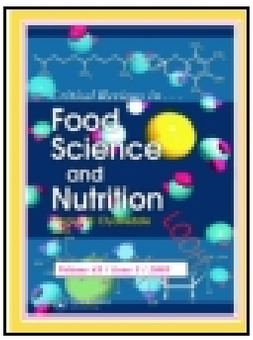

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

Role of calcium on lipid digestion and serum lipids: a review

Ana-Isabel Mulet-Cabero & Peter J. Wilde

To cite this article: Ana-Isabel Mulet-Cabero & Peter J. Wilde (2021): Role of calcium on lipid digestion and serum lipids: a review, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2021.1954873

To link to this article: https://doi.org/10.1080/10408398.2021.1954873

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2021.1954873

Taylor & Francis
Taylor & Francis Group

REVIEW

Role of calcium on lipid digestion and serum lipids: a review

Ana-Isabel Mulet-Cabero and Peter J. Wilde

Quadram Institute Bioscience, Norwich Research Park, Norwich, Norfolk, UK

ABSTRACT

Calcium is an essential nutrient for humans that can be taken as supplement or in a food matrix (e.g. dairy products). It is suggested that dietary calcium may have a beneficial effect on cardiovascular risk but the mechanism is not clear. In this review, the main mechanisms of the possible cholesterol-lowering effect of calcium, i.e. interaction with fatty acids and bile acids, are described and clinical evidences are presented. The observations from interventional studies of the possible cholesterol-lowering effect in terms of the main related mechanisms are variable and do not seem to fulfill all the related aspects. It seems that the interplay of calcium in blood lipid metabolism might be due to its complex and multiple roles in the lipid digestion in the small intestine. The interactions between calcium and, fatty acids and bile may lead to impaired mixed micelle formation and solubilization, which is crucial in the lipid absorption and metabolism. In addition, the calcium source and its surrounding matrix will have an influence over the physiological outcome. This research is important for the delivery and formulation of calcium, particularly with the move toward plant-based diets.

KEYWORDS

Calcium; lipid digestion; cholesterol; fatty acids; bile

Introduction

Calcium (Ca) is an essential nutrient for humans that is an important component of our diet through foods rich in Ca or can be taken as supplement, mainly in the form of citrate or carbonate. The major contributor of Ca in diet is dairy produce. Dairy products contain a high content in saturated fatty acids (FAs) and cholesterol and, therefore, have been associated with an increased risk of cardiovascular disease (CVD). However, it has been observed that dairy product consumption resulted in a neutral or even beneficial association with CVDs (Givens 2017). This paradox has been potentially associated to their high Ca content. CVDs are currently one of the leading causes of mortality worldwide (WHO 2015). A high plasma concentration of cholesterol has been considered as a causal risk of CVDs (Pekkanen et al. 1990). Therefore, much attention has been paid to find strategies for reduction of plasma cholesterol.

It is suggested that dietary Ca may have a beneficial effect on cardiovascular risk through its effect on serum lipid profile. Several intervention studies have showed that Ca supplementation may lower total and low-density lipoprotein (LDL)- cholesterol concentrations (Boon et al. 2007; Denke, Fox, and Schulte 1993; Ditscheid, Keller, and Jahreis 2005; Lorenzen and Astrup 2011; Reid et al. 2002; Shahkhalili et al. 2001). However, the consistency and magnitude of the effects were variable and not all the studies have confirmed this effect (Rajpathak et al. 2010; Reid et al. 2010). Some studies have observed the increased fecal fat excretion with the increase in Ca intake (Denke, Fox, and Schulte 1993; Govers et al. 1996; Lorenzen and Astrup 2011; Shahkhalili et al. 2001; Soerensen et al. 2014). The cholesterol-lowering effect of Ca has been attributed to increased FA excretion, in particular saturated FAs. However, the studies did not always show a significant increase in saturated FA excretion (Boon et al. 2007; Ditscheid, Keller, and Jahreis 2005). Saturated FAs have been linked to increased risk of CVD, through the increase of blood lipids in particular LDL-cholesterol (Griffin 2017). The mechanism underlying the increased LDL-cholesterol by saturated FA is not clear yet, but it has been suggested that it might be via suppression of LDL-receptor mediated clearance of LDL-cholesterol (Grundy and Denke 1990). In addition, other studies have shown an increased bile salts excretion with high Ca intake (Ditscheid, Keller, and Jahreis 2005; Govers et al. 1996; Lorenzen and Astrup 2011). This would lead to a lower amount of bile acid (BA) being reabsorbed in the intestine, causing a higher de novo synthesis of BAs from blood cholesterol. In general, there is not a clear understanding of the mechanisms underlying the effect of Ca on the serum lipid profile.

These mechanisms are intimately related to the lipid digestion process occurring in the small intestine. Lipid digestion is a dynamic process and Ca has been considered a controlling factor. Ca may act as both promoter and inhibitor of lipid digestion. Ca can promote the flocculation of the oil droplets (Ye and Singh 2000; Ye and Singh 2001), which results in less surface area thereby reducing lipid digestion. On the other hand, Ca can facilitate lipid digestion by aiding the removal and precipitation of the FFAs that can be accumulated at the surface of the droplet (Hu et

CONTACT Ana-Isabel Mulet-Cabero anabel.mulet-cabero@quadram.ac.uk

Table 1. Examples of food sources with high Ca content.

Source	Food	Amount (g)	Calcium content (mg)*
Dairy	Yogurt (plain, low fat)	245	448
	Cheese (Cheddar)	42	306
	Milk (whole)	244	276
Sea food	Sardine (canned in oil)	85	324
	Salmon (pink, canned in water)	85	235
Plant	Tofu (firm)	126	253
	Spinach (cooked)	85	122
	Turnip greens	72	99
	Soybeans (cooked)	86	88
	almonds (roasted)	28	75
	Kale (cooked)	85	47
	Broccoli (cooked)	78	31
	Brussel sprouts (cooked)	78	28

*Data from the USDA Nutrient Database for Standard Reference (US Department of Agriculture, Agricultural Research Service 2001)

al. 2010; Zangenberg et al. 2001). Moreover, it has been suggested that Ca forms complexes with phosphate and BAs (Van der Meer et al. 1997). During intestinal digestion, mixed micelles of bile salts, phospholipids and lipid digestion products are formed. Therefore, if high concentrations of Ca are present, the micellization process can be disrupted, changing the solubilizing properties of the mixed micelles.

The aim of this review is to provide an updated overview of the effect of Ca on lipid digestion and serum lipids. We first briefly consider the actual lipid digestion process and Ca sources. Then, the complex role of Ca in lipid digestion, mainly in in vitro studies, is illustrated. Finally, based on in vivo studies, we present the role of Ca in serum lipids discussing the main proposed mechanisms for the lipid-lowering effect.

Digestion process of dietary lipids

Lipids in food are mainly in the form of triacylglycerols (TAGs), constituing approximately 95% of dietary lipids. Lipid digestion starts in the stomach by gastric lipase, accounting for 5-40% overall lipolysis (Armand 2007). Gastric lipase has been reported to preferentially cleave short- and medium-chain fatty acid at sn-3 position of the ester bond of TAG resulting in sn-1,2 diacylglycerol (DAG) and free fatty acids (FFA) (Moreau et al. 1988). Lipids are further digested in the small intestine by the action of lipases and esterases, i.e. pancreatic lipase, cholesterol esterase and phospholipase, facilitated by the presence of colipase, bile salts and biliary lipids. The human pancreatic lipase is responsible for the hydrolysis of 40-70% of TAGs. (Armand 2007). Bile salts play a crucial role in lipid digestion, being involved in different steps of lipolysis. The DAGs and TAGs from the gastric phase are emulsified through the excellent emulsifying properties of bile salts, creating a large surface area for hydrolysis to continue (Bauer, Jakob, and Mosenthin 2005; Fave, Coste, and Armand 2004). Bile salts can interact and disrupt the interfacial emulsifier layer, facilitating lipase adsorption at the interface, thus further promoting hydrolysis. Pancreatic lipase acts at the surface of the lipid particle and hydrolyzes FAs at the sn-1 and sn-3 positions of the glycerol backbone and produces FFAs and 2-monoacylglycerol (MAG) (Rogalska, Ransac, and Verger 1990). Colipase absorbs to the TAG surface and interacts with lipase and bile salts to allow the TAG to interact with the active site of the lipase enzyme, facilitating the hydrolysis of FA from the TAG molecule. The short- and medium- chain FA ($\leq$ C12) released are more water-soluble and therefore easier to be removed from the interface for subsequent absorption in the intestinal mucosa. In contrast, long-chain FA and MAG are relatively less water-soluble and remain absorbed at the oil-water interface (Bauer, Jakob, and Mosenthin 2005). Bile salts facilitate the removal of the lipolysis products from the interface, by acting as a detergent, effectively solubilizing the lipolysis products into mixed micelles. These micelles are formed by the bile salts micelles and interactions with phospholipids, cholesterol and lipolytic products (FFA and MAG). These colloidal structures allow the solubilization and transport of lipids to the gut wall, facilitating the absorption of lipids.

Dietary components such as Ca affect the process of lipid digestion. Ca might interact with FFA, BAs and cholesterol, altering the course of lipolysis and lipid absorption, as described in the following sections.

Calcium sources and bioavailability

Ca is an essential nutrient that performs many important functions in the human body including bone and teeth mineralization, a cofactor for enzymes, neuronal transmission and muscle contraction. The average recommended dietary allowance of Ca is about 900 mg per day for adults, rising to 1,200 mg/day for adolescents and the elderly. The requirements are greater during certain life periods such as during pregnancy. Inadequate intake of Ca is a global problem, especially in aging populations, and it has been associated with several medical disorders such as osteoporosis. For more information see the review by Theobald (2005).

A certain level of Ca is naturally present in human digestive juices and additional amounts arise from ingested foods, particularly those containing high levels of this mineral.

