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Review

Systematic review of the mechanisms and evidence behind the hypocholesterolaemic effects of HPMC, pectin and chitosan in animal trials

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ABSTRACT

Dietary fibres have diverse mechanisms in reducing plasma cholesterol, which could be useful for treating high levels of low-density lipoprotein cholesterol (LDL-C). The objective of this review is to determine the state of the evidence for the cholesterol-lowering effects of three selected fibres and their mechanisms, using the most recent animal trials. Therefore, a systematic review was conducted for hydroxypropyl methylcellulose (HPMC), pectin and chitosan in Pubmed, Embase and the Cochrane Library. All fibres reviewed reduced total cholesterol, very low-density lipoprotein cholesterol (VLDL-C) and LDL-C. Pectin gave a small, and chitosan an impressive rise in high-density lipoprotein cholesterol (HDL-C). A limitation of this study is the variety of animal models, each with distinct cholesterol profiles. Possible publication bias was also detected. In conclusion, chitosan seems to be the most promising of the studied fibres. A dietary fibre could be designed that yields the best cholesterol-lowering effect, using experiences in tailoring physicochemical properties and primarily exploiting the biophysical mechanisms of action.

Contents

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1. Introduction

1.1. Relevance

In middle- and high-income countries, cardiovascular diseases are the leading causes of death and disability (Aller et al., 2004; Erkkila & Lichtenstein, 2006; Parolini et al., 2013; Theuwissen & Mensink, 2008). Relevant risk factors for these diseases are smoking and obesity (Choi et al., 2012; Erkkila & Lichtenstein, 2006), as well as a high serum concentration of low-density lipoprotein cholesterol (LDL-C) (Aller et al., 2004; Carr, Wood, Hassel, Bahl, & Gallaher, 2003; Erkkila & Lichtenstein, 2006; Maki et al., 2009; Marounek, Volek, Duskova, Tuma, & Taubner, 2013; Yokoyama et al., 2011). The diet-heart hypothesis links an intake of high quantities of saturated fat to a high LDL-C level, which increases the risk of cardiovascular diseases (Cos et al., 2001; Erkkila & Lichtenstein, 2006). In fact, a 1% reduction in total cholesterol is associated with an approximately 2.5% reduction in coronary heart disease incidence (Choi et al., 2012). Decreasing LDL-C levels would benefit the populations in many countries.

The hypocholesterolaemic effects of specific fibres have been known for over 40 years (Aller et al., 2004; Bartley et al., 2010; Cox et al., 2013; Hong, Turowski, Lin, & Yokoyama, 2007; Theuwissen & Mensink, 2008). The literature suggests that a 10 g per day increase in intake of dietary fibre can lower the risk of coronary events by 12% and deaths by 19% (Pereira et al., 2004). Most people, however, do not consume the recommended amount of fibre on a daily basis (Anderson et al., 2009; Aprikian et al., 2003; Bazzano, 2008; Gallaher & Gallaher, 2009; Grizard, Dalle, & Barthomeuf, 2001; Theuwissen & Mensink, 2008) which is considered to be 25–38 grammes per day (Department of Agriculture., National Agricultural Library., National Academy of Sciences., Institute of Medicine., & Food and Nutrition Board., 2005; EFSA Panel on Dietetic Products, 2010a).

1.2. Cholesterol

Cholesterol, in the human body, originates from two sources: it is synthesized in the liver (accounting for 700–900 mg per day), and taken in by diet (300–500 mg per day) (Gunness & Gidley, 2010).

Small amounts of cholesterol are used for the synthesis of steroid hormones (Gunness & Gidley, 2010) and cell membranes (Gunness & Gidley, 2010; Park, Choi, & Kim, 2000). Most of the cholesterol is used by the liver to synthesize bile acids, such as cholic acid (Choi et al., 2012; Gunness & Gidley, 2010), which are needed to increase absorption of hydrophobic nutrients (Gunness & Gidley, 2010). Bile acids are stored in the gall bladder and released into the duodenum and proximal jejunum after being stimulated by cholecystokinin (Gunness & Gidley, 2010).

Approximately 95% of excreted bile acids are reabsorbed and recycled by reuptake (Choi et al., 2012; Gunness & Gidley, 2010). The lost fraction is compensated by synthesis and diet uptake (Gunness & Gidley, 2010).

Cholesterol is transported by lipoproteins, which are small spheres containing phospholipids, apolipoproteins and cholesterol. These lipoproteins can be divided into subgroups: LDL-C, high-density lipoprotein cholesterol (HDL-C) and very low-density lipoprotein cholesterol (VLDL-C). LDL-C is associated with cardiovascular diseases and, as such, is considered 'bad' cholesterol that contributes to plaque formation (Aller et al., 2004; Bokura & Kobayashi, 2003; Carr et al., 2003; Erkkila & Lichtenstein, 2006; Maki, Carson, Miller et al., 2009; Marounek et al., 2013; Nordestgaard & Varbo, 2014; Yokoyama et al., 2011).

Conversely, HDL-C is considered 'good' cholesterol that protects against cardiovascular diseases (Ban, Rico, Um, & Kang, 2012; Bokura & Kobayashi, 2003; Gunness & Gidley, 2010; Rader & Hovingh, 2014; Yao & Chiang, 2002), and helps scavenge LDL-C from the arteries and carry it back to the liver, where it is broken down (Ban et al., 2012; Hossain et al., 2007; Zong et al., 2012).

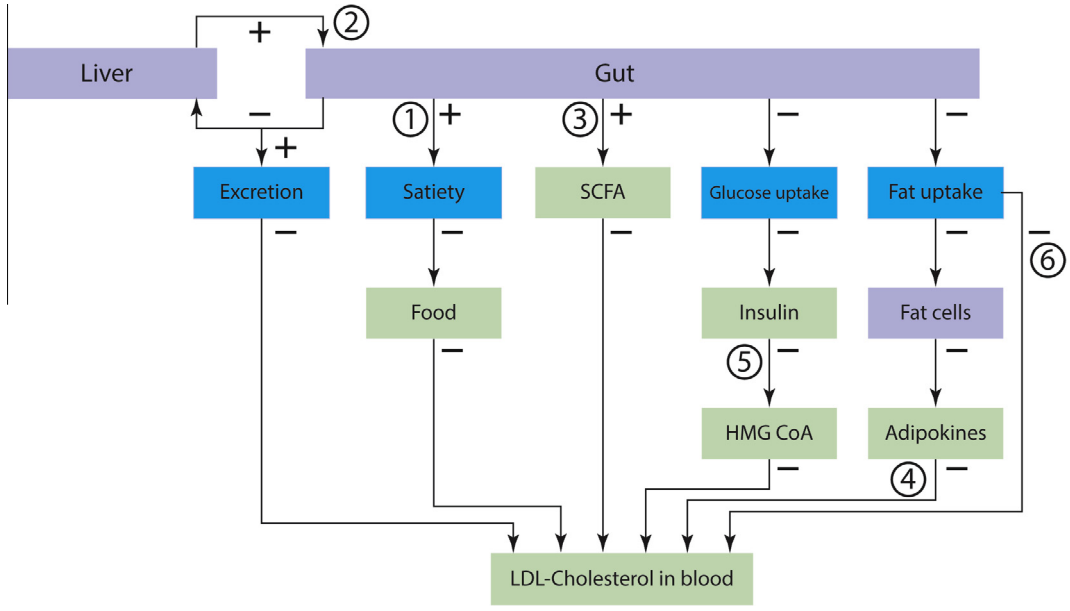

Fig. 1. Schematic overview of the proposed mechanisms of the hypocholesterolaemic effects of fibres. The influence of fibres on each process is indicated with a "+" (increasing due to fibre intake) or "−" (decreasing due to fibre intake) sign. A more detailed description of each mechanism is given in Section 1.5 *Mechanisms of action*, to which the numbers in the Figure refer.

