Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

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Abstract

High levels of total- and LDL-cholesterol in blood are risk factors related to cardiovascular disease. One of several measures to prevent this is consumption of the viscous soluble fibre β-glucan that in high amount is found in oats. In 2006, EFSA approved the health claim that a dose of at least 3 g β-glucan per day maintains normal blood cholesterol concentrations. The cholesterol-lowering properties of β-glucan have been shown to vary based on the processing and use in products. The aim of this literature review was to compare the cholesterol-lowering effect of oat β-glucan in ready-to-eat cereals and bread. These two products are easily accessible and common in the western diet. They can contain β-glucan of the same source but differ in several steps of manufacturing.

The cholesterol lowering property of oat β-glucan is primarily affected by the solubility and molecular weight distribution. In bread making, fermentation and the use of flour with low falling number can decrease molecular weight and increase solubility. In the manufacturing extruded ready-to-eat cereals, high molecular weight is retained and solubility increased. One of the possible cholesterol lowering mechanisms of β-glucan is by increased hepatic bile acid production from cholesterol either endogenously synthesized or taken from the circulation.

Six studies with ready-to-eat cereals and five studies with bread or muffins, cookies and buns were included for comparison. Eight out of the selected eleven studies reported a significant decrease in total- and LDL-cholesterol. Regarding dose of β-glucan and study subjects, the studies with cereals had more similarities and achieved more consistent results compared to the studies with bread. Ready-to-eat cereals seem to be a more favourable product than bread for oat β-glucan consumption in order to achieve a cholesterol reduction, due to more beneficial effects of processing on molecular weight and solubility of the β-glucan.

Keywords: Oat, β-glucan, total cholesterol, LDL-cholesterol, RTE cereals, bread

Sammanfattning

Höga nivåer av total- och LDL-kolesterol i blod utgör en riskfaktor för hjärt-kärlsjukdom. En av flera möjliga åtgärder för att förhindra insjuknande är konsumtion av den viskösa och lösliga fibern β-glukan, som i stora mängder finns i havre. EFSA godkände 2006 hälsopåståendet att en dos av minst 3 g β-glukan per dag upprätthåller normala blodkolesterolkoncentrationer. Bearbetning och användning i produkter har visat sig påverka graden av β-glukanets kolesterolsänkande effekt. Syftet men denna litteraturstudie var att jämföra kolesterolsänkning efter konsumtion av β-glukan i flingor respektive bröd. Dessa två livsmedel är lättillgängliga och vanliga i den västerländska kosten samt kan innehålla samma källa till β-glukan, men skiljer sig på flera punkter i tillverkningsprocessen.

β-Glukanets kolesterolsänkande egenskaper påverkas primärt av löslighet och molekylviktsfördelning. Vid brödbakning kan jäsning och användning av mjöl med lågt falltal minska molekylvikten och öka lösligheten. Vid tillverkning av extruderade flingor bibehålls hög molekylvikt och lösligheten ökar. En av de möjliga mekanismer genom vilken β-glukan sänker kolesterolnivåer är ökad gallsyraproduktion i levern där kolesterol som antingen syntetiseras endogent eller tas från cirkulationen används.

Sex studier med flingor och fem studier med bröd eller muffins, kakor och bullar jämfördes. Åtta av de elva utvalda studierna rapporterade en signifikant minskning av total- eller LDL-kolesterol. Studierna på flingor hade i jämförelse med studierna på bröd mer likheter gällande dos av β-glukan och försökspersoner. Analysen av studierna tyder på att flingor med β-glukan ger en större kolesterolminskning än bröd på grund av fördelaktigare processeffekter på molekylvikt och lösligheten hos β-glukanet.

Nyckelord: havre, β-glucan, total-kolesterol, LDL-kolesterol, flingor, bröd

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1 Introduction

Increased total- and LDL-cholesterol concentrations in blood are risk factors related to cardiovascular disease, which is one of today's most common cause of mortality in Sweden (Risérus *et al.*, 2013). Therefore, a lifestyle that prevents high cholesterol-levels is promoted. A healthy diet is important and this includes a recommended intake of of 25-35 g dietary fibre each day (NNR, 2012).