Dietary calcium

Ca can be consumed either in the form of a food matrix or as inorganic dietary supplement. Ca is present in a wide variety of foods. In a typical Western diet, it is estimated that approximately 72% of Ca is obtained from dairy products and the remaining Ca comes from small contributions from vegetables, grains, legumes, fruit, fish and eggs (Del Valle et al. 2011). Table 1 shows a summary of the main foods with a high Ca content from dairy, seafood and plant sources. However, a food source containing a high amount of Ca does not necessarily imply that it is a good source of Ca intake. For instance, Titchenal and Dobbs (2007) stated "The calcium content is similar in equal volumes of cooked spinach and milk. However, it would require approximately

6 L of cooked spinach (or 700 mL of milk) to obtain 300 mg of absorbable calcium".

In milk, Ca is in equilibrium between the aqueous and proteinaceous phases (large aggregated protein structures known as casein micelles). One-third of Ca is in the aqueous phase of milk, mainly, as ionic Ca (free ion) and a stable complex mainly with citrate and to a lesser extent with inorganic phosphate. In the casein micelle phase, Ca is bound to the phophoserine residue present in casein as well as to the colloidal inorganic phosphate (Gaucheron 2005). However, the detailed structure and interaction between calcium phosphate and caseins in the micelles is not clear yet.

The most common forms of supplemental Ca are Ca carbonate and Ca citrate. Some studies have tried to rank the bioavailability of Ca from different Ca salts. Ca citrate and Ca lactate seemed to be more efficiently absorbed than Ca carbonate (Nicar and Pak 1985). However, the bioavailability of Ca was not directly related to the solubility of the Ca salts (Sheikh et al. 1987). Other studies did not find differences in the utilization of Ca from various sources (Patton and Sutton 1952; Sheikh et al. 1987). The differences in results may reflect the variables measured (urine or fecal Ca analyses), the levels of Ca fed, the duration of the studies and the manner in which the Ca sources were administered (alone, or in meals).

Absorption of Ca is also dependent on the various components in a food matrix or present in a meal that can bind Ca or form insoluble Ca salts. For instance, oxalic acid and phytic acid, contained in plant- and cereal-based foods, have been reported to decrease Ca absorption by formation of insoluble complexes (Kennefick and Cashman 2000; Larsen et al. 2003), dietary fiber can have a similar effect through Ca binding or physical entrapment (Torre, Rodriguez, and Saura-Calixto 1991). In contrast, proteins and phosphopeptides have demonstrated a positive effect on Ca absorption. Phosphopeptides, such as those in dairy, can bind and increase the proportion of soluble Ca, thereby facilitating its absorption (Li, Tomé, and Desjeux 1989). The bioaccessibity of Ca is critical for its absorption. Some studies have shown that the bioaccessibility of Ca is dictated by matrix (Sahuquillo, Barberá, and Farré 2003) and Ca source (Lorieau et al. 2018). These studies highlight that the information of the Ca content in a food product is not necessary representative of the Ca that is actually absorbed.

Calcium in the human digestive tract

The Ca levels in the human gut depends on the nature of the food consumed and may vary from person-to-person. The fluids in the upper gastrointestinal (GI) tract contain electrolytes including Ca. In saliva, the total range of Ca concentration found in 11 individuals was 1.1-4.2 mmol/L (Larsen et al. 1999). In the fasted state, the average Ca concentrations in the stomach and upper intestine were reported as 0.6 ± 0.2 and 0.5 ± 0.3 mmol/L, respectively (Lindahl et al. 1997). Data after a meal, containing minimum amounts of Ca, showed the mean Ca value of 1.5 mmol/L and between 0.25-2 mmol/L in stomach and upper intestine, respectively (Fordtran and Walsh 1973). However, after a meal containing 250 mL of milk and 2 glazed donuts the content of Ca in the small intestine went up to 20 mmol/L.

The digestive conditions within the standardized in vitro static method for food digestion of INFOGEST are based on the fed state of human digestion (Brodkorb et al., 2019). Nevertheless, the concentration of Ca in oral, gastric and small intestinal phases are 0.75, 0.075 and 0.3 mmol/L, respectively.

Effect of calcium on lipid digestion

Ca plays a crucial role in lipid digestion. In vitro studies have shown that increased Ca concentration can promote the rate and extent of lipid digestion in the small intestinal phase (Hu et al. 2010; Li, Hu, and McClements 2011; Lin et al. 2017; Tan et al. 2020; Aiqian Ye et al. 2013; Zangenberg et al. 2001). This effect might be due to some physicochemical mechanisms. It has been shown that a certain level of Ca is required as a cofactor to activate pancreatic lipase (Kimura et al. 1982). Scow (1988) suggested that Ca ions enhance the binding and the activity of pancreatic lipase on the lipid surface by diminishing the electrostatic repulsions occurring between them. Therefore, at low levels of Ca, the enzyme may not be in its most active form, slowing down the rate of lipolysis. There is an alternative and/or complementary approach that has been considered as the most likely mechanism of the action of Ca on lipolysis which is that Ca ions bind and precipitate FFAs during intestinal digestion, leading to an increased lipolysis. Specifically, during lipid digestion, insoluble FAs, in particular long-chain FAs, accumulate at the lipid droplet surface. This hinders the access of pancreatic lipase to the surface of lipid droplets (Fave, Coste, and Armand 2004). Ca increases the partitioning of those FAs from the interface to the aqueous phase in the form of Ca soaps, which are insoluble under intestinal conditions, enhancing the access of substrate to the lipase. The formation of Ca soaps has been assessed by microscopy (Patton et al. 1985; Scow 1988) and interfacial rheology (Torcello-Gómez, Boudard, and Mackie 2018). An increase in lipolysis would be expected to provide an increase of FA bioaccessiblity. However, whilst the effect of Ca in precipitating FAs may increase lipolysis, the poor solubility of the Ca soap could actually reduce lipid bioaccessibility and absorption (Lin et al. 2017; Lorenzen et al. 2007). This is explained in more detailed in the next sections.

In a recent study by Tan et al. (2020) the effect of Ca levels (0.525 to 10 mmol/L) on lipid digestion was studied in corn oil-in-water nanoemulsions using the pH-stat method for quantification of small intestinal lipolysis. The FFA released in the small intestinal digestion increased linearly with increasing concentrations of Ca, from around 39% at 0.525 mmol/L Ca to around 95% at 10 mmol/L Ca. The authors performed a back titration at pH 9 at the end of the intestinal phase. A full hydrolysis of lipids, at levels even higher than 100% FFA, was obtained in all the samples, regardless of the Ca levels used. The authors suggested that

a high proportion of the FFAs generated during the small intestinal digestion were non-ionized at neutral pH when low Ca levels were used, thereby these were not titrated by NaOH used in the pH-stat method. Therefore, it seems that the Ca concentration used in this study did not impact the total amount of FFAs generated but the ionization state of the FFAs. This was supported by Sassene et al. (2014) and explained by the effect of Ca on the equilibrium between unionized and ionized FFAs affecting the carboxylic acid groups, thereby releasing the titratable protons. The addition of Ca pushed the equilibrium toward ionized FFAs according to the Le Chatelier's principle by removing ionized FFAs by formation of insoluble Ca soaps. At high Ca concentrations (e.g. 10 mmol/L), the FAs are mainly detected by continuous titration at neutral pH when the ratio of unionized:ionized FA is below 1. In contrast, at ratios above 1, the majority of FFAs are detected by back titration to pH 9, this is at low Ca concentrations (e.g. 1.4 mmol/L).

The influence of Ca on lipid digestion in an emulsion system has been seen to be dependent on the emulsifier used to stabilize the lipid droplets and any dietary components or food additives that can bind Ca. For instance, Hu et al. (2010) assessed the effect of ethylenediaminetetraacetic acid (EDTA), high-methoxyl pectin and alginate on the extent of lipid digestion in presence of 20 mmol/L of $CaCl_2$ in the digestion of corn oil-in-water emulsion. As the concentration of EDTA increased there was a substantial decrease in lipid digestion rate (from 98% to 32% when EDTA was added at 0 and 5 mmol/L respectively). This effect was attributed to the ability of EDTA to sequester Ca ions and thereby, preventing them from either activating the lipase and/or precipitating the long-chain FAs. Similarly, the authors tested the effect of two anionic polysaccharides i.e. alginate and high-methoxyl pectin on the effect of lipid digestion rate when Ca was present (20 mmol/L). These polysaccharides present different Ca-binding properties; alginate binds more strongly than pectin. The presence of small amounts of alginate (0.05 or 0.1%) considerably reduced the rate and extent of lipid digestion in the emulsion in contrast to the weak effect observed with pectin. However, the effect of alginate could be also due to the entrapment of lipid droplets inhibiting the lipase activity. Similarly, the addition of KH_2PO_4 in soya oil-in water emulsion in presence of 20 mmol/L of $CaCl_2$ led to a reduction of extent of lipid digestion, which as attributed to the formation of insoluble precipitate between Ca and phosphate (Aiqian Ye et al. 2013). In the latter study, the influence of different Ca salts on the kinetics of in vitro digestion was assessed. The soluble salts tested (Ca gluconate, Ca acetate and Ca chloride) had a greater effect on increasing lipolysis than insoluble salts (CaO or $CaSO_4$). These studies suggest the critical role of the ionic state of Ca on lipolysis.

Similarly, Ye et al. (2013) showed how an increase in lipolysis was dependent on the emulsifying agent used, during digestion in presence of Ca. The authors showed that the emulsion stabilized by lecithin reduced the effect of added Ca on lipolysis when compared to the emulsions stabilized by sodium caseinate, whey proteins and Tween 20. This study also showed that the emulsions stabilized by whey proteins and caseins influenced the rate of lipid digestion, in which Ca may also act as inhibitor to lipid digestion. The addition of Ca has been seen to destabilize milk-protein stabilized emulsions by inducing flocculation and aggregation (Ye and Singh 2000; Aiqian Ye and Singh 2001). This effect is attributed to Ca forming bridges between adsorbed layers on different emulsion droplets. This can lead to an increased droplet aggregation and reduced surface area, which may lead to lower the lipid digestion rates. Similarly, Li, Hu, and McClements (2011) showed, in a β-lactoglobulin stabilized emulsion, a lower lipid digestion at 20 mmol/L Ca compared to high lipolysis rates at lower concentrations of Ca. The authors showed that high levels of Ca induced extensive flocculation as seen by the increase in mean particle and less negative zeta-potential. Therefore, in flocculated emulsions induced by Ca, Ca has to two opposing effects: promoting the lipolysis rate by precipitating FFA and reducing the lipolysis rate by flocculation, which could lead to an unchanged or slow down lipid digestion profile.