1.3. Cholesterol reduction

Statins, in combination with lifestyle modifications, are used as first-line drug treatment for hypercholesterolaemia (Maki, Carson, Miller et al., 2009; Metzger, Barnes, & Reed, 2009; Parolini et al., 2013). Statins inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme in cholesterol biosynthesis (Gunness & Gidley, 2010; Maki, Carson, Miller et al., 2009; Marounek et al., 2013). However, statins are often ineffective for achieving the treatment goal for LDL-cholesterol (Jaffer & Sampalis, 2007; Maki, Carson, Miller et al., 2009; Marounek et al., 2013). This is partially due to side effects that many patients suffer, including headache, nausea, vomiting and muscle pain (Bokura & Kobayashi, 2003; Jaffer & Sampalis, 2007; Marounek et al., 2013), causing reluctance of clinicians to use high doses (Maki, Carson, Miller et al., 2009). Given that specific dietary fibres show cholesterol-lowering effects, they have the potential to assist in reaching the desired cholesterol-lowering target with fewer side effects (Jaffer & Sampalis, 2007; Maki, Carson, Miller et al., 2009).

1.4. Fibres

Dietary fibres consist of non-digestible carbohydrates and lignin, including non-starch polysaccharides, that are intrinsic and intact in plants (Department of Agriculture. et al., 2005; EFSA Panel on Dietetic Products, 2010a). Primary sources of fibres include fruits, vegetables, legumes, nuts and cereal products (Anderson et al., 2009; Bazzano, 2008; Erkkila & Lichtenstein, 2006). These substances resist digestion by enzymes produced in the human gastrointestinal system (Aller et al., 2004; Bazzano, 2008; Brown, Rosner, Willett, & Sacks, 1999; Chang, Yao, & Chiang, 2012; Dharmarajan, Ravunniarath, & Pitchumoni, 2003). Whether fibres from sources other than plants can be considered dietary fibres is questionable (Dakhara, Anajwala, & Selote, 2012). These are sometimes referred to as functional fibres (Department of Agriculture. et al., 2005) or seen as dietary fibre too, given their comparable digestibility (EFSA Panel on Dietetic Products, 2010a).

Fibres can be categorised as soluble and insoluble fibres (Anderson et al., 2009; Brown et al., 1999; Erkkila & Lichtenstein, 2006; Theuwissen & Mensink, 2008). Examples of soluble fibres include gums, pectins, hydroxypropyl methylcellulose and mucilages (Aller et al., 2004; Anderson et al., 2009; Bazzano, 2008; Brockman, Chen, & Gallaher, 2012; Erkkila & Lichtenstein, 2006; Theuwissen & Mensink, 2008). Most are fermented in the large intestine (Anderson et al., 2009). Some have gel-forming properties with increasing viscosity and these are often referred to as viscous fibres (Erkkila & Lichtenstein, 2006).

Examples of insoluble fibres, or structural or matrix fibres, are cellulose and lignin (Aller et al., 2004; Bartley et al., 2010; Bazzano, 2008; Erkkila & Lichtenstein, 2006; Theuwissen & Mensink, 2008). They are fermented to a limited extent, but are beneficial because of a bulking action that promotes bowel regularity (Anderson et al., 2009; Dharmarajan et al., 2003; Theuwissen & Mensink, 2008).

The health-promoting effects of soluble and insoluble fibres differ similarly with respect to their characteristics. Insoluble fibres have been proven to reduce cardiovascular incidence more than do soluble fibres (Erkkila & Lichtenstein, 2006), while soluble fibres have shown the best results in reducing cardiovascular risk factors, such as cholesterol levels (Aller et al., 2004; Brown et al., 1999; Erkkila & Lichtenstein, 2006; Eussen et al., 2010; Parolini et al., 2013). This discrepancy is not yet understood.

1.5. Mechanisms of action

Literature describes various proposed mechanisms of action by which fibres are thought to influence plasma cholesterol values. A brief overview of these mechanisms is shown in Fig. 1.

The first proposed mechanism indicates that fibres are able to decrease overall energy intake (Brown et al., 1999; Eussen et al., 2010; van Bennekum, Nguyen, Schulthess, Hauser, & Phillips, 2005). Food high in fibres often contains fewer calories than does the nutrient-dense food it replaces, and takes longer to ingest and digest than do its low-fibre counterparts (Anderson et al., 2009; Erkkila & Lichtenstein, 2006). Soluble fibres increase viscosity and bind water, which increases bulk forming, and thus satiety.

Fibres also delay gastric emptying and slow down food's progress through the small intestine, which promotes satiety (Anderson et al., 2009; Brockman et al., 2012; Eussen et al., 2010; Gunness & Gidley, 2010; Krzysik, Grajeta, Prescha, & Weber, 2011; van Bennekum et al., 2005). In the small intestine, dietary fibres modulate incretin secretion, which stimulates insulin release (Anderson et al., 2009) and can also reduce appetite (Anderson et al., 2009; Brockman et al., 2012). Overall, increased satiety and reduced appetite can reduce consumption of cholesterol-containing food, leading to lower cholesterol levels.

The second mechanism of action is an inhibition of bile acids reuptake (Anderson et al., 2009; Bartley et al., 2010; Hur, Lee, & Lee, 2015). Bile acids are synthesised in the liver from cholesterol (Bartley et al., 2010). They facilitate the absorption of cholesterol, lipids and vitamins that are soluble in intestinal fat (Bartley et al., 2010). By entrapping bile acids by means of viscosity (Grizard et al., 2001; Gunness & Gidley, 2010) or adsorption (Gunness & Gidley, 2010; Parolini et al., 2013; Theuwissen & Mensink, 2008) and inhibiting micelle formation (Brouns et al., 2012; Grizard et al., 2001; Gunness & Gidley, 2010; Hur et al., 2015; Marounek, Volek, Synytsya, & Copikova, 2007), soluble fibres increase faecal excretion of cholesterol and bile acids (Anderson et al., 2009; Erkkila & Lichtenstein, 2006; Eussen et al., 2010; Hung, Anderson, Albers, Langhorst, & Young, 2011; Hur et al., 2015). Other research suggests that soluble fibres form a viscous layer of water in the lumen, thus preventing the permeation of bile acids and cholesterol (Erkkila & Lichtenstein, 2006; Eussen et al., 2010; Maki et al., 2009; Theuwissen & Mensink, 2008). Both mechanisms lead to an inhibited reuptake of bile acids in the small intestine, decreasing the enterohepatic pool of bile acids (Bartley et al., 2010; Brufau, Canela, & Rafecas, 2007; Dongowski & Lorenz, 2004; Erkkila & Lichtenstein, 2006; Sanchez et al., 2008). This decrease needs to be compensated; therefore, hepatic enzymes, such as cholesterol 7-α-hydroxylase, also known as CYP7A1 (the rate-limiting enzyme involved in bile acid production (Zong et al., 2012), are upregulated (Bartley et al., 2010; Eussen et al., 2010; Kim et al., 2011; Marounek et al., 2007; Zong et al., 2012). CYP7A1 uses cholesterol, leading to lower cholesterol levels in liver cells (Ausar et al., 2003; Eussen et al., 2010). This, in turn, upregulates LDL-receptors (Bazzano, 2008; Zong et al., 2012) and CYP51 and HMG-CoA reductase production in the liver cells (Bartley et al., 2010; Eussen et al., 2010), which increases uptake of LDL-cholesterol from the blood (Erkkila & Lichtenstein, 2006; Eussen et al., 2010; Theuwissen & Mensink, 2008).

This second mechanism can also be stimulated in a more direct way. Some fibres can increase bile acid production from cholesterol by upregulating hepatic LDL receptor mRNA, enhancing LDL uptake from the blood (Anandan et al., 2013).

