) at iron:carotenoid molar ratios: from 1:0.3 to 1:2 iron:beta-carotene; at 1:2 and 1:1 for lycopene; from 1:1 to 1:0.13 for lutein, and from 1:1 to 1:0.03 for zeaxanthin (García-Casal & Leets, 2014). However, the exact mechanism behind the effects of carotenoids on iron absorption remains to be elucidated. Though these former studies suggested that carotenoids enhance iron absorption, it might come at the expense of carotenoid oxidation as reported by Sy, Dangles, Borel, and Caris-Veyrat (2013). The authors, investigating the reactivity of (all-E)-beta-carotene in the presence of heme (0.5–500 mM) and nonheme iron (0.05–25 mM) found that under the acidic conditions (pH = 4) of a simulated gastric digestion, beta-carotene (15 μM) was quickly oxidized, while the conversion of Fe^{II} to Fe^{III} was inhibited, which may thus also be hypothesised as the underlying mechanism for enhancing iron absorption by carotenoids.

3.2.2. *In vivo studies with carotenoids – Influence on minerals*

Similar results as reported above *in vitro* have also been encountered *in vivo* by the same authors. García-Casal and colleagues (2006) tested the effect of adding lycopene (3.6 mg), lutein (1.8 mg) and zeaxanthin (1.8 mg) to a wheat- or corn-based radioactive labelled meal, and iron bioavailability was assessed by measuring the incorporation of radioactive iron into the bloodstream 15 d after the test meals intake. The authors found that iron absorption significantly increased from 8.1% to 22.5%, 15.8%, and 16.5% when 3.6 mg of lycopene, 1.8 mg of lutein, or 1.8 mg of zeaxanthin, respectively, were added to a wheat-based breakfast. Similarly, the same amounts of carotenoids added to a corn-based breakfast led to a significant increase of iron bioavailability from 5.4% to 18.0%, 12.9%, and 11.1%, respectively. Curiously, the investigated carotenoids were also capable of overcoming the coffee-mediated inhibition of iron absorption, which was additionally tested in the human study. However, it is hard to discriminate whether carotenoids *per se* had an effect on iron absorption, or if the trial results were somehow influenced by the supplement formulation. Thus, despite the *in vitro* and *in vivo* results, the mechanism behind the effect of carotenoids on iron absorption is yet to be elucidated, though a protective effect against oxidation of Fe^{II} into Fe^{III} appears plausible.

Other studies have in turn reported that iron supplementation is able to improve vitamin A status, while zinc supplementation appears to improve carotenoid bioavailability. Kana-Sop et al. (2015) looked at the influence of iron (20 mg of iron fumarate) and zinc (20 mg of zinc sulphate) supplementation (11 days) on the bioavailability of retinol and provitamin A carotenoids from papaya. Participants were assigned to 1 of the 3 testing groups – 1) iron alone, 2) zinc alone, or 3) zinc + iron – and followed a vitamin A free diet and provitamin A carotenoid free diet, from day 6 to day 11 of the trial period. On day 11, participants were given a papaya based test meal for breakfast, and the postprandial concentrations of beta- and alpha-carotene, beta-cryptoxanthin and retinol in the plasma chylomicron fraction were determined over a period of 7 h. While postprandial concentration of retinol was significantly higher in the groups taking iron or iron + zinc, compared to zinc alone, the postprandial provitamin A carotenoid concentration in chylomicrons was higher in the groups taking either zinc or zinc + iron, compared to iron alone. Given these results, the authors proposed that iron supplementation promoted the conversion of the provitamin A carotenoids into retinol, hence the former lower chylomicron appearance when compared to the zinc supplement group. However, the study design did not include a placebo group, so it is difficult to evaluate the real influence of zinc and iron status/supplementation on the bioavailability of carotenoids and retinol metabolism. It is possible that subjects suffered in part from iron deficiency anaemia, characterized by chronic inflammation (Rubin, Ross, Stephensen, Bohn, & Tanumihardjo, 2017), which is known to reduce retinol transport (and thus showing an apparent reduced conversion into retinol).