The effect of Ca on lipid digestion can therefore influence the solubility of other lipophilic compounds. Tan et al. (2020) showed that the bioaccessibility of β-carotene decreased with increasing levels of Ca (from 65.5% at 0.525 mmol/L to 23.7% at 10 mmol/L Ca). The authors attributed this effect to the ability of Ca ions to precipitate the β-carotene contained in the mixed micelles since the concentration of β-carotene increased in the precipitate. However, the concentrations of bile salts in the different phases of the digesta were not measured to confirm the precipitation of bile micelles. Similarly, Lin et al. (2017) showed that the bioaccessibility of β-carotene from a nanoemulsion stabilized by modified starch decreased with the increase of Ca levels, despite the increased lipolysis. The authors showed a positive linear relationship between bioaccessibility of the β-carotene and bile salts ($r = 0.896$) and FFA ($r = 0.943$) concentrations in the micellar phase. The amounts of bile salts and lipolytic products in the micellar phase decreased with increase in the concentration of Ca. Therefore, the reduced solubility of lipophilic component can be due to the precipitation of FFA, bile salts and mixed micelles by Ca. These studies show that Ca also affects the capacity of self-assembly into mixed micelles for the solubilization of lipolytic products, which influence the uptake of components of the micelles, but the mechanisms are not fully understood.

Possible mechanisms of calcium-induced effect on serum lipids

Ca has been mainly associated to a beneficial effect on serum lipids in humans. In an observational study of data from 470 people, Jacqmain et al. (2003) showed that daily intake of Ca significantly lowered total, LDL- and the ratio of total: high density lipoprotein (HDL)- cholesterol in women and men. Many intervention studies in humans have focused on the effect of supplementary Ca (900-2,000 mg per day) on plasma lipid profile. Most of these

studies showed some kind of positive effect of Ca, although the consensus of the results does not seem to be firm. The outcomes concerning the effect of Ca supplementation on LDL-cholesterol are contradictory. Some studies reported a decreasing effect of 4.4-15% (Bell et al. 1992; Ditscheid, Keller, and Jahreis 2005; Shahkhalili et al. 2001), in contrast to other studies showing not statistically significant effect (Chai et al. 2013; Reid et al. 2002). Similarly, there is inconsistency in the effect of total cholesterol and HDL-cholesterol, showing a decrease of 5-7% (Chai et al. 2013; Denke, Fox, and Schulte 1993; Ditscheid, Keller, and Jahreis 2005) and an increase of 4.1-7% (Bell et al. 1992; Reid et al. 2002), respectively, which has been not observed in other studies (Karanja et al. 1994; Rajpathak et al. 2010). Furthermore, Chai et al. (2013) observed the significant TAG lowering effect of Ca ($p < 0.05$), which was not obtained in other studies. However, there are other studies that did not show statistical difference in any serum lipids and lipoproteins levels when compared with a control group (Karanja et al. 1994; Rajpathak et al. 2010; Reid et al. 2010). These conflicting results may be related to different study design, subject number, Ca dosing and source, habitual diet and interaction with other nutrients across the different intervention studies as well as the adjustment of the results for energy intake.

The mechanism by which Ca might affect blood cholesterol remains unclear. However, there are some predominate mechanisms: (1) inhibition of the absorption of saturated FAs, (2) inhibition of the reabsorption of bile salts and (3) inhibition of the absorption of cholesterol.

Interaction of calcium with lipids during digestion

Clinical observations

Several human studies have shown that increased dietary Ca intake leads to increased fecal fat excretion (Table 2). The mechanism behind this effect is probably an interaction between Ca and FAs, resulting in the formation of insoluble Ca-FA soaps, and hence reduction of fat absorption. As illustrated in Table 2, fecal fat excretion in humans increased by an average of 2 g when dietary Ca intake was increased. In a meta-analysis study of 15 randomized controlled trials, it was showed that Ca intake significantly increased fecal fat excretion and impaired the absorption of dietary fat (Christensen et al. 2009). The authors highlighted that this effect might be minor when considering the impact on body-weight regulation. The daily excretion of 2 g of fat (~ 18 kcal) is equivalent to 1 kg body weight in a year. Moreover, it seems that a plateau is reached for the effect of Ca at high concentrations of Ca. For example, Boon et al. (2007) found an increase fecal fat excretion from 4.8 to 7.2 g/day corresponding to an increase of 894 mg/day Ca, similarly to the ingestion of 2,197 mg/day Ca (from 4.8 to 7.5 g/day fecal fat). In a meta-analysis study, dairy products and Ca supplements were compared for their effect on fecal fat excretion showing no significant differences between the sources of Ca (Christensen et al. 2009). However, the investigated dairy trials showed consistency among these studies whereas in the Ca supplement studies there was moderate heterogeneity ($I^2 = 49.5\%$) indicating some inconsistency. The authors attributed this inconsistency to confounding factors such as methods used for fat analyses, Ca sources, matrix in which Ca is provided and interaction with other nutrients. Similarly, Boon et al. (2007) compared the effect of Ca from Ca carbonate with that in dairy products but the authors did not find significant differences in fat excretion.

Formation of calcium-fatty acid soap

The main mechanism suggested behind the fat excretion is the formation of a complex between FFAs, released after hydrolysis of TAGs in the GI tract, and Ca in its ionized form. This concept was first proposed by Givens (1917). One molecule of Ca can bind two molecules of FAs forming the complex called Ca-FA soap or Ca soap. This complex has been shown to be largely insoluble in water and simulated intestinal fluids (Graham and Sackman 1983) and, then, poorly absorbed as determined in rats (Gacs and Barltrop 1977). It has also been suggested that FAs may bind indirectly to Ca via insoluble calcium-phosphate complexes formed in the small intestine (Van der Meer et al. 1997). Therefore, the formation of Ca soap could be the responsible for the impact of Ca on FA digestibility. However, the formation of Ca soaps and its effects has been little studied. The investigation of direct evidence of the presence Ca soaps in feces is scarce due to the complexity of extracting the Ca soaps from the fecal material and low recoveries (Owen et al. 1995). This is mainly because Ca soaps are comprised of different FA that have different levels of solubility in organic solvents. Therefore, the current measurement is based on the extraction of the total fat.

Factors influencing calcium soaps formation

The interaction between Ca and FAs depends on several factors such as the release of Ca from the food matrix, the solubility of the dietary Ca source within the GI tract, the fat source and its FA composition, the pH of the intestinal environment and the presence of other dietary compounds (Kies 1985; Kopic and Geibel 2013).

Dietary fiber and protein. In free living, normal-weight and overweight individuals, the association between the habitual Ca intake and fecal fat excretion was studied (Kjølbaek et al. 2017). The authors showed that dietary Ca intake (1000 mg/10 MJ per day) was positively associated with excretion of fecal fat and energy. However, this association was no longer obtained after taking into consideration the adjustment for intake of fiber. Dietary fiber has been reported to affect lipid digestion and some mechanisms have been proposed (Grundy et al. 2016). The intake of dietary fiber can decrease fat absorption by increasing the fecal fat excretion and should be taken into account when considering the effect of Ca. Similarly, a high protein intake has been suggested to improve Ca bioavailability by keeping Ca in solution and, then, preventing the interaction with FAs. To illustrate the latter, Jacobsen et al. (2005) studied the effect on fecal fat excretion in three type of diets; low Ca and

Table 2. Examples of clinical studies showing the effect of calcium on fecal fat excretion and blood lipids.

	Participants					Calcium Intervention				Outcome			
						Dosing (mg/d)			Fat Energy(%)	Fecal fat excretion (g/d)			
Study	Type	Age (years)[1]	N	Design	Duration	Ca (c)	Ca (+)	Source	(%saturated)	Ca (c)	Ca (+)	Ca measures	Blood lipids
Denke, Fox, and Schulte (1993)	Healthy men with moderate hypercholesterolemia	43 ± 4	13	Randomized crossover single-blind	10 days	410	2,200	Citrate malate in juice and muffins, and tablets	34 (13% saturated)	2.6	5.4†	↑Renal†	↓total and LDL-C† ↑HDL-C* ↑TAG* ↔Lipoprotein A
Welberg et al. (1993)	Healthy men and women	27 ± 1	24	Randomized, parallel, doble-bind	1 week	1,320	3,800 5,040	Calcium carbonate supplements	∼35(ns)	5.4	7.0* 7.6*	↑Renal + fecal †	nm
Govers et al. (1996)	Healthy men	38 ± 2	13	Randomized, doble blind, cross-over, placebo-controlled	1 week	764	1,820	Dairy products	35 (15% saturated)	6.7	9.3†	↑Renal + fecal† ↑Fecal† ↑Fecal water*	nm
Shahkhalili et al. (2001)	Healthy men	34 ± 6	10	Randomized, double-blind, crossover study	2 weeks	950	1,855	Calcium carbonate in chocolate matrix	39% (16.6%saturated)	4.4	8.4†	nm	↓total-C* ↓LDL-C † ↔HDL-C and TAGs
Jacobsen et al. (2005)	Men and women, healthy and moderately overweight	24.2 ± 2	10	Randomized crossover	1 week	474	1,735 1,869	Dairy products	30%(ns)	6.0	14.2† 5.9*	nm	↔Total, HDL-C ↑LDL-C* ↓TAG*
Ditscheid, Keller, and Jahreis (2005)	Men and women, Healthy	25 ± 2	31	Placebo-controlled, double-blind, crossover study	4 weeks	1,193	2,204	Pentacalcium hydroxy-triphosphate in a bread matrix	35(18% sat)	3.9	4.3*	↑Fecal† ↑Renal†	↓ Total-C† ↓LDL-C* ↓LDL/HDL *
Boon et al. (2007)	Healthy mean and women	28 ± 2	10	Randomized, crossover	7 days	348	1,242 2,545 1,242	Dairy products CaCO3 supplements	35% (ns)	4.8	7.2* 7.5* 6.7*	↓Plasma* ↑Renal† ↓Fecal*	↓HDL-C* ↓Total-C* ↓TAG(†only at the highest conc)
Bendsen et al. (2008)	healthy, moderately overweight men and women	range 25–47	11	Randomized crossover	7 days	698	2,287	Dairy products	30% (12%)	5.4	11.5†	↑Fecal† ↑Renal†	nm
Lorenzen and Astrup (2011)	Healthy men	32.8 ± (SEM 1.2)	9	Randomized cross-over	10 days	500	1,971 1,977	Dairy products (milk)	49.5 (60%) 25(54%)	∼6	11.3† 8†	↑Fecal† ↑Renal†	↓Total-C†, ↓LDL-C†, ↔HDL-C
Soerensen et al. (2014)	Healthy men	27.7 ± 4.8	15	Randomized crossover	2 weeks	500	1,700 1,700	Dairy products (milk vs cheese)	32 (19%)	3.9	5.2† 5.7†	Nm	↓ total- and LDL-C † ↔TAG and HDL-C