The third mechanism is the inhibition of cholesterol synthesis by short-chain fatty acids (SCFAs), such as propionate, acetate and butyrate, which are products of soluble fibre fermentation from intestinal bacteria (Bartley et al., 2010; Bazzano, 2008; Brockman et al., 2012; Eussen et al., 2010; Gunness & Gidley, 2010; Krzysik et al., 2011; Santas, Espadaler, Mancebo, & Rafecas, 2012; Theuwissen & Mensink, 2008). After absorption, each SCFA has a specific function: acetate can be used as a substrate for cholesterol synthesis (Yao, Huang, & Chiang, 2008); propionate inhibits this process through HMG-CoA reductase inhibition (Bartley et al., 2010; Bazzano, 2008; Brufau et al., 2007; Dongowski & Lorenz, 2004; Dvir et al., 2000; Gunness & Gidley, 2010; Maki et al., 2000; Park et al., 2000; Terpstra, Lapre, De Vries, & Beynen, 2002b), and butyrate serves as an energy source for enterocytes (Marounek et al., 2007; Park et al., 2000). However, evidence suggests that HMG-CoA reductase inhibition is not the primary mechanism (Gunness & Gidley, 2010), and studies in humans are scarce and contradictory (Gunness & Gidley, 2010; Theuwissen & Mensink, 2008).

SCFAs also stimulate intestinal hormone secretions, for example, peptide tyrosine-tyrosine (PYY) and glucagon-like peptide-1 (GLP-1), which increase satiety (Brockman et al., 2012). Furthermore, SCFAs lower colonic pH, which decreases bile acid solubility, thus preventing reabsorption (Dongowski & Lorenz, 2004).

The fourth proposed mechanism is found in adipokines, substances produced by fat cells. High fat diets result in increases of leptin, resistin and TNF-α levels and a decrease of adiponectin (Ban et al., 2012; Hung et al., 2009; Liu et al., 2012). Adipokines are important for glucose regulation and lipid metabolism, including cholesterol (Ban et al., 2012; Bartley et al., 2010; Brockman et al., 2012; Hsieh, Yao, Cheng, & Chiang, 2012; Hung et al., 2009).

A more beneficial profile of these adipokines and cholesterol has been found in hamsters and mice with high fibre intake as compared to lower fibre intake (Ban et al., 2012; Hung et al., 2009). The proposed mechanism is the reduction of adipose tissue, leading to a more advantageous adipokine production and secretion (Ban et al., 2012), which in turn is thought to improve plasma cholesterol concentration.

The fifth mechanism indicates that fibres delay intestinal absorption of glucose, due to increased viscosity of the intestinal content, which leads to lower insulin levels (Gunness & Gidley, 2010; Sanchez et al., 2008; Schwab, Louheranta, Torronen, & Uusitupa, 2006; Theuwissen & Mensink, 2008). Insulin activates HMG-CoA reductase; therefore lower insulin could result in lower cholesterol production (Gunness & Gidley, 2010; Theuwissen & Mensink, 2008). Whether this effect is relevant has not been proven, but increases in levels of HMG-CoA reductase have been shown in human trials (Theuwissen & Mensink, 2008).

The sixth mechanism relies on fatty acids. Lauric, myristic and palmitic acid (C12:0, C14:0, C16:0, respectively) decrease LDL-receptor production and activity, which leads to an increase in LDL-C (Fernandez & West, 2005; Santas et al., 2012). Inhibition of the absorption of these fatty acids through fibre intake would lower LDL-C (Santas et al., 2012).

Probably, multiple mechanisms are valid for each fibre, but the relevance of the proposed mechanisms varies. Current research is focussed on determining the importance of each mechanism for all dietary fibres (Carr et al., 2003; Eussen et al., 2010; Hung et al., 2009;).

To determine the state of the evidence of the cholesterol-lowering effects of fibres and their mechanisms, three specific fibres will be reviewed in this article: the semisynthetic hydroxypropyl methylcellulose (HPMC), pectin (originates from fruits and vegetables), and chitosan (found in shellfish).

2. Method

2.1. General

HPMC, chitosan and pectin were selected for review, based on: (1) the evidence of their cholesterol-lowering effect. Many studies on these fibres have been published, so information is likely to be available; (2) their distinct origins, which might indicate differences in mechanisms of action; (3) chemical modifiability.

While meta-analyses in human populations have already been conducted, they don't provide information on the mechanisms behind the observed effect. However, animal research provides for more measurements and can provide more insight into the relationship between the structure of a fibre and its effect. Therefore this study performed a quantitative analysis on animal studies only.

2.2. Objective

The aim of this review is to gain insight in the mechanisms that cause the cholesterol-lowering effect of selected fibres and present an overview of the effects that are seen in different animal models. This could also provide recommendations for future research.

2.3. Data sources

A systematic review was conducted, using articles retrieved from Pubmed, Embase and the Cochrane Library. "[Fibre name] AND cholesterol" was used as the search string, on September 2, 2014. Publications were included in the systematic search if they were published in peer-reviewed journals between January 1, 2000 and the date of the search. No further limits were used.

2.4. Exclusion criteria

- *In vitro* studies.
- Studies done in non-vertebrate animals.
- Studies in which the fibres were not individually evaluated.
- Articles published in languages other than English.
- Articles published before January 1, 2000.
- All types other than primary peer-reviewed articles.

2.5. Study selection

Articles were assessed for their eligibility by reviewing the title and abstract. When further information was needed to determine relevance, the full text was evaluated. Additional articles were selected for inclusion in the review where deemed necessary, for example to provide information on the chemical structure of the fibres or to clarify the relevance of cholesterol. Fig. 2 provides an overview of the selection procedure.

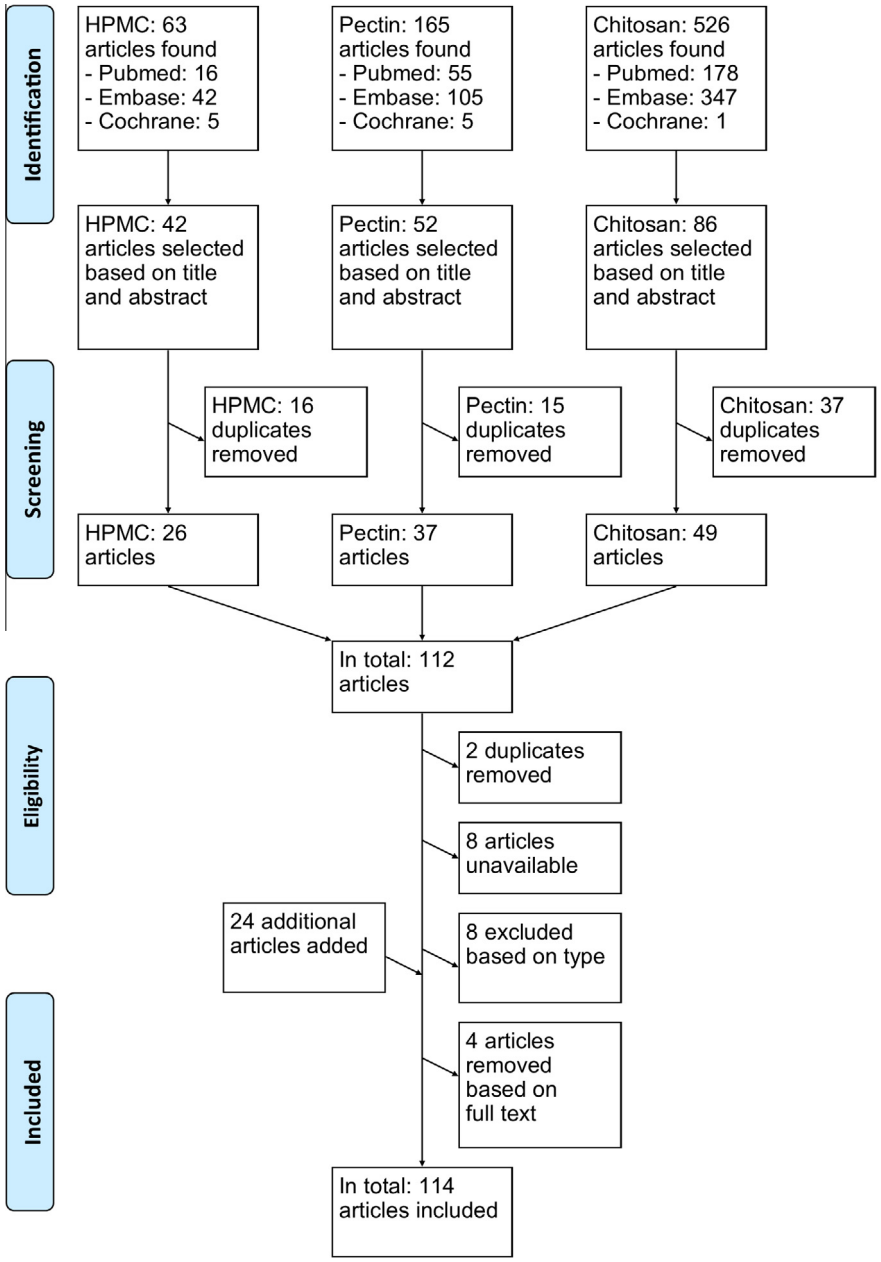

Fig. 2. Schematic overview of the study selection process.