In another human trial with pregnant women receiving iron (30 mg) and folic acid (0.4 mg), participants were additionally supplemented daily with zinc alone (30 mg), beta-carotene (4.5 mg) alone, or zinc plus beta-carotene, for their entire pregnancy period. Micronutrient status was assessed 1 and 6 months postpartum. Six months after partum, women who were supplemented with zinc plus beta-carotene showed significantly higher plasma concentrations of beta-carotene compared to the control group (i.e. women taking iron and folic acid only), while no significance was seen when women were supplemented with either zinc or beta-carotene alone (Dijkhuizen, Wieringa, West, & Muhilal, 2004). While it is not clear why zinc supplementation together with beta-carotene improved the plasma concentration of the latter, authors suggested that zinc could have enhanced beta-carotene uptake from the intestinal lumen, for instance, by affecting the excretion of pancreatic enzymes or the composition of bile acids. Studies have also reported that supplementation with zinc significantly improved plasma concentration of vitamin A in the participants (Dijkhuizen et al., 2004; Munoz, Rosado, Lopez, Furr, & Allen, 2000), which could be due to an effect of zinc on an increased synthesis of retinol binding protein (RBP) in the liver with a consequent higher production of vitamin A. Indeed, a lack of zinc is related to general effects on the human metabolism, slowing down many essential processes, resulting e.g. in impaired physical growth and altered vitamin A metabolism (Hambidge, 2000). Despite these existing studies, indicating a role for vitamin A on iron status and vice versa, it should be pointed out that many of the trial designs looking at these interactionsare not without certain flaws, including the following: the absence of a placebo group; lack of randomization; no assessment of the baseline status of either iron or vitamin A; and the lack of assessment of additional markers of anaemia and inflammation in iron and/or vitamin A deficient populations.

3.2.3. *In vivo studies with minerals – influence on carotenoids*

Two recent human trials have reported contrarily effects of dietary calcium on carotenoid bioavailability. In the first published study, lycopene (19 mg) bioavailability from tomato paste was assessed in a randomized cross-over study with 10 subjects (Borel et al., 2016). In this postprandial trial, area-under the plasma concentration-time curve suggested that 500 mg of dissolved calcium (as carbonate) was able to reduce lycopene bioavailability by 83%. A limitation of this study was the limited follow-up in the time of plasma-measurements, which was limited to 7 h and may have been insufficient to differentiate any later influences on absorption kinetics.

A second randomized cross-over placebo controlled trial with 24 subjects receiving either a placebo or 500 or 1000 mg calcium as carbonate in gelatine capsules with a spinach rich meal (23 mg total carotenoids) was recently published (Corte-Real, Guignard et al., 2017). Following area-under time curves of plasma triacylglycerol rich lipoprotein (TRL) fractions, no significant differences in the bioavailability of beta-carotene, lutein, beta-cryptoxanthin, or plasma triglycerides were found. It is possible that the high fibre content of spinach compromised the availability of carotenoids, or that the dissolution of calcium carbonate required too much time for resulting in negative interactions with the released carotenoids. It also is possible that, when comparing both human intervention studies, the more lipophilic lycopene in the first trial was more strongly influenced in its solubility than the more polar carotenoids (mostly lutein) present in spinach.

Thus, these 2 studies demonstrate that effects such as type of carotenoid, matrix, kinetic of the mineral, and measured endpoints could possibly influence interactions and results between minerals and carotenoids, and point out that more human trials in this domain are warranted.

4. Conclusions and perspectives

Calcium supplementation has been regarded as a potential treatment for lowering total blood lipids and blood pressure (Cronin et al., 2016; Vaskonen, Mervaala, Sumuvuori, Seppanen-Laakso, & Karppanen, 2002), and some epidemiological evidence even supports its use for dietary management of obesity (Schrager, 2005). Given the evidence of the positive effects of millimolar mineral concentrations on lipolysis, and of high mineral intake on the increased faecal excretion of fats, it is yet to be determined whether other divalent minerals act in a similar fashion, and what their effect on the bioavailability of lipophilic food microconstituents over a wide range of physiological concentrations may be.

However, taken together the observational trials on animal and *in vitro* mechanistic investigations, it is likely that high concentrations of divalent minerals are able to interfere with digestive processes, resulting in the precipitation of lipids and bile salts required for the emulsification of liposoluble constituents, though significant interactions have only been suggested for carotenoids, cholesterol, fatty acids, and minerals themselves. Nevertheless, this effect of reducing available fatty acids and bile acids may have repercussions on the availability of other liposoluble microconstituents, in addition to limiting the absorption of fatty acids itself.

While for many subjects following a regular and diverse diet, the potential interactions between divalent minerals and liposoluble dietary constituents is likely of no concern, this could differ for subjects taking large quantities of supplements, and/or for those with malabsorption conditions, such as pancreatitis, inflammatory bowel diseases such as Crohn's disease or ulcerative colitis (Kaulmann & Bohn, 2016), or subjects with a critical status of the aforementioned liposoluble micronutrients, for example subjects with low vitamin A status (Bhutta, Salam, & Das, 2013). Therefore, the investigation of potential negative influences of high doses of divalent minerals and liposoluble microconstituents is warranted.

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Conflict of interest

The authors have no conflict of interest to declare.

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