[1]Mean ± SD, otherwise specified. ns, not specified. nm, not measured. C, cholesterol. LDL, low-density lipoprotein. HDL, high-density lipoprotein †Significantly different from calcium diet control (Ca (-)) ($P < 0.05$). * Not statistically significantly different from the calcium diet control

normal protein [500 mg Ca, 15% of energy (E%) from protein], high calcium and normal protein (1800 mg Ca, 15 E% protein), and high Ca and high protein (1800 mg Ca, 23 E% protein). The authors showed that the fecal fat excretion increased 2.5- fold when the dietary Ca content increase and the protein content remained the same (15 E%). However, there was no increase in fat excretion when the increased dietary Ca intake was incorporated together with an increase in the protein content. Moreover, the Ca excreted in urine was 60% higher for the high Ca/high-protein diet compared to the high Ca/normal-protein diet. This suggested that more Ca was absorbed and, consequently, there was less Ca available for Ca soap formation. Similarly, Boon et al. (2007) did not show significant differences in fecal fat excretion in diets varying in Ca (400- 2,500 mg Ca/day) with a daily protein intake of 20% of energy. Ca might bind to the phosphoserine residues of proteins, for instance casein (Scholz-Ahrens and Schrezenmeir 2000). The Ca-protein complexes would lead to less Ca available for the formation of Ca soaps, explaining the lower excretion of FA in the presence of high protein. Therefore, fiber and protein are examples of dietary confounding factors that can modulate the effect of a high Ca diet.

Fatty acid chemistry. In vivo studies have suggested that the FA chain length and the degree of saturation are a major contributing factor that determined the extent of Ca soap formation. Greater formation of soaps has been observed with saturated long-chain FAs than unsaturated FAs and shorter-chain FAs. For example, Shahkhalili et al. (2001) showed that Ca supplementation in chocolate increased fecal fat excretion by ~2-fold. The FA excretion was significantly much higher in the saturated FAs, especially of those of the main FA in cocoa butter i.e. 16:0 and 18:0, having increased from 0.55 to 1.75 g/day and from 0.78 to 3.0 g/day, respectively. Similarly, Denke, Fox, and Schulte (1993) showed that the content of saturated FAs of 18:0, 16:0 and 14:0 in Ca soaps was higher in a high Ca diet compared to a low Ca diet, increasing in total from 6% to 13% whereas the FA 18:1 presented a lower increase (from 2 to 4%). The higher capacity of saturated FAs to form Ca soaps might be because these FAs are absorbed more slowly from the intestine compared to unsaturated FAs since they take longer to be incorporated into the biliary micelles (Ockner, Pittman, and Yager 1972). Hence, the saturated FAs have a higher probability of interaction with dietary Ca. Moreover, it has been reported that the Ca soaps that are formed by saturated long chain FAs are poorly soluble in the GI conditions. Gacs and Barltrop (1977) showed in in vitro conditions that stearic acid (C18:0) presented the lowest solubility follow by medium-chain FA (C10-C14) and short-chain FA (C8) soaps. In contrast, Bendsen et al. (2008) showed that monounsaturated FAs were more affected by Ca than saturated FAs. It is important to note that the fat in the latter study was from dairy products in which saturated FAs are mainly located in the position sn-2. The FA in the extreme position of the TAG, i.e. sn-1 and sn-3 are more susceptible to lipase hydrolysis during intestinal digestion, hence, they might be more susceptible to interact with Ca. This study illustrates that the TAG structure of the dietary fat can be another controlling factor in the degree of FA excretion.

Effects of calcium-fatty acid soaps on serum lipids

As seen in Table 2, most of the studies showing a significant increase in fecal fat excretion following increased Ca consumption have also reported a cholesterol-lowering effect. Soerensen et al. (2014) showed that fecal fat excretion was correlated to changes in LDL- cholesterol and explained a significant proportion of the changes in total- and LDL-cholesterol in a multiple linear regression. Denke, Fox, and Schulte (1993) reported that Ca fortification resulted in a reduction in cholesterol serum of 0.34 mmol/L. This decrease was probably due to the decreased absorption of fat, in particular saturated FAs. The saturated FAs inhibit the receptor-mediated uptake of LDL into liver cells, consequently decreased the clearance of LDL particles from the circulation (Grundy and Denke 1990). In an in vitro study, Vinarova et al. (2016) explained the reduction of solubilized (bioaccessible) cholesterol in presence of Ca by the reduced solubility capacity of the mixed micelles caused by the precipitation between Ca ions and FAs.

In the Denke, Fox, and Schulte (1993) study, it was predicted, using the Keys equation, that the total serum cholesterol lowering effect should result in a 0.05-0.06 mmol/L reduction for a 2-3 g decrease of saturated fat intake, which is much lower than the outcome. Hence, increased fecal fat excretion might be insufficient to explain the observed effect on cholesterol, which was also suggested in other studies (Shahkhalili et al. 2001). It can be therefore suggested that Ca has an additional cholesterol lowering effect, which may be due to the interaction with bile salts explained in the following section. It has also been suggested that fecal fat excretion might be a poor estimate of fat absorption due to the subsequent degradation of unabsorbed FAs by the gut bacteria (Denke, Fox, and Schulte 1993).

The possible interaction of FA and Ca should be reflected in their absorption and excretion. As seen in Table 2, most of the studies have shown a significant increase of Ca excretion in feces and urine. For instance, Lorenzen and Astrup (2011) presented significant fat excretion as well as a higher excretion of Ca in feces and urine during the high Ca diet periods compared with the low Ca diet. However, the intake of Ca did not significantly affect the Ca concentration in plasma even though there was an increased Ca excretion in urine and feces (Boon et al. 2007). A decrease in the postprandial increase in chylomicron TAG would be expected since Ca partly inhibits fat absorption. Findings from human studies of circulating TAGs have been inconsistent. Most of the studies that have measured plasma TAG level did not show any significant effect of Ca intake, despite the increased fat excretion (Denke, Fox, and Schulte 1993; Shahkhalili et al. 2001; Soerensen et al. 2014). However, the consumption of Ca from dairy products resulted in the attenuation of postprandial lipidemia, as seen by the reduction in chylomicron TAG concentration (Lorenzen et al.

2007). This effect was significant in dairy Ca, in contrast to supplemental Ca. The authors suggested that this was probably linked to a reduced fat absorption indicating that Ca from dairy sources might be more effective than supplemental Ca in augmenting the fecal excretion of fat. However, studies are scarce to draw any conclusion. Previous studies have indicated that cheese could be less hypercholesterolemic than other dairy products (Tholstrup et al. 2004), which could be associated with the ability to form Ca soaps by the different behavior of Ca in the matrix. Milk fat is organized differently in dairy products, for instance fat in milk is surrounded by a membrane whereas it is entrapped by a protein structure of different consistency in cheese and yogurt. Ca is bound to milk proteins, in particular caseins. Therefore, the formation of Ca soaps in the intestine may be enhanced in dairy products such as cheese since Ca and fat might present higher interaction. Hence, the effect of milk fat intake may depend on whether the fat is bound within a cheese matrix or not. Similarly, the coagulation of milk in the stomach can bring the fat and Ca structurally closer. Moreover, the concentration of both Ca and fat is higher in cheese compared to milk. Soerensen et al. (2014) compared milk- and cheese- diets (1,700 mg/day Ca) with a control diet (~500 mg/day Ca). The two dairy diets increased fecal fat excretion as well as lowered the total and LDL- cholesterol when compared with the control diet. Cheese diet presented higher fecal fat and lower total and LDL- cholesterol compared with the milk diet, however, the effects were not significant.

As observed in the previous studies, there is a substantial variation in the consistency and magnitude of effects observed, and not all studies were able to confirm the effect on the serum lipid profile in relation to the interaction of Ca with FAs.

Interactions of calcium with bile acids during digestion

Clinical observations

Fecal BA excretion. As observed in Table 3, human studies have generally shown that high-Ca intakes decrease fat digestibility and significantly increase fecal BA excretion between 11 and 53% (Ditscheid, Keller, and Jahreis 2005; Govers et al. 1996; Lorenzen and Astrup 2011; Van der Meer et al. 1990). For instance, in a study by Govers et al. (1996), supplementation with Ca produced not only the expected 2 g/day increase in fecal fat but an increase in BAs (from 492 to 692 µmol/day). However, other studies did not observe a statistically significant effect (Bendsen et al. 2008; Denke, Fox, and Schulte 1993; Lupton et al. 1996). Some studies have shown a concomitant increase in fecal phosphate as well as fecal Ca, even though very few studies have measured this parameter. This could indicate the precipitation of Ca phosphate that could bind BAs, as explained below. Therefore, the lack of consistency of the effect of high Ca intake and fecal BA excretion might be explained by the lack of a sufficient amount of phosphate necessary for the formation of Ca phosphate, which could be insufficient to induce the binding of BAs and hence any detectable increase in fecal BA excretion. However, phosphate in human diets is thought to be in excess of Ca to form that complexation. The intestinal microbiota has been seen to be involved in the metabolism of Ca, which might influence these outcomes (Skrypnik and Suliburska 2018).

Lorenzen and Astrup (2011) showed the effect of high and low levels of Ca in combination with high- or low-fat diets. High Ca diets presented higher total BA excretion, regardless the fat content. However, the BA increase was more pronounce in the low-fat diet (from 178 to 346 µmol/day) compared the high-fat diet (from 274 to 393 µmol/day). This might be due to a competitive effect for Ca ions of the formation of Ca soaps.