2.6. Study analysis

To analyse the results of each study, the relative difference in total cholesterol, LDL-C, HDL-C and VLDL-C levels between the group on a high-fat diet and the group fed with a high-fat diet enriched with one specified fibre was calculated, where data was available. The weighted mean was calculated by correcting for the number of animals that had been included in each study. These results were standardised for the relative quantity of fibre added to the diet, so that the figures give a consistent estimate of the effect a 1% fibre enriched diet would have had. To assess the possibility of publication bias, a funnel plot, based on outcomes versus sample size, was drawn. Microsoft Excel was used for all calculations and graphs.

3. Results

3.1. HPMC

3.1.1. General

Hydroxypropyl methylcellulose (HPMC) is a non-fermentable semi-synthetic dietary fibre, based on cellulose (Burdock, 2007), which is a carbohydrate consisting of anhydroglucose units (Reppas, Swidan, Tobey, Turowski, & Dressman, 2009). It is commonly used as a stabiliser, emulsifier and thickener (Ban et al., 2012; Burdock, 2007; Hung et al., 2011). After ingestion, HPMC forms a viscous solution in the gastrointestinal tract (Burdock, 2007; Maki, Carson, Kerr Anderson et al., 2009; Maki et al., 2000; Maki, Reeves et al., 2009).

3.1.2. Mechanisms

HPMC is non-fermentable due to its cellulose backbone (Hung et al., 2009); it does not yield SCFA production. In animal studies, a relationship has been found between the viscosity of used HPMC and a hypocholesterolaemic effect of bile acid and cholesterol excretion (Bartley et al., 2010). Therefore, HPMC's action is thought to increase the viscosity of intestinal content (Ban et al., 2012; Bartley et al., 2010; Hung et al., 2009), which increases faecal excretion of bile acids (Cox et al., 2013; Hung et al., 2011; Yokoyama et al., 2011) (*second mechanism*).

This action can cause either direct adsorption of bile acids on HPMC, or the formation of a barrier that decreases permeability (Ban et al., 2012; Maki, Carson, Kerr Anderson et al., 2009; Maki et al., 2000; Yokoyama et al., 2011). This last option was shown when *in vivo* permeability was determined, using a fluorescein isocyanate-labelled dextran polymer: the polymer was decreased by 50% in the group taking HPMC supplementation (Kim, Bartley, Young, Seo, & Yokoyama, 2013). This also suggests that the timing of HPMC consumption is important; ingesting HPMC during a meal increases its effectiveness (Maki et al., 2000).

The mRNA level of CYP7A1 (involved in bile acid synthesis (Kim et al., 2011)) was found to be upregulated after HPMC administration (Kim et al., 2013). This is to be expected, since bile acid production needs to increase to compensate for the lower reuptake.

HPMC has been shown to have a beneficial effect in regulating adipokine production (Ban et al., 2012; Bartley et al., 2010). High fat diets resulted in increases of leptin, resistin and TNF-α levels and a decrease of adiponectin, but the addition of HPMC counteracted this outcome (Ban et al., 2012; Hung et al., 2009). This suggests that the cholesterol-lowering effects of HPMC could also partially be due to a decrease in adipose tissue through diminished absorption of fat and lower food intake. This would lead to a more advantageous production of adipokines, thus leading to better cholesterol levels (Ban et al., 2012; Hung et al., 2009) (*fourth mechanism*).

3.1.3. Animal studies

Several studies in animals have shown the hypocholesterolaemic effects of HPMC, which can cause weight reduction (Kim et al., 2013). The results of the eight studies that were found and selected in this search are given in Table 1 (in the Supplementary material) and Fig. 3.

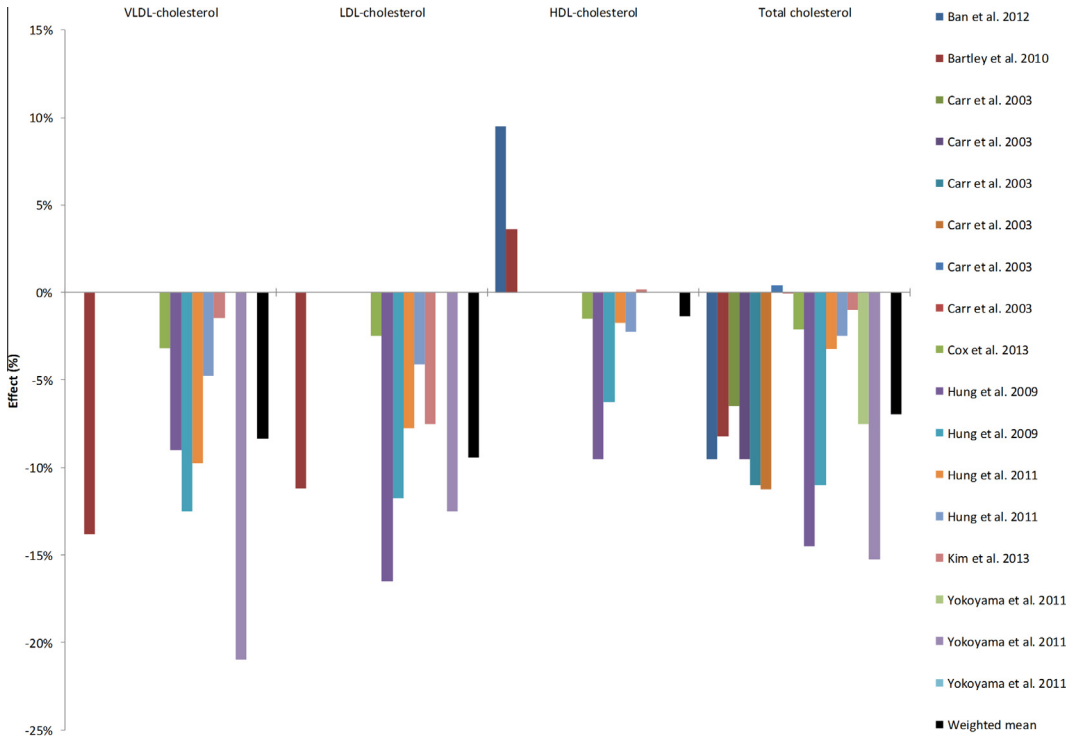

Fig. 3. The average effect of HPMC on cholesterol levels by percent administered in diet. To correct for differences in the amount of fibre added to the diet, all determinations are standardised so that the results correspond to 1% of the diet being fibre. All reviewed studies, including not significant results, are displayed. The weighted mean was calculated for each cholesterol class, using all studies that reported on that specific class.