Lowered cholesterol in response to consumption of rolled oats was observed already in 1963 (De Groot *et al.*, 1963). Since then and until today, further evidence of this connection has been disclosed. The polysaccharide β-glucan is proposed to be responsible for the effect. β-Glucan is a viscous soluble fibre present in the endosperm cell walls and of which the highest amounts are found in oats and barley (Åman & Bryngelsson, 2009).

This link between oat β-glucan and favourable cholesterol reductions opens up for the use of health claims on food products. In 2006, the European Food Safety Authority (EFSA) published a scientific opinion on health claims related to β-glucans and maintenance of normal blood cholesterol concentrations. EFSA stated that the majority of human intervention trials found significant reductions in LDL-cholesterol in response to a dose of at least 3 g β-glucan per day (EFSA, 2009), which is equal to 100 g rolled oats (Sonestedt, 2013). The U.S Food and Drug Administration (FDA) published a similar statement already in 1997 but included a requirement of 0.75 g β-glucan per portion in addition to the total daily dose of 3 g (FDA, 1997).

The cholesterol-lowering properties of β-glucan have been shown to vary based on the processing and use in products. Processing of cereals is a necessity for human consumption but some procedures have been reported to alter important characteristics of β-glucan, which resulted in a lacking effect on cholesterol levels (Andersson *et al.*, 2014). This information can be valuable in the manufacturing of new products and choice of processing, as well as in the use of health claims and for individuals who want to control their blood cholesterol concentrations.

1.1 Aim

The purpose of this literature review was to compare the cholesterol-lowering effect of oat β-glucan in ready-to-eat cereals and bread. Differences in β-glucan content and characteristics in the products due to processing in relation to cholesterol reduction were investigated.

1.2 Method

Information used in this literature review was collected from scientific articles, books and web data bases such as Scopus and Web of Science. Studies were delimitated to human trials with oat β-glucan incorporated to ready-to-eat cereals and bread or muffins, since these two products are easily accessible and common in the western diet. They can contain β-glucan of the same source but differ in several steps of manufacturing. Treatment diets with cookies or buns in addition to bread and muffins were included as well.

2 Background

2.1 Properties of β-glucan

The main underlying reason for the cholesterol-lowering effect of β-glucan is suggested to be viscosity. The viscosity is primarily influenced by solubility and molecular weight distribution (Åman & Bryngelsson, 2009). Information about these factors is therefore interesting in studies where the link between β-glucan and cholesterol is being examined.

Solubility can be defined as the property of a solid polysaccharide, either present in a solid food substance or not, to homogenously disperse in a liquid medium. The solubility is affected by factors such as temperature, digestive enzymes and pH. Conditions similar to the gut environment is preferred when the solubility of β-glucan is analysed (Wang & Ellis, 2014).

Dietary fibre and their components can be analysed by a variety of methods. β-Glucans differ in molecular structure, size and solubility and the outcome of analysis is often difficult to compare, especially when different analytical methods are used. Difference in β-glucan molecular weight have also been shown to vary between oats of different growing conditions (Andersson *et al.*, 2014). Some studies have as well reported variation in molecular weight between different varieties, while other have not observed any significant differences of this (Wood, 2001).

β-Glucan consist of linear chains of about 30 % (1→3)- and 70 % (1→4)-linked β-D-glucopyranosyl units. The molecular weight of oat β-glucan is about 1-3 × 10^6 g/mol. The (1→3)-linkages force the molecule to bend and prevent aggregation, which increase the molecule's solubility in water. Longer series of (1→4)-linkages on the other hand probably lower solubility due to formation of junctions (Andersson *et al.*, 2014). The extractability of β-glucan is a measure of the solubility (Wood, 2001). Oat β-glucan is 36-75 % extractable in hot water but might also be extracted by dilute acid or alkali. This can be more effective but increase degradation (Andersson *et al.*, 2014). Molecular weight, thickness of the cell and entanglement with other cell-wall components are examples of factors that also affect extractability (Wood, 2001).