High concentrations of Ca intake have observed to change the BA profile in the feces. Ditscheid, Keller, and Jahreis (2005) showed that secondary BAs were more excreted in feces than primary BAs, the latter did not differ between the control and high Ca intake diets. Although Bendsen et al. (2008) did not observe any effect of Ca on the total fecal BA excretion, the degree of BA conjugation was significantly higher in the high Ca diet, with greater fecal excretion of both taurine- and glycine-conjugated BAs. Similarly, Van der Meer et al. (1990) showed a 2-fold increase in the ratio of dihydroxy to trihydroxy BAs in the high Ca diet, as seen by the increase in cholic acid and decrease of both chenodeoxycholic and deoxycholic acid. This effect might be influenced by the higher hydrophobicity present in the dihydroxy BAs compared to the trihydroxy BAs. Govers et al. (1996) showed that Ca intake decreased the concentration of secondary BAs as well as hydrophobic FAs in fecal water, which reduced the cytotoxicity of fecal water. This soluble fraction of the total fecal concentration determines the toxicity to colonic epithelial cells. It has been suggested that secondary BAs and hydrophobic long-chain FAs may damage the colonic epithelium and stimulate the proliferation of colonic crypt cells, which enhances the risk of colon cancer (Zimmerman 1993). Therefore, dietary Ca can be seen as a strategy to reduce cytotoxic effects due to its effects in precipitating soluble acids (Buset et al. 1990; Lapre, De Vries, and Van der Meer 1991).

Calcium and bile acids binding

The increase of fecal bile excretion in higher Ca intake could be due to the formation of insoluble Ca salts of BAs or by a less direct mechanism proposed by Van der Meer and De Vries (1985), in which BA anions adsorb to insoluble Ca phosphate particles.

Regarding a possible direct interaction between BA and Ca ions, the precipitation of the insoluble Ca salts of BAs or complexation between BAs and Ca is controlled by the activity of Ca ions, pH and, the concentration and structure of the monomeric BA anion (Gu et al. 1992). In turn, the activity of Ca ions in the GI tract is controlled by several factors including presence of sodium, phosphate, carbonate and FA anions. Hofmann and Mysels (1992) concluded that "although the Ca salts of unconjugated and glycine-conjugated BAs are quite insoluble, they do not precipitate under healthy conditions because of the low monomer

Table 3. Examples of clinical studies showing the effect of calcium on fecal bile excretion and blood lipids.

	Participants					Calcium Intervention					Outcome		
						Dosing (mg/day)			Dietary Phosphate (mmol/day)				
Study	Type	Age[1]	N	Design	Duration	Ca (c)	Ca (+)	Source	Ca (c)	Ca (+)	Bile excretion	Phosphate excretion	Blood lipids
Denke, Fox, and Schulte (1993)	Healthy men with moderate hypercholesterolemia	43 ± 4	13	Randomized crossover single-blind	10 days	410	2,200	Citrate malate in juice and muffins, and tablets	ns	ns	↑Fecal BAs*	nm	↓ C†, ↑TAG*, ↓LDL-C†, ↑HDL-C*
Govers et al. (1996)	Healthy men	38 ± 2	13	Randomized, doble blind, cross-over, placebo-controlled	1 week	764	1,820	Dairy products	75.8	78.6	↑Fecal BAs† ↓ BA in fecal water	↑Total, organic and inorganic fecal P† ↑Total urinary + fecal P* ↓Inorganic P fecal water†	nm
Ditscheid, Keller, and Jahreis (2005)	Men and women, healthy	25 ± 2	31	Placebo-controlled, double-blind, crossover	4 weeks	1,193	2,204	Pentacalcium hydroxy-triphosphate in a bread matrix	1528 (phosphorous, mg/d)	1998† (phosphorous, mg/d)	↑Total fecal BA† ↑Secondary BA† ↔Primary BA*	↑Fecal phosphorous†	↓Total-C†, ↓LDL-C*, ↓LDL-C/HDL-C*
Lupton et al. (1996)	Men and woman, with history of resected colon adenocarcinoma	58.5 ± 1.3	22	Randonised	16 weeks	1,317	+2,000	Calcium carbonate or citrate	ns	ns	↓Fecal Total BA*	↓Fecal inorganic P* ↑Fecal organic phosphate†	
Bendsen et al. (2008)	Healthy, moderately overweight men and women	range 25–47	11	Randomized crossover	7 days	698	2,287	Dairy products	142 (mg, normalized per MJ)	207(mg, normalized per MJ)	↓Total fecal BA* ↑Conjugated BA† ↓Unconjugated BA†	nm	nm
Lorenzen and Astrup (2011)	Healthy men	32.8 ± (SEM 1.2)	9	Randomized crossover	10 days	500	1,971 1,977	Dairy products (milk)	815 (mg/10 MJ)	1996(mg/10 MJ) 2050(mg/10 MJ)	↑Total fecal BA† ↑Fecal Conjugated BA† ↑Fecal deconjugated BA*	nm	↓Total-C†, ↓LDL-C†, ↔HDL-C

[1]Mean ± SD, otherwise specified. ns, not specified. nm, not measured. C, cholesterol. P, phosphate. BA, bile acid. LDL, low-density lipoprotein. HDL, high-density lipoprotein †significantly different from calcium diet control (Ca (-)) ($P < 0.05$). * Not statistically significantly different from the calcium diet control.

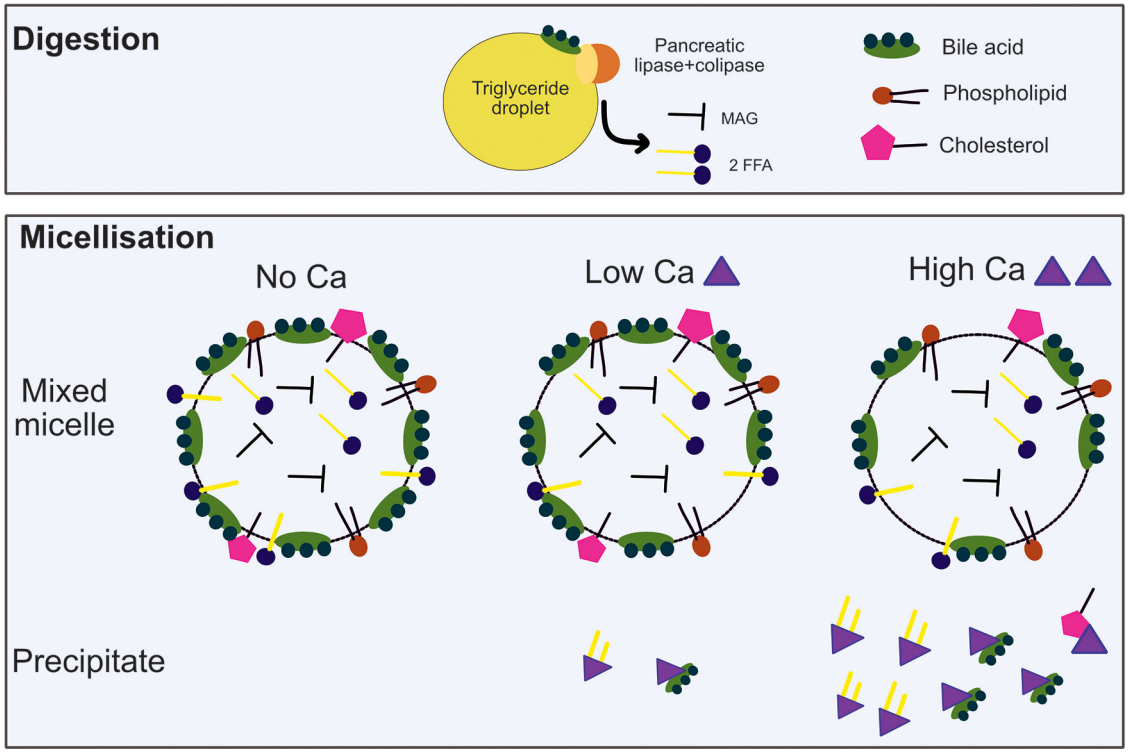

Figure 1. Schematical representation of the effect of Ca in the micellization process.

concentration of bile anions, as well as the low activity of Ca ions". Despite that the formation of insoluble salts between soluble Ca and BAs seems to be unlikely, Ca has been observed to present high affinity binding with bile salts forming soluble complexes that induced the enhancement of intestinal Ca ions uptake (Sanyal, Hirsch, and Moore 1992). These complexes should be important to consider because they might change the physical properties of the micelles.

Some studies have proved that the effect of Ca on excretion of BAs is due to the adsorption of BAs to Ca phosphate. In vitro binding experiments showed that formation of insoluble Ca phosphate is a prerequisite for binding glycine-conjugate and unconjugated BAs (Van der Meer and De Vries 1985). Moreover, Van der Meer et al. (1990) observed in humans the increased concentration of Ca and phosphate as well as BAs in feces, showing that the precipitated phosphate is associated with Ca. Therefore the mechanism proposed implies the molecular interactions between Ca, phosphate and BAs. Ca ions can be precipitated by phosphate forming amorphous Ca phosphate (ACP) (Govers et al. 1996; Van der Meer and De Vries 1985; Van der Meer et al. 1990; Welberg et al. 1993) at pH higher than 5.6 (Van der Meer and De Vries 1985) with a calcium:phosphate molar ratio of 3:2 (Govers et al. 1994; Termine, Peckauskas, and Posner 1970). This insoluble ACP can bind and precipitate BAs (Govers et al. 1994; Van der Meer, Termont, and De Vries 1991; Van der Meer and De Vries 1985). This occurs at pH higher than 5.6, with optimum at pH 6.1 as observed in the in vitro study of Van der Meer and De Vries (1985). It has been proposed that an ionic adsorption occurs between the negative charged carboxylic headgroups of BA and the positive charged Ca (Govers et al. 1994). The differential interaction with Ca is determined by the physicochemical properties of the BAs; the type of conjugation and hydrophobicity of BA are important determinants of adsorption to ACP. For instance, glycine-conjugated BA (predominant in the human duodenal bile) and unconjugated BA were preferentially bound to ACP, whereas taurine-conjugated BA showed little binding (Govers et al. 1994; Van der Meer and De Vries 1985). This could be explained by the low affinity for Ca of the sulfonate anion in taurine-conjugated BAs compare to the carboxyl anion contained in glycine-conjugated BAs. Moreover, these in vitro binding studies showed, in human duodenal bile, that conjugated dihydroxy BAs were precipitated to a greater extent by ACP than the trihydroxy BAs (Govers et al. 1994). In the latter study the critical binding concentration of the BAs was much lower than their critical micellar concentration, suggesting that the monomers of BAs bind to the ACP. However, in the same study, it was also shown that some kind of hydrophobic aggregation facilitates the absorption to the ACP surface. This is in agreement with the studies by Qiu et al. (1991) and Van der Meer and De Vries (1985), in which the presence of BAs in micellar state was considered a prerequisite for adsorption to ACP. Therefore, it is possible that a combination of BA states is involved in the binding, a first adsorption of BA monomers and subsequent formation of hydrophobic aggregates on the ACP surface. These in vitro studies also supported the idea that Ca ions do not have the ability to precipitate BAs, in contrast to that of ACP (Govers et al. 1994; Van der Meer, Termont, and De Vries 1991). The increasing concentration of soluble Ca (up to 15 mmol/L $CaCl_2$) did not precipitate some BAs (deoxycholic acid, glycochenodeoxycholic acid and taurochenodeoxycholic acid) whereas equimolar concentration of Ca and phosphate from 5 mmol/L decreased the solubility of

BAs (Van der Meer, Termont, and De Vries 1991). Interestingly, Govers et al. (1994) studied the adsorption properties of glycochenodeoxycholate to other Ca salts. There was a slight adsorption onto $Ca_3(PO_4)_2$, i.e. the crystalline form with a similar ratio as ACP, whereas the binding was negligible in the rest of insoluble salts tested, $CaCO_3$, $CaHPO_4{\cdot}2H_2O$, $Ca(H_2PO_4)_2{\cdot}H_2O$ and hydroxyapatite. This clearly shows the unique binding properties of the ACP.