3.1.4. Human studies

In human subjects, HPMC has been tested in doses of 5–30 g per day (Anderson et al., 2009; Cox et al., 2013; Maki et al., 2000, 2007; Reppas et al., 2009; Maki, Carson, Kerr Anderson et al., 2009). Various clinical trials have indicated that HPMC lowers LDL-C (Anderson et al., 2009; Maki, Reeves et al., 2009; Maki et al., 2000; Reppas et al., 2009; Maki, Carson, Kerr Anderson et al., 2009) without changing HDL-cholesterol (Anderson et al., 2009; Maki, Carson, Kerr Anderson et al., 2009; Reppas et al., 2009) or triglyceride concentrations (Anderson et al., 2009; Maki, Carson, Kerr Anderson et al., 2009), and has a hypoglycaemic effect (Maki et al., 2007). An 8.5% reduction in LDL-C has been found, using 5 g per day of HPMC (Anderson et al., 2009). The highly viscous HPMC was more effective than its low-viscous counterpart in human studies (Reppas et al., 2009).

Another trial was performed to determine whether adding HPMC to statin therapy would prove beneficial. The addition of HPMC resulted in a total reduction of LDL-C (10.5%) and total cholesterol (7.4%), compared to statin therapy alone (Maki, Carson, Miller et al., 2009).

3.1.5. Conclusion

HPMC has only mild side effects (Maki et al., 2000) and effectively decreases total and LDL-C levels in animals and humans. It might be useful as an additive in food products for human use, or as an add-on to statin therapy, as demonstrated in a human trial. (Maki, Carson, Miller et al., 2009). However, HPMC lacks the ability to increase HDL-C. This casts doubts on the actual health-promoting effect of HPMC, namely whether it can actually prevent cardiovascular diseases. Further research on hard outcomes, e.g. the effect on coronary events and death, needs to be performed before conclusions can be drawn.

3.2. Pectin

3.2.1. General

Several subtypes of pectin exist, but all are polymers consisting of blocks of polar D-galacturonic acid and rhamnogalacturonan units (Thakur, Singh, & Handa, 1997). It is found in the plant cell walls of fruits and vegetables (Thakur et al., 1997). Pectin can be used as a fat replacement in food (Brouns et al., 2012; Marounek et al., 2007; Theuwissen & Mensink, 2008). The molecular weight and the degree of esterification of the carboxyl groups determine its physicochemical properties (e.g. whether it dissolves (Dongowski & Lorenz, 2004; Marounek et al., 2007; Thakur et al., 1997), and its effectiveness in lowering cholesterol (Brouns et al., 2012; Marounek et al., 2007). If more than 50% of the galacturonic acid residues are esterified it is called high methoxyl pectin (Theuwissen & Mensink, 2008). Pectins with a high degree of hydrophobic substitution are less soluble (Marounek et al., 2007), and are thought to be more effective in lowering plasma cholesterol (Brufau et al., 2007; Dongowski & Lorenz, 2004; Marounek et al., 2007; Sanchez et al., 2008).

3.2.2. Mechanisms

The fact that the cholesterol-lowering effect of pectin depends on the ability to form a viscous gel (Brouns et al., 2012) is an important clue about the mechanism of action. It is assumed that pectin binds to cholesterol and bile acids in the gut, both reducing reabsorption and promoting their excretion (Brufau et al., 2007; Dongowski & Lorenz, 2004; Hur et al., 2015; Marounek et al., 2007; Park et al., 2000). Pectin also disturbs micelle formation (Dongowski & Lorenz, 2004), thus inhibiting absorption of cholesterol (Brufau et al., 2007) (*second mechanism*).

Pectin is not degraded by enzymes during passage through the human small intestine (Dongowski & Lorenz, 2004; Marounek et al., 2007). In the large intestine, however, it is almost completely fermented to SCFAs (Dongowski & Lorenz, 2004; Kirat, 2010; Marounek et al., 2007) of which acetate is most predominant (Kirat, 2010; Marounek et al., 2007; Thakur et al., 1997). Acetate is a cholesterol precursor (Kirat, 2010; Marounek et al., 2007), whereas propionate inhibits hepatic cholesterol synthesis (Dongowski & Lorenz, 2004). These data indicate that the pectin-induced changes in an acetate:propionate ratio are unlikely to significantly contribute to pectin's cholesterol-lowering effect (*third mechanism*).

It is recognised that pectin reduces the blood glucose rise after a meal (EFSA Panel on Dietetic Products, 2010b). This results in lower insulin levels, leading to lower HMG-CoA reductase activity (Gunness & Gidley, 2010; Theuwissen & Mensink, 2008) (*fifth mechanism*).

3.2.3. Animal studies

Various types of pectin have been used in animal studies, with differing results (Krzysik et al., 2011; Marounek et al., 2007; Terpstra et al., 2002b). An overview of the results is given in Table 2 (in the Supplementary material) and Fig. 4.

3.2.4. Human studies

The effect of pectin ingestion by humans has been evaluated (Brouns et al., 2012; Schwab et al., 2006). A meta-analysis of the effect of pectin in humans showed a 13% reduction of LDL-C when consuming 12–24 g of pectin per day (Anderson et al., 2009). The type of pectin seems crucial because highly viscous and highly esterified pectin appears to be more effective (Brouns et al., 2012).

The European Panel on Dietetic Products, Nutrition and Allergies concluded that consumption of 6 g of pectin per day contributes to the maintenance of normal cholesterol levels (EFSA Panel on Dietetic Products, 2010b).

3.2.5. Conclusion

The reviewed literature shows a diverse pattern of lowering cholesterol, which can be explained by the multiple types of pectins tested and the specific animal models. The molecular composition of pectin used for human consumption is thought to be crucial for its gel-forming properties, and thus for the cholesterol-lowering effect (Brouns et al., 2012; Marounek et al., 2007; Sanchez et al., 2008; Terpstra, Lapre, De Vries, & Beynen, 2002a). Not all of the studies we included mentioned the supplement's characteristics, and our analysis did not test for this, so the cholesterol-lowering effect cannot be concluded from our data set.

Overall, pectin seems to reduce LDL-C and total cholesterol, and possibly VLDL-C, but the effect on HDL-C remains uncertain. Although the consumption of some pectins appears to make HDL-C levels rise, this effect is small and the clinical significance is unclear. Again, more research is needed before the health effect of pectins on humans can be fully established.

3.3. Chitosan

3.3.1. General

Second only to cellulose, chitosan is the most abundant and easily obtained natural polymer known (Hossain et al., 2007; Rizzo et al., 2013). It is found in shellfish, e.g. clams and krill oysters, squid, fungi, yeasts and insects (Anandan et al., 2013; Baker, Tercius, Anglade, White, & Coleman, 2009; Hsieh et al., 2012). Soluble chitosan is derived from insoluble chitin by de-N-acetylation and is a polymer of N-acetyl-D-glucosamine and D-glucosamine (Tharanathan & Kittur, 2003). Relevant parameters for chitosan's ability to lower cholesterol are molecular weight (Chang et al., 2012), degree of acetylation – deacetylation makes chitosan less