2.2 Effects of processing

EFSA's opinion on the cholesterol-lowering effect of β-glucan is applicable on non-processed or minimally processed oat β-glucan (EFSA, 2009). However, the meaning of minimal processing is not defined, but the health claim generally remains relevant as long as the viscosity is maintained (Åman & Bryngelsson, 2009). Oat bran and oat bran concentrate is often used in products to achieve a high content of β-glucan. Oat bran is produced by sieving away starchy endosperm (Kulp, 2000). A high content of the subaleurone layer is thereby achieved and results in an oat bran that can contain as much as 10 % β-glucan. Oat bran concentrate is made by defatting and sieving. It can contain more than 20 % β-glucan. The solubility and molecular weight distribution of β-glucan remains rather unchanged by quick and dry processes, e.g. production of oat bran. The β-glucan in extruded breakfast cereals, porridge and muffins are also relatively unaffected. However, wet and longer processes, such as bread making, increase the degradation of the quite sensitive linear chains of β-glucan (Åman & Bryngelsson, 2009).

A decrease in molecular weight (MW) and an increase in the relative amount of short-chained β-glucan have been observed during the making of both soft and crisp breads (Andersson *et al.*, 2009). Fermentation of dough contributes to the breakdown but oven baking has no effect. Dough made of flour with high falling number retains MW of β-glucan to a greater extent. This suggests that endogenous enzymes play a role in the degradation (Andersson *et al.*, 2008). β-Glucanases are present in wheat flour, which often is added in the making of oat bread and muffins. These enzymes can increase the depolymerisation of β-glucan (Whitehead *et al.*, 2014). Tosh *et al*. (2008) observed a reduction in MW of β-glucan in muffins treated with β-glucanase. Average MW decreased from 2200 kDa to 400 kDa. Simultaneously, solubility of β-glucan increased from 44 % to 57 %. When MW was further reduced to 120 kDa, solubility went down to 26 %.

Extractability have been shown to increase during fermentation (Andersson *et al.*, 2008). A higher content of rye β-glucan in crisp bread than soft bread, but with a lower extractability, was observed by Rakha *et al*. (2010). In the crisp bread, fermentation and extrusion increased extractability whilst oven baking did not have an effect. In a study by Beer *et* al. (1997), extractability and MW of oat bran β-glucan in muffins was investigated. The baking decreased molecular weight but increased extractability. Various muffins recipes resulted as well in different extractability of β-glucan. Frozen storage of muffins decreased extractability but MW was retained (Beer *et al.*, 1997). Reduced solubility during storage in low temperatures with low water availability has been detected in other studies as well. This might be due to reorganisation of β-glucan chains and an increased structural arrangement (Wang & Ellis, 2014).

Commercial extruded ready-to-eat cereals retain a high MW of β-glucan and have possibly an increased bioactivity. This is because of disruption of the cell wall and dispersion of β-glucan during the process (Tosh *et al.*, 2010). Tosh *et al.* (2010) observed maintained high MW and increased solubility, from 39 % to 67 %, in an extruded cereal. A high viscosity was thereby achieved and a greater cholesterol-lowering effect than of the original bran product was expected.

2.3 Cholesterol and lipoproteins

Cholesterol is a fat soluble alcohol, a sterol. It consists of a 17 carbon sterol structure with a hydrocarbon chain, two methyl groups and is sometimes esterified with a fatty acid. The cholesterol is used for production of steroid hormones, membranes, vitamin D and bile acids. Cholesterol metabolism is carefully controlled in healthy individuals. Large amounts of cholesterol in the diet lower endogenous production and small amounts increase it (Becker, 2013).

Lipids are transported in the blood as lipoproteins. These consist of an amphiphilic outer layer of apolipoproteins and phospholipids, and a water insoluble centre of triglycerides and esters of cholesterol. Two of the main groups of lipoproteins are low density lipoprotein (LDL) and high density lipoprotein (HDL). The higher the density of the lipoprotein, the more protein and less triglyceride content. LDL has about 45 % content of cholesterol and provides cholesterol to cells when the endogenously synthesized amount is not enough. Residual cholesterol is transported in the form of HDL to the liver, which is the only organ that can discard cholesterol. HDL consist of about 22 % cholesterol and apart from transport to the liver, it carries proteins and excess breakdown material (Becker, 2013).