Effect of calcium-bile acid interactions on serum lipids

The study by Ditscheid, Keller, and Jahreis (2005) showed a decrease in total cholesterol with high Ca intake. Since there was no effect on fecal fat excretion, the lowering-effect of cholesterol was solely attributed to the increased fecal bile excretion. The formation of ACP might be responsible for the lack of an effect of Ca supplementation on fecal fat excretion. If Ca is used up by the formation of ACP, it would not be available for the formation of Ca-FA soaps. However, Denke, Fox, and Schulte (1993) did not present differences in fecal BA excretion but fecal fat excretion, showing lower total- and LDL-cholesterol in a high Ca diet. The kinetics and extent of Ca usage for the formation of Ca soaps and ACP is not defined therefore it is difficult to distinguish the contribution of each process to any cholesterol-lowering effect. Significant differences in fecal excretion in both BAs and fat were observed in the study by Lorenzen and Astrup (2011), leading to a lower total- and LDL- cholesterol. This shows the possible contribution of both processes to the observed cholesterol-lowering effect.

The precipitation of BAs due to the binding with ACP in the small intestine can result in the expected increase in fecal BAs. This leads to less BA available in solution, decreasing their solubilizing activity and therefore the formation of mixed micelles. This would imply a reduced availability of BAs for the process of fat digestion thereby impairing fat digestion. Moreover, this effect would also decrease the re-absorption of BAs, thus, their recycling through the enterohepatic circulation, which may result in increased cholesterol uptake from the circulation into the liver for the de novo synthesis of BAs. This would lead to a decrease in serum total- and LDL- cholesterol concentrations (Färkkilä and Miettinen 1990). For example, cholestyramine, i.e. a BA sequestrant, resulted in a five to six- fold increase in BA secretion, showing a 17% reduction in serum cholesterol concentrations (Moutafis et al. 1977). It is possible, then, that high Ca intake causing around two-fold increase in BA secretion could result in a 5% reduction in serum cholesterol concentrations.

Conclusions

Dietary Ca intake has been generally observed to be linked to reduced serum total- and LDL- cholesterol concentrations. However, the results from interventional studies of the effect of Ca, both dairy and supplementary, on lipid profile are not consistent. The observations of the possible cholesterol-lowering effect in terms of fecal fat and BAs are variable and do not seem to fulfill all the related-aspects. For instance, in most of the studies, the serum TAG concentrations did not show the corresponding reduction following high Ca intake, despite an increased fecal fat excretion. It would appear therefore that the effect of Ca is probably through multiple and possible interconnected mechanisms, affecting different metabolic and homeostatic processes, hence the impact is more complex than a simple flux between various physiological pools of cholesterol.

The interplay of Ca in blood lipid metabolism might be due to its complex and multiple roles in lipid digestion in the small intestine. Ca can act as inhibitor of lipid digestion by inducing flocculation and increasing the surface area of lipase action. On the other hand, Ca can enhance lipid digestion through the removal of FAs from the interface, leading to the formation of Ca-FA soaps. Moreover, Ca has been shown to interact with BAs and cholesterol in the small intestinal conditions. Apart from these two main mechanisms, some authors suggested a possible interaction between Ca and cholesterol itself (Ditscheid, Keller, and Jahreis 2009), however this has not been proven in vivo. At present, most of the studies investigating lipid digestion of food matrices during the intestinal phase have used the pH-titration method as methodology to obtain the rate and extent of FFA released. However, the conditions used are not standardized, which makes difficult to compare results between laboratories. Therefore, a standard protocol for a pH-stat methodology, considering the limitations of the method, would be helpful.

The interactions between Ca and, FA and bile and cholesterol may lead to impaired mixed micelle formation and solubilization, which is crucial for lipid absorption and metabolism. Figure 1. shows a representation of the effect of Ca in the micellization process. In addition, the form of calcium is important, not simply the concentration. Therefore the calcium source, its surrounding matrix and chemical form will have an influence over the physiological outcome. In further studies of this relationship, therefore, special attention should be paid to the investigation of the interplay of how the particular form of Ca affects the solubilization and physicochemical properties of mixed micelles. These factors should also be tightly controlled and fully characterized when designing human or animal studies. Only then will we be able to fully understand the role of calcium in our diets on lipid digestion, metabolism and health outcomes.

Acknowledgements

The authors gratefully acknowledge the support of the Biotechnology and Biological Sciences Research Council (BBSRC, UK); this research was funded by the BBSRC Institute Strategic Programme Food Innovation and Health BB/R012512/1 and its constituent project(s) BBS/E/F/000PR10343 (Theme 1, Food Innovation) and BBS/E/F/000PR10345 (Theme 2, Digestion in the Upper GI Tract).

Disclosure statement

No potential conflict of interest was reported by the authors.

Abbreviations

CVD	cardiovascular diseases
Ca	calcium
LDL	low-density lipoprotein
FA	fatty acid
TAG	triacylglycerol
DAG	diacylglycerol
FFA	free fatty acid
MAG	monoacylglycerol
HDL	high-density lipoprotein
BA	bile acid
EDTA	ethylenediaminetetraacetic acid

References

Armand, M. 2007. Lipases and lipolysis in the human digestive tract: Where do we stand? *Current Opinion in Clinical Nutrition and Metabolic Care* 10 (2):156–64. doi: 10.1097/MCO.0b013e3280177687.

Bauer, E., S. Jakob, and R. Mosenthin. 2005. Principles of physiology of lipid digestion. *Asian-Australasian Journal of Animal Sciences* 18 (2): 282–95. doi: 10.5713/ajas.2005.282.

Bell, L., C. E. Halstenson, C. J. Halstenson, M. Macres, and W. F. Keane. 1992. Cholesterol-lowering effects ohypercholesterolemiain patients with mild to moderate hypercholesterolemia. *Archives of Internal Medicine* 152 (12):2441–4.

Bendsen, N. T., A. Hother, S. Jensen, J. K. Lorenzen, and A. Astrup. 2008. Effect of dairy calcium on fecal fat excretion: A randomized crossover trial. *International Journal of Obesity* 32 (12):1816–24. doi: 10.1038/ijo.2008.173.

Boon, N., G. Hul, J. Stegen, W. Sluijsmans, C. Valle, D. Langin, N. Viguerie, and W. Saris. 2007. An intervention study of the effects of calcium intake on faecal fat excretion, energy metabolism and adipose tissue mRNA expression of lipid-metabolism related proteins. *International Journal of Obesity* 31 (11):1704–12. doi: 10.1038/sj.ijo.0803660.

Brodkorb, A., L. Egger, M. Alminger, P. Alvito, R. Assuncao, S. Ballance, T. Bohn, C. Bourlieu-Lacanal, R. Boutrou, F. Carriere, et al. 2019. INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols* 14 (4):991–1014. doi: 10.1038/s41596-018-0119-1.

Buset, M., P. Galand, M. Lipkin, S. Winawer, and E. Friedman. 1990. Injury induced by fatty acids or bile acid in isolated human colonocytes prevented by calcium. *Cancer Letters* 50 (3):221–6. doi: 10.1016/0304-3835(90)90269-4.

Chai, W., R. V. Cooney, A. A. Franke, and R. M. Bostick. 2013. Effects of calcium and vitamin D supplementation on blood pressure and serum lipids and carotenoids: A randomized, double-blind, placebo-controlled, clinical trial. *Annals of Epidemiology* 23 (9):564–70. doi: 10.1016/j.annepidem.2013.07.003.

Christensen, R., J. K. Lorenzen, C. R. Svith, E. Bartels, E. Melanson, W. Saris, A. Tremblay, and A. Astrup. 2009. Effect of calcium from dairy and dietary supplements on faecal fat excretion: A meta-analysis of randomized controlled trials. *Obesity Reviews* 10 (4):475–86. doi: 10.1111/j.1467-789X.2009.00599.x.

Del Valle, H. B., A. L. Yaktine, C. L. Taylor, and A. C. Ross. 2011. *Dietary reference intakes for calcium and vitamin D*. Washington DC: National Academies Press.

Denke, M. A., M. M. Fox, and M. C. Schulte. 1993. Short-term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men. *The Journal of Nutrition* 123:1047–53.

Ditscheid, B., S. Keller, and G. Jahreis. 2005. Cholesterol metabolism is affected by calcium phosphate supplementation in humans. *The Journal of Nutrition* 135 (7):1678–82. doi: 10.1093/jn/135.7.1678.

Ditscheid, B., S. Keller, and G. Jahreis. 2009. Faecal steroid excretion in humans is affected by calcium supplementation and shows gender-specific differences. *European Journal of Nutrition* 48 (1):22–30. doi: 10.1007/s00394-008-0755-2.

Färkkilä, M., and T. A. Miettinen. 1990. Lipid metabolism in bile acid malabsorption. *Annals of Medicine* 22 (1):5–13. doi: 10.3109/07853899009147233.

Fave, G., T. Coste, and M. Armand. 2004. Physicochemical properties of lipids: New strategies to manage fatty acid bioavailability. *Cellular and Molecular Biology* 50:815–32.

Fordtran, J. S., and J. H. Walsh. 1973. Gastric acid secretion rate and buffer content of the stomach after eating. Results in normal subjects and in patients with duodenal ulcer. *The Journal of Clinical Investigation* 52 (3):645–57. doi: 10.1172/JCI107226.