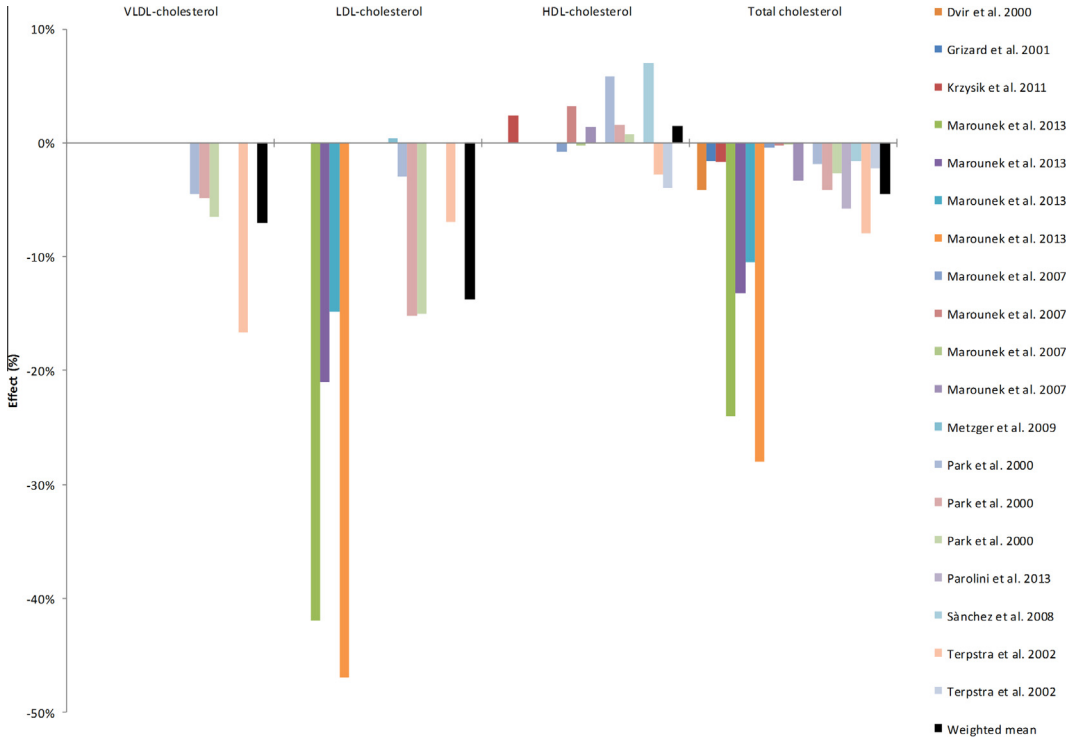

Fig. 4. The average effect of pectin on cholesterol levels by percent administered in diet. All reviewed studies that mentioned the outcome, including not significant results, are displayed. The weighted mean was calculated for each cholesterol class, using all the studies that reported on that specific class.

susceptible to degradation (Yao & Chiang, 2006a; Yao et al., 2008) – and pK_a – which determines the ability to dissolve (Lee, Park, Choi, Yi, & Shin, 2003; Ni et al., 2004; Yao & Chiang, 2006; Ylitalo, Lehtinen, Wuolijoki, Ylitalo, & Lehtimaki, 2002; Choi et al., 2012; Hernandez-Gonzalez, Gonzalez-Ortiz, Martinez-Abundis, & Robles-Cervantes, 2010; Liu et al., 2012; Yao et al., 2008). Viscosity appears to be less relevant (Chiang, Yao, & Chen, 2000).

Chitosan is strictly not a dietary fibre, because it does not have a vegetable origin, but it does have the same chemical and physiological properties as have vegetable fibres (Sumiyoshi & Kimura, 2006; Tharanathan & Kittur, 2003; Yao & Chiang, 2006a; Zhang, Liu, Li, & Xia, 2008). Since chitosanase is not produced in most animal intestines (Kim, Park, Yang, & Han, 2001), chitosan is indigestible for mammals (Asha & Nair, 2005; Ausar et al., 2003; Gallaher, Munion, Hesslink, Wise, & Gallaher, 2000; Sumiyoshi & Kimura, 2006). However, chitosan oligosaccharide, the soluble hydrolysis product of chitosan, is water-soluble and can be partially digested and absorbed by mammals (Kim et al., 2001; Wang et al., 2011; Zong et al., 2012).

3.3.2. Mechanisms

Chitosan is thought to act in several ways. First, where chitosan is soluble, it lowers cholesterol levels by increasing the viscosity of stomach content (Ausar et al., 2003; Gallaher et al., 2000), which inhibits uptake of cholesterol (*second mechanism*). This action delays gastric emptying (Chang et al., 2012), thus leading to a decrease in food intake by inducing satiety (van Bennekum et al., 2005) (*first mechanism*).

Next, chitosan acts as a cationic polysaccharide in an acidic environment, e.g. the stomach (Tang et al., 2005; Ylitalo et al., 2002), so the positive amino groups of the fibre bind to negatively charged molecules, such as bile acids and fatty acids (Anandan et al., 2013; Anraku et al., 2010; Ausar et al., 2003; Baker et al., 2009; Choi et al., 2012; Jaffer & Sampalis, 2007; Jun et al., 2012; Kim et al., 2001; Tai, Sheu, Lee, Yao, & Chiang, 2000). This leads to higher activity of the LDL-receptor and thus lowers LDL-C plasma levels (Santas et al., 2012) (*sixth mechanism*). Neutrally charged triglycerides are not affected here (Hossain et al., 2007).

In the intestine, a higher pH makes the complex precipitate with bound fatty and bile acids and cholesterol (Tang et al., 2005; Yao & Chiang, 2006b; Zhang, Zhang, Mamadouba, & Xia, 2012; Zhang et al., 2008). After precipitation, the bound fatty- and bile acids are inaccessible to enzymes (Hossain et al., 2007; Tai et al., 2000) and are excreted with the stool (Anandan et al., 2013; Anraku et al., 2010; Ausar et al., 2003; Baker et al., 2009; Choi et al., 2012; Sumiyoshi & Kimura, 2006). The lack of cholesterol impairs emulsifications and decreases triglyceride uptake (Jun et al., 2012; Ylitalo et al., 2002). *In vitro*, chitosan can bind approximately four times its own weight in lipids (Guha et al., 2005).

Another way of action could be dependent on chitosan's antioxidant activity. Removal of abnormalities in lipid metabolism that are associated with oxidative phenomena creates better cholesterol values (Anraku et al., 2009, 2010). Reduced oxidative stress has been found to be due to chitosan in animals (Ahmed et al., 2014; Anraku et al., 2010) and humans (Anraku et al., 2009). However, other studies indicate that chitosan causes increased oxidative stress (Hossain et al., 2007), making antioxidant supplementation advisable. This requires more research.

Finally, the inhibition of pancreatic lipase activity has been shown *in vitro* and in mice (Choi et al., 2012; Sumiyoshi & Kimura, 2006). This decreases lipid and cholesterol absorption. Chitosan's inhibition of liver and plasma lipase has also been proposed as a relevant mechanism (Zhang et al., 2008).

3.3.3. Animal studies

In animal studies, chitosan has been shown to be effective in reducing LDL-C and total plasma cholesterol and increasing

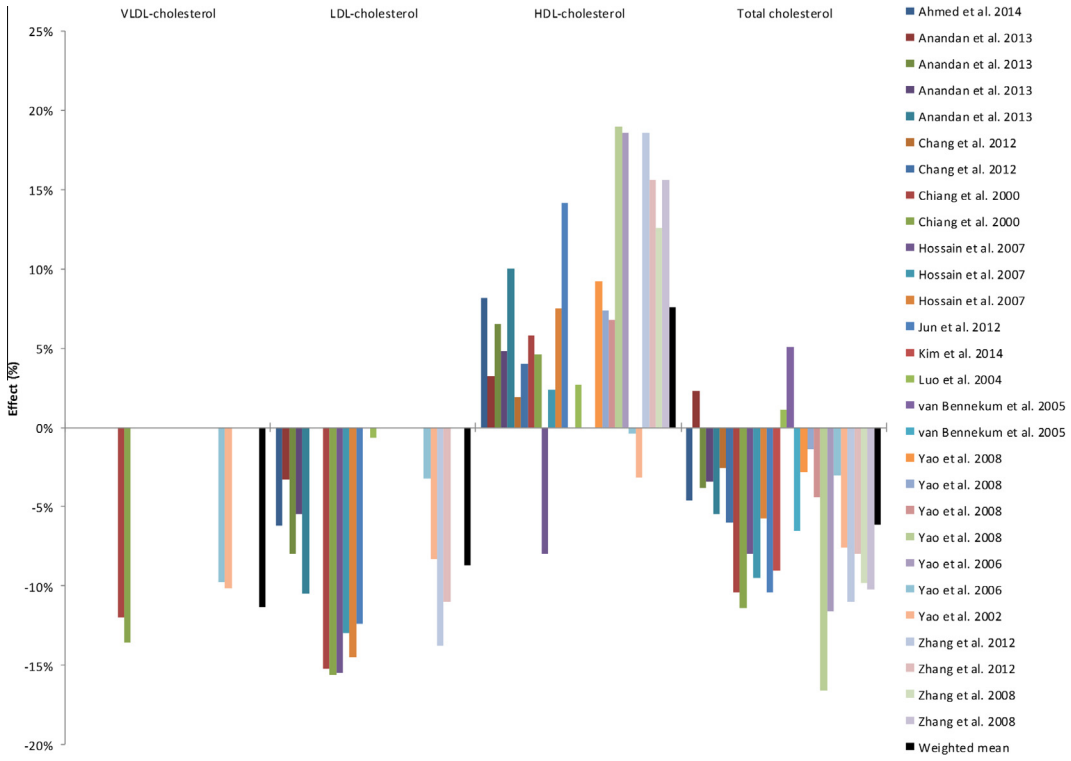

Fig. 5. The average effect of chitosan on cholesterol levels by percent administered in diet. All reviewed studies, including not significant results, are displayed. Studies that were administered through water are not displayed. The weighted mean was calculated for each cholesterol class, using all the studies that reported on that specific class. (See above-mentioned references for further information.)