LDL-cholesterol increase accumulation of fat-laden macrophages in the wall of veins and is therefore commonly called “bad” cholesterol. HDL-cholesterol, as well as a small ratio of total cholesterol and HDL-cholesterol, have on the other hand been shown to decrease the risk of cardiovascular disease and is called the “good” cholesterol (Becker, 2013). Individuals with cardiovascular disease or other risk factors are recommended a total cholesterol level of 5.0 mmol/l or lower. The LDL-cholesterol level should be less than 3.0 mmol/l and HDL-cholesterol more than 1.0 mmol/l (Touminen, 2015).

LDL-cholesterol and HDL-cholesterol levels are affected by several factors. A diet with a lot of saturated fat increase LDL-cholesterol in plasma. Premenopausal women generally have higher HDL-cholesterol levels than men (Becker, 2013).

Smoking and large amounts of alcohol have a negative effect while physical activity has a positive impact. Type 2-diabetics often have high levels of triglycerides and low levels of HDL-cholesterol (Touminen, 2015).

2.4 Cholesterol lowering mechanism

The mechanism by which β-glucan lowers serum cholesterol is not completely established. In a review by Gunness and Gidley (2010) three possible modes of actions are summarised. It is suggested that soluble fibre bind to bile acids and increase their excretion by inhibiting re-absorption. This results in an increased hepatic bile acid production from cholesterol either endogenously synthesized or taken from the circulation (Ellegård & Andersson, 2007; Lia *et al.*, 1997). Microbiotic fermentation of β-glucan in the colon might also have an impact. The fermentation results in short-chained fatty acids (SCFAs). One of probably many effects of SCFAs, mainly propionate, is inhibition of HMG-CoA reductase. This is the rate-controlling enzyme of cholesterol synthesis (Levrat *et al.*, 1994). However, studies in this area have shown varying results (Gunness & Gidley, 2010). Another possible mode of action is lowered glycaemic response by the delay of gastric emptying, influence on nutrient absorption in the small intestine and reduced insulin levels (Sonestedt, 2013). Insulin can contribute to lowered cholesterol by also inhibiting HMG-CoA reductase. Since insulin levels are affected by several factors, it is difficult to differentiate its effects with the influence of β-glucan viscosity and above mentioned mechanisms (Gunness & Gidley, 2010).

3 Studies

3.1 Cereals

Six studies on the cholesterol lowering effect of oat β-glucan incorporated to ready-to-eat cereals were selected for comparison and are presented in Table 1. Characteristics of the study, product with β-glucan and any significant change in blood lipid concentrations are featured in the table.

In a study by Lovegrove *et al*. (2007), 31 women and 31 men consumed 3.0 g of β-glucan from oat bran concentrate (OBC) per day in oat bran cereal for 8-weeks. The trial was finished by a 4-week follow up period. Mean age of study objects was 56.6 years and mean baseline body mass index (BMI) 26.0. In the beginning of the trial, TC and LDL-C levels in the β-glucan group were 6.5 and 4.3 mmol/l, respectively, and 6.4 and 4.3 for the control group. The control group consumed a wheat cereal product. No significant change in fasting plasma cholesterol or LDL-C between the two groups was observed. HDL-C levels were lowered.

RTE oat flakes with 1.45 g of β-glucan per day were consumed in a study by Charlton *et al*. (2012). This was one of three diet arms that lasted for 6 weeks and 26 subjects completed the β-glucan treatment. The control group consisted of 31 subjects and received cornflakes. Mean age was 51 years and mean BMI 27.3. At baseline, TC and LDL-C levels in the β-glucan group were 6.12 and 3.84 mmol/l, respectively, and 6.03 and 3.86 for the control group. The peak molecular weight of the β-glucan was 760 kDa and the solubility (of total β-glucan) was 90.62 %. Compared to control, the β-glucan group lowered TC by 3.8% and LDL-C by 8.5 %.