Gacs, G., and D. Barltrop. 1977. Significance of Ca-soap formation for calcium absorption in the rat. *Gut* 18 (1):64–8. doi: 10.1136/gut.18.1.64.

Gaucheron, F. 2005. The minerals of milk. *Reproduction Nutrition Development* 45 (4):473–83. doi: 10.1051/rnd:2005030.

Givens, D. I. 2017. Saturated fats, dairy foods and health: A curious paradox? *Nutrition Bulletin* 42 (3):274–82. doi: 10.1111/nbu.12283.

Givens, M. H. 1917. Studies in calcium and magnesium metabolism: III. The effect of fat and fatty acid derivatives. *Journal of Biological Chemistry* 31 (2):441–4. doi: 10.1016/S0021-9258(18)86702-3.

Govers, M. J., D. S. Termont, J. A. Lapré, J. H. Kleibeuker, R. J. Vonk, and R. Van der Meer. 1996. Calcium in milk products precipitates intestinal fatty acids and secondary bile acids and thus inhibits colonic cytotoxicity in humans. *Cancer Research* 56:3270–5.

Govers, M. J., D. S. Termont, G. A. Van Aken, and R. Van der Meer. 1994. Characterization of the adsorption of conjugated and unconjugated bile acids to insoluble, amorphous calcium phosphate. *Journal of Lipid Research* 35 (5):741–8.

Graham, D. Y., and J. W. Sackman. 1983. Solubility of calcium soaps of long-chain fatty acids in simulated intestinal environment. *Digestive Diseases and Sciences* 28 (8):733–6. doi: 10.1007/BF01312564.

Griffin, B. 2017. Serum low-density lipoprotein as a dietary responsive biomarker of cardiovascular disease risk: Consensus and confusion. *Nutrition Bulletin* 42 (3):266–73. doi: 10.1111/nbu.12282.

Grundy, S. M., and M. A. Denke. 1990. Dietary influences on serum lipids and lipoproteins. *Journal of Lipid Research* 31 (7):1149–72.

Grundy, M. M. L., C. H. Edwards, A. R. Mackie, M. J. Gidley, P. J. Butterworth, and P. R. Ellis. 2016. Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. *British Journal of Nutrition* 116 (5):816–33. doi: 10.1017/S0007114516002610.

Gu, J. J., A. F. Hofmann, H. T. Ton-Nu, C. D. Schteingart, and K. J. Mysels. 1992. Solubility of calcium salts of unconjugated and conjugated natural bile acids. *Journal of Lipid Research* 33 (5):635–46.

Hofmann, A. F., and K. J. Mysels. 1992. Bile acid solubility and precipitation in vitro and in vivo: The role of conjugation, pH, and Ca2+ ions. *Journal of Lipid Research* 33 (5):617–26.

Hu, M., Y. Li, E. A. Decker, and D. J. McClements. 2010. Role of calcium and calcium-binding agents on the lipase digestibility of emulsified lipids using an in vitro digestion model. *Food Hydrocolloids* 24 (8):719–25. doi: 10.1016/j.foodhyd.2010.03.010.

Jacobsen, R., J. K. Lorenzen, S. Toubro, I. Krog-Mikkelsen, and A. Astrup. 2005. Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. *International Journal of Obesity* 29 (3):292–301. doi: 10.1038/sj.ijo.0802785.

Jacqmain, M., E. Doucet, J.-P. Després, C. Bouchard, and A. Tremblay. 2003. Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. *The American Journal of Clinical Nutrition* 77 (6):1448–52. doi: 10.1093/ajcn/77.6.1448.

Karanja, N., C. D. Morris, P. Rufolo, G. Snyder, D. R. Illingworth, and D. A. McCarron. 1994. Impact of increasing calcium in the diet on nutrient consumption, plasma lipids, and lipoproteins in humans. *The American Journal of Clinical Nutrition* 59 (4):900–7. doi: 10.1093/ajcn/59.4.900.

Kennefick, S., and K. Cashman. 2000. Inhibitory effect of wheat fibre extract on calcium absorption in Caco-2 cells: Evidence for a role of associated phytate rather than fibre per se. *European Journal of Nutrition* 39 (1):12–7. doi: 10.1007/s003940050071.

Kies, C. 1985. Effect of dietary fat and fiber on calcium bioavailability. In *Nutritional bioavailability of calcium*, ed. C. Kies, Vol. 275, 175–87. Washington DC: American Chemical Society.

Kimura, H., Y. Futami, S.-i. Tarui, and T. Shinomiya. 1982. Activation of human pancreatic lipase activity by calcium and bile salts. *The Journal of Biochemistry* 92 (1):243–51. doi: 10.1093/oxfordjournals.jbchem.a133920.

Kjølbaek, L., J. K. Lorenzen, L. H. Larsen, and A. Astrup. 2017. Calcium intake and the associations with faecal fat and energy excretion, and lipid profile in a free-living population. *Journal of Nutritional Science* 6:e50. doi: 10.1017/jns.2017.55.

Kopic, S., and J. P. Geibel. 2013. Gastric acid, calcium absorption, and their impact on bone health. *Physiological Reviews* 93 (1):189–268. doi: 10.1152/physrev.00015.2012.

Lapre, J. A., H. T. De Vries, and R. Van der Meer. 1991. Dietary calcium phosphate inhibits cytotoxicity of fecal water. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 261 (6):G907–G912. doi: 10.1152/ajpgi.1991.261.6.G907.

Larsen, M., A. Jensen, D. Madsen, and E. Pearce. 1999. Individual variations of pH, buffer capacity, and concentrations of calcium and phosphate in unstimulated whole saliva. *Archives of Oral Biology* 44 (2):111–7. doi: 10.1016/s0003-9969(98)00108-3.

Larsen, T., S. H. Thilsted, S. K. Biswas, and I. Tetens. 2003. The leafy vegetable amaranth (Amaranthus gangeticus) is a potent inhibitor of calcium availability and retention in rice-based diets. *British Journal of Nutrition* 90 (3):521–7. doi: 10.1079/BJN2003923.

Li, Y., M. Hu, and D. J. McClements. 2011. Factors affecting lipase digestibility of emulsified lipids using an in vitro digestion model: Proposal for a standardised pH-stat method. *Food Chemistry* 126 (2):498–505. doi: 10.1016/j.foodchem.2010.11.027.

Lindahl, A., A.-L. Ungell, L. Knutson, and H. Lennernäs. 1997. Characterization of fluids from the stomach and proximal jejunum in men and women. *Pharmaceutical Research* 14 (4):497–502. doi: 10.1023/a:1012107801889.

Lin, Q., R. Liang, A. Ye, H. Singh, and F. Zhong. 2017. Effects of calcium on lipid digestion in nanoemulsions stabilized by modified starch: Implications for bioaccessibility of β-carotene. *Food Hydrocolloids* 73:184–93. doi: 10.1016/j.foodhyd.2017.06.024.

Li, Y., D. Tomé, and J. Desjeux. 1989. Indirect effect of casein phosphopeptides on calcium absorption in rat ileum in vitro. *Reproduction Nutrition Développement* 29 (2):227–33. doi: 10.1051/rnd:19890210.

Lorenzen, J. K., and A. Astrup. 2011. Dairy calcium intake modifies responsiveness of fat metabolism and blood lipids to a high-fat diet. *British Journal of Nutrition* 105 (12):1823–31. doi: 10.1017/S0007114510005581.

Lorenzen, J. K., S. Nielsen, J. J. Holst, I. Tetens, J. F. Rehfeld, and A. Astrup. 2007. Effect of dairy calcium or supplementary calcium intake on postprandial fat metabolism, appetite, and subsequent energy intake. *The American Journal of Clinical Nutrition* 85 (3):678–87. doi: 10.1093/ajcn/85.3.678.

Lorieau, L., L. Le Roux, F. Gaucheron, A. Ligneul, E. Hazart, D. Dupont, and J. Floury. 2018. Bioaccessibility of four calcium sources in different whey-based dairy matrices assessed by in vitro digestion. *Food Chemistry* 245:454–62. doi: 10.1016/j.foodchem.2017.10.108.

Lupton, J. R., G. Steinbach, W. C. Chang, B. C. O'Brien, S. Wiese, C. L. Stoltzfus, G. A. Glober, M. J. Wargovich, R. S. McPherson, and R. J. Winn. 1996. Calcium supplementation modifies the relative amounts of bile acids in bile and affects key aspects of human colon physiology. *The Journal of Nutrition* 126 (5):1421–8. doi: 10.1093/jn/126.5.1421.

Moreau, H., Y. Gargouri, D. Lecat, J.-L. Junien, and R. Verger. 1988. Purification, characterization and kinetic properties of the rabbit gastric lipase. *Biochimica et Biophysica Acta* 960 (3):286–93. doi: 10.1016/0005-2760(88)90036-7.

Moutafis, C. D., L. A. Simons, N. B. Myant, P. W. Adams, and V. Wynn. 1977. The effect of cholestyramine on the faecal excretion of bile acids and neutral steroids in familial hypercholesterolaemia. *Atherosclerosis* 26 (3):329–34. doi: 10.1016/0021-9150(77)90085-5.

Nicar, M. J., and C. Y. Pak. 1985. Calcium bioavailability from calcium carbonate andcalcium citrate. *The Journal of Clinical Endocrinology & Metabolism* 61 (2):391–3. doi: 10.1210/jcem-61-2-391.

Ockner, R. K., J. P. Pittman, and J. L. Yager. 1972. Differences in the intestinal absorption of saturated and unsaturated long chain fatty acids. *Gastroenterology* 62 (5):981–92.

Owen, R. W., U. M. Weisgerber, J. Carr, and M. H. Harrison. 1995. Analysis of calcium-lipid complexes in faeces. *European Journal of Cancer Prevention* 4 (3):247–56. doi: 10.1097/00008469-199506000-00006.

Patton, M. B., and T. Sutton. 1952. The utilization of calcium from lactate, gluconate, sulfate and carbonate salts by Young College Women: One figure. *The Journal of Nutrition* 48 (4):443–52. doi: 10.1093/jn/48.4.443.

Patton, J. S., R. D. Vetter, M. Hamosh, B. Borgstrom, M. Lindstrom, and M. C. Carey. 1985. The light microscopy of triglyceride digestion. *Food Structure* 4:5.

Pekkanen, J., S. Linn, G. Heiss, C. M. Suchindran, A. Leon, B. M. Rifkind, and H. A. Tyroler. 1990. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. *New England Journal of Medicine* 322 (24):1700–7. doi: 10.1056/NEJM199006143222403.