HDL-C, and its safety has been proven (Zhang, Zhong, Tao, Wu, & Su, 2012). An overview of the results is presented in Table 3 (in the Supplementary material) and Fig. 5.

3.3.4. Human studies

Multiple studies have tested the effect of chitosan on cholesterol in humans. Some have found positive effects (Bokura & Kobayashi, 2003; Choi et al., 2012; Jaffer & Sampalis, 2007; Ni et al., 2004; Ylitalo et al., 2002), but others none (Hernandez-Gonzalez et al., 2010; Ho, Tai, Eng, Tan, & Fok, 2001). A meta-analysis conducted solely in patients with hypercholesterolaemia – those who would benefit from the effect most – found a significant reduction of total cholesterol, although LDL-C and HDL-C were not significantly affected (Baker et al., 2009). A Cochrane review included various studies and found that chitosan produced a significant increase in HDL-C and a reduction of LDL-C and total cholesterol (Jull, Ni, Bennett, Dunshea-Mooij, & Rodgers, 2008); however, clinical relevance was questioned.

To test whether chitosan actually binds fat in the human intestine, as it does in animal models, several studies measured faecal fat excretion. Results varied, as some found an effect while others did not (Gallaher et al., 2002; Ni et al., 2004). Bile acid excretion, however, was increased in all studies (Gallaher et al., 2002).

3.3.5. Conclusion

The use of many types of chitosan with various properties complicates the comparison of studies. However, the claim that chitosan has a positive influence on the cholesterol profile in animals and humans is supported by the presented data. The high HDL-C increase is particularly impressive. The downside of high chitosan doses is the diminished uptake of essential fatty acids, fat-soluble vitamins and minerals (Kim et al., 2001). It is hypothesised that chitosan oligosaccharide would not have this unwanted effect (Kim et al., 2001), but there are not enough data available to support this hypothesis.

3.4. Other fibres

As previously stated, this paper provides a systematic but time-limited overview of the field of fibre research. Far more studies than those mentioned in this article have been performed. Other fibres, e.g. psyllium, polysaccharides, such as inulin, polydextrose, β-glucan, and guar gum, as well as fibre-rich foods, such as corn bran and oat bran, are not discussed in this article, although they too might have beneficial effects by lowering cholesterol (Choi et al., 2012; Dakhara et al., 2012).

Most of those studies reviewed confirm the cholesterol-lowering effects in humans of the fibres under survey in this review. In general, 6 g of water-soluble viscous fibre daily intake was found to lower LDL-C by approximately 5.4% (Anderson et al., 2009). An analysis on hard outcome parameters for fibre use, such as coronary events (a 14% lower risk after an increase of 10 g of dietary fibres per day) and mortality (a 27% lower risk), has already been performed (Pereira et al., 2004). These results were independent of other dietary factors, age, sex, baseline BMI, smoking, blood pressure, diabetes and hypercholesterolaemia.

4. Discussion

4.1. General

Pereira et al. (2004) calculated, using a pooled analysis of cohort studies, that for each 10 g per day increment in total, cereal, or fruit fibre intake, coronary risk decreased by 10–30%. The struggle, in these calculations, is correcting the results for bias; humans with high fibre intake are more likely to lead a healthier life, with more

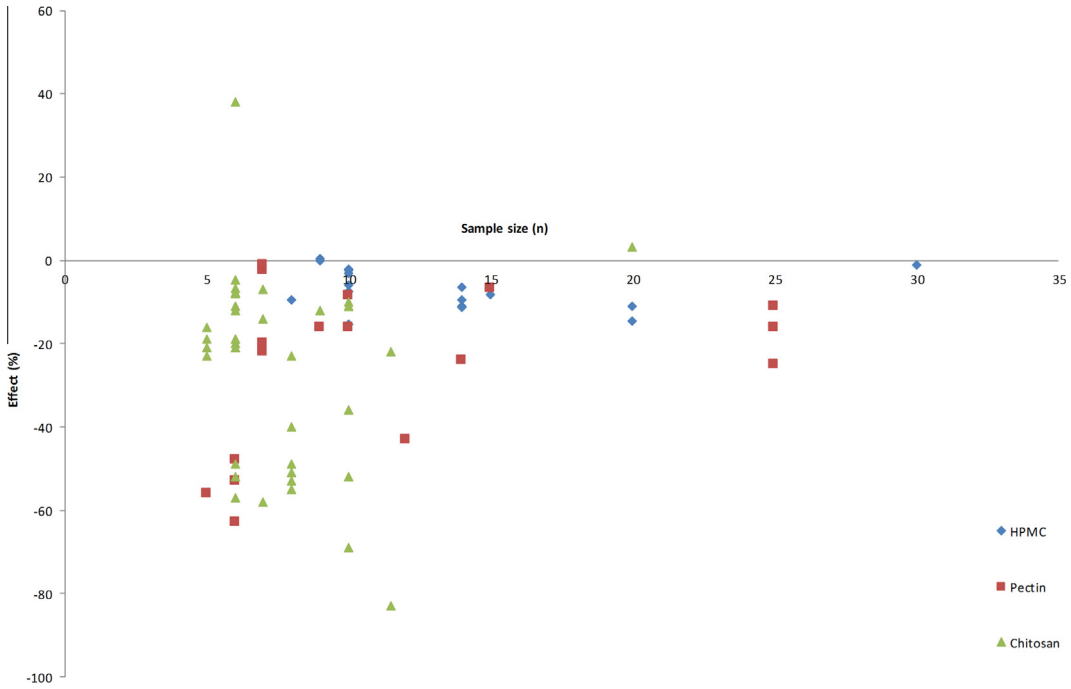

Fig. 6. Funnel plot for the reviewed studies: the number of subjects per arm were plotted against the found effect on total cholesterol relative to the high-fat group. For LDL-C, HDL-C and VLDL-C, the same shape was found, but with a lower density because fewer studies measure these outcomes.

physical activity, less smoking, less fat and more vitamin intake, which yields confounding factors (Erkkila & Lichtenstein, 2006). Testing specific fibres in real-life situations requires subjects to use the dietary fibres for longer periods than those currently investigated, as studies have shown that the cholesterol-lowering effect builds up over time (Anderson et al., 2009).

4.2. Limitations

Animal models for studying the cholesterol-lowering effect of fibres and the mechanisms thereof offer several benefits over human studies. In animal models, environment and food intake are controllable and costs are relatively low. However, extrapolation of the experimental outcomes to humans requires careful translational interpretations. In rodents, the majority of cholesterol is carried in HDL rather than in LDL, which is the case for humans (Bergen & Mersmann, 2005; Vitic & Stevanovic, 1993; Xiangdong et al., 2011). Rodents show relatively limited plasma cholesterol changes as a reaction to fibres, and they are more resistant to atherosclerosis than are humans (Jokinen, Clarkson, & Prichard, 1985). As a replacement parameter for plasma cholesterol in rats, liver cholesterol can be analysed (Aprikian et al., 2003; Carr et al., 2003; Gallaher et al., 2000; Marounek et al., 2007). In addition to rats and mice, hamsters, guinea pigs and swine are used; the advantage of these animals is that their lipoprotein metabolism is more comparable to humans (Fernandez, Wilson, Conde, Vergara-Jimenez, & Nicolosi, 1999; Spady & Dietschy, 1983).