Wolever *et al*. (2010) studied the effect of oat β-glucan of different molecular weights incorporated to extruded RTE cereal. During 4 weeks, subjects were assigned to one of five treatments; 3 g β-glucan/day of high-MW (2210 kDa, n=86, y=52), 4 g β-glucan/day of medium-MW (850 kDa, n=67, y=52), 3 g β-glucan/day of medium-MW (530 kDa, n=64, y=52), 4 g β-glucan/day of low-MW (210 kDa, n=63, y=53) or control (wheat cereal, n=87, y=52). Products of lower MW was made by increased temperature and shear and decreased moisture content during extrusion. Mean baseline BMI was 27.4. Initially, subjects had TC levels between 5.0 and 8.0 mmol/l, and LDL-C levels between 3.0 and 5.0 mmol/l. After treatment and compared to the control group, all β-glucan cereals, except 4 g low-MW, significantly lowered LDL-C. The 3 g high-MW reduced TC as well.

In a study by Johnston *et al.* (1998), 40 women and 22 men consumed 2.9 g oat soluble fibre per day through whole grain oat RTE cereal, Cheerios, for 6 weeks. A 6-week pre-treatment period was completed before the intervention. The control group consisted of 62 people that consumed cornflakes. Mean age of subjects was 57 years. Subjects taking medications such as androgens and lipid lowering drugs were not included in the study. Subjects taking e.g. oestrogen and beta blockers that possibly affect lipid levels were accepted if doses were stable. In the beginning of the trial, TC and LDL-C levels in the β-glucan group were 6.19 and 4.10 mmol/l, respectively, and 6.14 and 4.15 for the control group. Compared to the control group, TC was lowered by 3.8 % and LDL-C by 4.2 % in the β-glucan group.

In a 6-week study by Karmally *et al*. (2005), 73 Hispanic Americans consumed 3.0 g oat soluble fibre per day through RTE oat cereal, Cheerios. The control group consisted of 79 subjects that consumed corn cereal. Both groups completed a 5-week diet run in period before the intervention. Mean age was 49 years and mean baseline BMI 29.3. Initially, TC and LDL-C levels in the β-glucan group were 5.41 and 3.67 mmol/l, respectively, and 5.16 and 3.45 for the control group. The β-glucan group lowered TC by 4.5 % and LDL-C by 5.3 % compared to baseline. No significant changes were observed in the control group.

In a 12-week study by Maki *et al*. (2010), 58 women and 19 men consumed a whole-grain RTE cereal, providing 3.0 g β-glucan per day, as part of a dietary program for weight loss. Diet recommendations were set to aim for an energy deficit of about 500 kcal/day, for a weight loss of around 0.5 kg/week. The control group consisted of 67 subjects and received a low fibre diet with e.g. corn cereal and white toast. The mean age was 49 years and mean BMI 32.0. At baseline, TC and LDL-C levels in the β-glucan group were 6.0 and 4.0 mmol/l, respectively, and 5.9 and 4.0 for the control group. No difference in weight loss was observed between the groups, but the waist circumference in the β-glucan group decreased more. Compared to baseline, TC was lowered by 5.4 % and LDL-C by 8.7 % in the β-glucan group.

Table 1. Characteristics of studies with cereal products.

Study	Product (source of β-glucan)	Dose of β-glucan (g/d)	MW of β-glucan (kDa)	Study design	Subjects [1]	Change in blood lipids (%)
Lovegrove *et al*. (2007)	Oat bran cereal (OBC[3])	3.0		8-week double-blind, controlled, randomized parallel trial	n=62 y=56.6	HDL -7.6
Charlton *et al*. (2012)	Oats low: Extruded RTE[4] oat flakes	1.45	760	6-week parallel, randomized, controlled, single-blind trial	n=57 y=51	TC -7.2 LDL-C -8.5
Wolever *et al*. (2010)	Extruded RTE[4] cereal[5] (oat bran)	3H: 3.0 4M: 4.0 3M: 3.0 4L: 4.0	3H: 2210 4M: 850 3M: 530 4L: 210	4-week randomized, controlled, double-blind, parallel trial	3H: n=86, y=52 4M: n=67, y=52 3M: n=64, y=52 4L: n=63, y=53 W[6]: n=87, y=52	3H: LDL-C -5.5 TC -3.9 4M: LDL-C -6.5 3M: LDL-C -4.7 4L: LDL-C -2.3[7]
Johnston *et al*. (1998)	RTE[4] oat cereal (whole grain oat flour)	2.9[2]		6-week parallel, randomized, controlled trial	n=124 y=57	TC -3.8 LDL-C -4.2
Karmally *et al*. (2005)	RTE[4] oat cereal (whole grain oat flour)	3.0[2]		6-week, randomized, controlled trial	n=152 y=49.0	TC -4.5 LDL-C -5.3
Maki *et al*. (2010)	RTE[4] oat cereal (whole grain oat flour)	3.0		12-week randomized, parallel-arm, controlled trial	n=144 y=48.9	TC -5.4 LDL-C -8.7