Qiu, S. M., G. Wen, N. Hirakawa, R. D. Soloway, N. K. Hong, and R. S. Crowther. 1991. Glycochenodeoxycholic acid inhibits calcium phosphate precipitation in vitro by preventing the transformation of amorphous calcium phosphate to calcium hydroxyapatite. *The Journal of Clinical Investigation* 88 (4):1265–71. doi: 10.1172/JCI115430.

Rajpathak, S. N., X. Xue, S. Wassertheil-Smoller, L. Van Horn, J. G. Robinson, S. Liu, M. Allison, L. W. Martin, G. Y. Ho, and T. E. Rohan. 2010. Effect of 5 y of calcium plus vitamin D supplementation on change in circulating lipids: Results from the Women's Health Initiative. *The American Journal of Clinical Nutrition* 91 (4):894–9. doi: 10.3945/ajcn.2009.28579.

Reid, I. R., R. Ames, B. Mason, M. J. Bolland, C. J. Bacon, H. E. Reid, C. Kyle, G. D. Gamble, A. Grey, and A. Horne. 2010. Effects of calcium supplementation on lipids, blood pressure, and body composition in healthy older men: A randomized controlled trial. *The American Journal of Clinical Nutrition* 91 (1):131–9. doi: 10.3945/ajcn.2009.28097.

Reid, I. R., B. Mason, A. Horne, R. Ames, J. Clearwater, U. Bava, B. Orr-Walker, F. Wu, M. C. Evans, and G. D. Gamble. 2002. Effects of calcium supplementation on serum lipid concentrations in normal older women: A randomized controlled trial. *The American Journal of Medicine* 112 (5):343–7. doi: 10.1016/s0002-9343(01)01138-x.

Rogalska, E., S. Ransac, and R. Verger. 1990. Stereoselectivity of lipases. II. Stereoselective hydrolysis of triglycerides by gastric and pancreatic lipases. *Journal of Biological Chemistry* 265 (33):20271–6. doi: 10.1016/S0021-9258(17)30500-8.

Sahuquillo, A., R. Barberá, and R. Farré. 2003. Bioaccessibility of calcium, iron and zinc from three legume samples. *Die Nahrung* 47 (6):438–41. doi: 10.1002/food.200390097.

Sanyal, A. J., J. I. Hirsch, and E. W. Moore. 1992. High-affinity binding is essential for enhancement of intestinal Fe2+ and Ca2+ uptake by bile salts. *Gastroenterology* 102 (6):1997–2005. doi: 10.1016/0016-5085(92)90325-S.

Sassene, P., K. Kleberg, H. D. Williams, J.-C. Bakala-N'Goma, F. Carrière, M. Calderone, V. Jannin, A. Igonin, A. Partheil, D. Marchaud, et al. 2014. Toward the establishment of standardized in vitro tests for lipid-based formulations, part 6: Effects of varying pancreatin and calcium levels. *The AAPS Journal* 16 (6):1344–57. doi: 10.1208/s12248-014-9672-x.

Scholz-Ahrens, K. E., and J. Schrezenmeir. 2000. Effects of bioactive substances in milk on mineral and trace element metabolism with

special reference to casein phosphopeptides. *British Journal of Nutrition* 84 (S1):147–53. doi: 10.1017/S0007114500002373.

Scow, R. O. 1988. Effect of sodium taurodeoxycholate, CaCl2 and albumin on the action of pancreatic lipase on droplets of trioleoylglycerol and the release of lipolytic products into aqueous media. *Biochimie* 70 (9):1251–61. doi: 10.1016/0300-9084(88)90192-7.

Shahkhalili, Y., C. Murset, I. Meirim, E. Duruz, S. Guinchard, C. Cavadini, and K. Acheson. 2001. Calcium supplementation of chocolate: Effect on cocoa butter digestibility and blood lipids in humans. *The American Journal of Clinical Nutrition* 73 (2):246–52. doi: 10.1093/ajcn/73.2.246.

Sheikh, M. S., C. A. Santa Ana, M. J. Nicar, L. R. Schiller, and J. S. Fordtran. 1987. Gastrointestinal absorption of calcium from milk and calcium salts. *New England Journal of Medicine* 317 (9):532–6. doi: 10.1056/NEJM198708273170903.

Skrypnik, K., and J. Suliburska. 2018. Association between the gut microbiota and mineral metabolism. *Journal of the Science of Food and Agriculture* 98 (7):2449–60. doi: 10.1002/jsfa.8724.

Soerensen, K. V., T. K. Thorning, A. Astrup, M. Kristensen, and J. K. Lorenzen. 2014. Effect of dairy calcium from cheese and milk on fecal fat excretion, blood lipids, and appetite in young men. *The American Journal of Clinical Nutrition* 99 (5):984–91. doi: 10.3945/ajcn.113.077735.

Tan, Y., R. Li, H. Zhou, J. Liu, J. M. Mundo, R. Zhang, and D. J. McClements. 2020. Impact of calcium levels on lipid digestion and nutraceutical bioaccessibility in nanoemulsion delivery systems studied using standardized INFOGEST digestion protocol. *Food & Function* 11 (1):174–86. doi: 10.1039/c9fo01669d.

Termine, J. D., R. A. Peckauskas, and A. S. Posner. 1970. Calcium phosphate formation in vitro. II. Effects of environment on amorphous-crystalline transformation. *Archives of Biochemistry and Biophysics* 140 (2):318–25. doi: 10.1016/0003-9861(70)90072-x.

Theobald, H. E. 2005. Dietary calcium and health. *Nutrition Bulletin* 30 (3):237–77. doi: 10.1111/j.1467-3010.2005.00514.x.

Tholstrup, T., C.-E. Høy, L. N. Andersen, R. D. K. Christensen, and B. Sandström. 2004. Does fat in milk, butter and cheese affect blood lipids and cholesterol differently? *Journal of the American College of Nutrition* 23 (2):169–76. doi: 10.1080/07315724.2004.10719358.

Titchenal, C. A., and J. Dobbs. 2007. A system to assess the quality of food sources of calcium. *Journal of Food Composition and Analysis* 20 (8):717–24. doi: 10.1016/j.jfca.2006.04.013.

Torcello-Gómez, A., C. Boudard, and A. R. Mackie. 2018. Calcium alters the interfacial organization of hydrolyzed lipids during intestinal digestion. *Langmuir* 34 (25):7536–44. doi: 10.1021/acs.langmuir.8b00841.

Torre, M., A. Rodriguez, and F. Saura-Calixto. 1991. Effects of dietary fiber and phytic acid on mineral availability. *Critical Reviews in Food Science and Nutrition* 30 (1):1–22. doi: 10.1080/10408399109527539.

US Department of Agriculture, Agricultural Research Service. 2001. USDA Nutrient Database for Standard Reference, Release 14: Nutrient Data Laboratory Home Page: US Department of Agriculture, Agricultural Research Service.

Van der Meer, R., and H. T. De Vries. 1985. Differential binding of glycine- and taurine-conjugated bile acids to insoluble calcium phosphate. *Biochemical Journal* 229 (1):265–8. doi: 10.1042/bj2290265.

Van der Meer, R., J. Lapre, M. Govers, and J. Kleibeuker. 1997. Mechanisms of the intestinal effects of dietary fats and milk products on colon carcinogenesis. *Cancer Letters* 114 (1–2):75–83. doi: 10.1016/s0304-3835(97)04629-6.

Van der Meer, R., D. S. Termont, and H. T. De Vries. 1991. Differential effects of calcium ions and calcium phosphate on cytotoxicity of bile acids. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 260 (1):G142–G147. doi: 10.1152/ajpgi.1991.260.1.G142.

Van der Meer, R., J. W. M. Welberg, F. Kuipers, J. H. Kleibeuker, N. H. Mulder, D. S. M. L. Termont, R. J. Vonk, H. T. De Vries, and E. G. E. De Vries. 1990. Effects of supplemental dietary calcium on the intestinal association of calcium, phosphate, and bile acids. *Gastroenterology* 99 (6):1653–9. doi: 10.1016/0016-5085(90)90471-c.

Vinarova, L., Z. Vinarov, S. Tcholakova, N. D. Denkov, S. Stoyanov, and A. Lips. 2016. The mechanism of lowering cholesterol absorption by calcium studied by using an in vitro digestion model. *Food & Function* 7:151–63.

Welberg, J. W. M., J. H. Kleibeuker, R. Van der Meer, F. Kuipers, A. Cats, H. V. Rijsbergen, D. S. M. L. Termont, W. B.-V. Ek, R. J. Vonk, N. H. Mulder, et al. 1993. Effects of oral calcium supplementation on intestinal bile acids and cytolytic activity of fecal water in patients with adenomatous polyps of the colon. *European Journal of Clinical Investigation* 23 (1):63–8. doi: 10.1111/j.1365-2362.1993.tb00719.x.

WHO. 2015. Cardiovascular diseases (CVDs): Fact sheet No. 317. 2015. Tillgänglig via. http://www.who.int/mediacentre/factsheets/fs317/en/. *Hämtad, 25*.

Ye, A., J. Cui, X. Zhu, and H. Singh. 2013. Effect of calcium on the kinetics of free fatty acid release during in vitro lipid digestion in model emulsions. *Food Chemistry* 139 (1–4):681–8. doi: 10.1016/j.foodchem.2013.02.014.

Ye, A., and H. Singh. 2000. Influence of calcium chloride addition on the properties of emulsions stabilized by whey protein concentrate. *Food Hydrocolloids* 14 (4):337–46. doi: 10.1016/S0268-005X(00)00010-2.

Ye, A., and H. Singh. 2001. Interfacial composition and stability of sodium caseinate emulsions as influenced by calcium ions. *Food Hydrocolloids* 15 (2):195–207. doi: 10.1016/S0268-005X(00)00065-5.

Zangenberg, N. H., A. Müllertz, H. G. Kristensen, and L. Hovgaard. 2001. A dynamic in vitro lipolysis model. I. Controlling the rate of lipolysis by continuous addition of calcium. *European Journal of Pharmaceutical Sciences* 14 (2):115–22. doi: 10.1016/s0928-0987(01)00169-5.

Zimmerman, J. 1993. Does dietary calcium supplementation reduce the risk of colon cancer? *Nutrition Reviews* 51 (4):109–12. doi: 10.1111/j.1753-4887.1993.tb03083.x.