The use of various animal species further complicates comparison, but also enables the possibility of studying the effects of fibres on multiple mechanisms in more detail (if the same fibre is used in both animal species). The overview of the reviewed HPMC articles (Table 1, suppl.) confirms a difference in cholesterol metabolism between rats/mice and hamsters. It shows that the effect in rodent serum is relatively low (0–20% decrease in total cholesterol) compared to the studies in hamsters (24–61% decrease in total cholesterol). The pectin (Table 2, suppl.) and chitosan (Table 3, suppl.) studies are mainly performed in rodents, so no comparison with hamsters can be made. In studies with hamsters, the use of males is preferred over females because of their greater responsiveness (Robins et al., 1995). In humans this difference has not been found (Pereira et al., 2004).

LDL can be divided into seven subclasses, based on size, density, physicochemical composition, metabolic behaviour and atherogenicity. Over one hundred studies have found that specifically small, dense LDL (sdLDL) is associated with cardiovascular risk (Gazi, Tsimihodimos, Tselepis, Elisaf, & Mikhailidis, 2007; Mikhailidis et al., 2011). Also, the main apolipoproteins present in LDL are apoB and apoE, while HDL has the major apolipoprotein apoA-I. The exact composition of apolipoproteins influences the class and metabolism of cholesterol (Wang et al., 2011). Although many studies have measured the various types of cholesterol (HDL-C, LDL-C, VLDL-C), subclasses are not often measured. It has been shown that not all types of LDL have the same predictive power for cardiovascular risk assessment (Gazi et al., 2007; Mikhailidis et al., 2011; Rizzo et al., 2013), so using an undivided LDL-C measurement will not give the most accurate prediction for effects on mortality or morbidity endpoints. By measuring the specific cholesterol subclasses, the predictive power of the surrogate parameter for cholesterol could be improved. There are indications that similar subclasses could be measured in animals, but further research is needed to establish a firm base for translational studies. This review does not take the various types of LDL into account, given that very few studies report on them. There are indications, however, that dietary fibres influence the composition of LDL particles (Fernandez, Abdel-Fattah, & McNamara, 1993; Fernandez et al., 1999).

4.3. Bias

The present article points out the benefits of published studies in animals, but cannot exclude possible publication bias. An indication of the likelihood of this phenomenon can be found by creating a funnel plot, plotting the outcome of each study against the sample size. Studies with fewer subjects should lead to variable results,

while larger studies should have more consistent results, thus creating the shape of a funnel (Lau, Ioannidis, Terrin, Schmid, & Olkin, 2006). The funnel plot of the selected trials is shown in Fig. 6. Given that the studies with larger sample sizes are probably closest to the real effect, and smaller studies should spread around that effect based on coincidence, in the obtained Figure a gap is found where the small studies should report negative outcomes. For all three fibres, this means that the cholesterol-lowering effect might be slightly overestimated.

4.4. Combination with statins

Statins are believed to reduce total cholesterol by 25–40% (Baker et al., 2009). However, the reduction caused by fibres in humans is much lower. Fibres may be beneficial as an add-on to statin therapy, since they could further reduce plasma cholesterol by mechanisms different from statins (Eussen et al., 2010; Guha et al., 2005; Maki, Carson, Miller et al., 2009). However, this would require more research into the interaction between fibres and statins. Fibre drug interactions have been shown in the case of chitosan and warfarin (Rizzo et al., 2013), pectins and gums combined with digoxin and acetaminophen (Dharmarajan et al., 2003), and wheat bran together with levothyroxine (Dharmarajan et al., 2003). With atorvastatin therapy, however, higher HDL-levels (Guha et al., 2005; Ho et al., 2001) and weight loss have been found by adding chitosan (Guha et al., 2005). Also, HPMC and several statins have been combined well in one study (Maki, Carson, Miller et al., 2009). This shows that these specific combinations could be advantageous for treatment of hypercholesterolaemia.

Another point of attention in combining statins and fibres is that both modulate bile acid metabolism. Statins are indicated for decreasing bile acid production, while fibres might decrease bile acid reuptake. A lack of bile salts can cause side effects, such as decreased fat-soluble vitamin uptake. Moreover, some fibres are indicated for blunting HDL-cholesterol, thus increasing the effects of statins (Erkkila & Lichtenstein, 2006; Eussen et al., 2010).

5. Conclusion

The overview of the results of the selected studies shows that all three fibres under review are capable of reducing LDL-C, VLDL-C and total cholesterol. HPMC and pectin had limited or inconclusive results for HDL-C modulation, which is in line with the findings in human studies (Anderson et al., 2009; Reppas et al., 2009; Maki, Carson, Kerr Anderson et al., 2009). However, chitosan increased HDL-C, despite the high basal HDL-C levels present in rats (the animal used in most of the studies).

An inverse association between fibre intake and coronary heart disease risk underlines the importance of adequate fibre consumption (Erkkila & Lichtenstein, 2006; Pereira et al., 2004). General lifestyle advice of regular physical exercise and a diverse diet with a great variety of fruits and vegetables (Bokura & Kobayashi, 2003; Dharmarajan et al., 2003; Ho et al., 2001; Ni et al., 2004; Pereira et al., 2004) supports the intake of fibre-rich foods. These foods are also richer in vitamins, minerals and antioxidants in general, and low in cholesterol and saturated fat (Ahmed et al., 2014; Brown et al., 1999).

The amount of fibre currently needed to effectively reduce cholesterol is high compared to statins. These high levels are generally acceptable for food, but might impair actual use as treatment. Before fibres can gain a place in therapy against hypercholesterolaemia, they need to become more effective. At present, optimisation of existing fibres (changing the molecular weight, viscosity, degree of hydrophobic substitution) has not yet led to a fibre that is active enough to be comparable to statin treatment. However, this approach holds significant promise for future research. Several studies suggest that the cholesterol-modulating effect can be enhanced by combining several fibres (Gallaher et al., 2002; Parolini et al., 2013), because every fibre has a unique profile of mechanisms and effects (Carabin et al., 2009; Reimer et al., 2013).

Of the three studied fibres, the effect of HPMC is most dependent on viscosity. It has been shown that the effectiveness of pectin is also related to its molecular shape and the degree of esterification. In the case of chitosan, the molecular charge adds to the cholesterol-modulating effect. Using these facts, it should be possible to design a dietary fibre that yields an optimal cholesterol-lowering effect, using experiences in tailoring physicochemical properties and exploiting the primarily biophysical mechanisms of action. The optimal fibre should be highly viscous and have side chains with a positive charge, so they allow for adsorption of cholesterol and bile acids. It should also be stable in a gastrointestinal environment and not be too easily digested by bacteria, since digestion would decrease adsorption and faecal excretion of bile acids and cholesterol, and lead to side effects. Fermentation of pectin is thought to be responsible for side effects, such as abdominal cramping and flatulence (Maki et al., 2000), and the explanation for the better tolerability of HPMC, which is not fermentable (Carr et al., 2003; Maki et al., 2000; Reppas et al., 2009; Yokoyama et al., 2011).

For now, the challenge is how to package a sufficient fibre load in an acceptable and palatable way in designer foods. For the general population, adding a mix of selected fibres to products used daily, e.g. noodles (Jitpukdeebodintra & Jangwang, 2009), bread (Ausar et al., 2003) or orange juice (Maki et al., 2000), could be an option.

In conclusion, soluble dietary fibres are a promising tool for cholesterol reduction. However, fibres that are currently being used are not adequately potent in typical pharmacological dosages and need optimisation and/or to be combined with other substances.

Author contribution

TvdG, AH, CvH, HP and TP designed the research, TvdG, AH and CvH provided essential materials, conducted the research, analysed data and performed the statistical analysis. TvdG, AH, and TP wrote the paper. TvdG, AH, HP and TP had primary responsibility for final content. All authors have read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.12.050.

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