[1] n: number of subjects receiving treatment or control, y: mean age, [2] soluble fibre (g/d), [3] OBC: oat bran concentrate, [4] RTE: ready-to-eat, [5] Four cereal products made during different extrusion conditions giving high (H), medium (M) or low (L) molecular weight, [6] Control group consuming wheat cereal, [7] not statistically significant

3.2 Bread

Five studies on the cholesterol lowering effect of oat β-glucan incorporated to bread, muffins and cookies were selected for comparison and are presented in Table 2. Characteristics of the study, the product with β-glucan and any significant change in blood lipid concentrations are featured in the table.

In a 4-week study by Robitaille *et al.* (2005), 18 premenopausal women consumed oat bran muffins giving a total 2.31 g β-glucan each day. Muffins were prepared by the study objects at home. Eggs, oil and water was supposed to be added to the mix of pre-packaged dry ingredients. The control group consisted of 16 premenopausal women receiving no supplement. A 2-week run-in period was completed before the treatment. Mean age was 38.3 years and mean baseline BMI 28.8. Initially, TC and LDL-C levels in the β-glucan group were 4.79 and 2.76 mmol/l, respectively, and 4.80 and 2.75 for the control group. Compared to the control group, HDL-C levels increased by 11.2 %. No significant change was observed for TC and LDL-C levels.

The effect of β-glucan incorporated in bread and cookies was investigated in study 1 by Kerckhoffs *et al*. (2003). During the 4-weeks of treatment, which was done after a 3-week run-in period, 25 subjects consumed the β-glucan products and 23 subjects got control bread and cookies with wheat fibre. Mean age was 51.5 years and mean baseline BMI 25.6. After baking, cookies and bread were frozen. Bread that had been frozen had a molecular weight distribution of 15 % > 1000 kDa, 30 % 250-1000 kDa and 55 % <250 kDa. Cookies that had been frozen had a molecular weight distribution of 40 % > 1000 kDa, 45 % 250-1000 kDa and 15 % <250 kDa. At baseline, TC and LDL-C levels in the β-glucan group were 5.98 and 3.96 mmol/l, respectively, and 6.00 and 4.09 for the control group. Out of the average consumption of 5.9 g β-glucan per day, 5.1 g came from bread and 0.8 g from cookies. After treatment, no significant difference was observed between the two groups.

The impact of oat soluble fibre in bread, buns and muffins was studied by Pick *et al*. (1996). The 24-week crossover study consisted of two 12-week periods, without any additional time in-between. Study subjects were 8 men with a mean age of 45 years and mean baseline BMI of 27.6, type 2-diabetes and initial TC levels were less than 7 mmol/l. Four subjects consumed the oat soluble fibre products for the first period, while the other four consumed the control white bread. At the second period diets switched. TC and LDL-C levels resulted in a 14 % and 23 % decrease, respectively, after the oat soluble fibre period in comparison to the white bread period.

In a study by Liatis *et al.* (2010), 12 men and 11 women with type 2-diabetes consumed bread enriched with 3.0 g β-glucan from OBC per day for 3 weeks. The control group consisted of 18 subjects who received a similar wheat bread. Mean age was 63 and mean baseline BMI 29.6. In the beginning of the trial, TC and LDL-C levels in the β-glucan group were 6.25 and 4.18 mmol/l, respectively, and 5.88 and 4.06 for the control group. After treatment and compared to the control group, TC and LDL-C levels decreased by 5 and 10 %, respectively, in the β-glucan group.

Kestin *et al*. (2012) compared the effect of oat bran to rice bran and wheat bran in a cross-over trial. Oat soluble fibre, 5.8 g each day, was provided through bread and muffins. Study subjects were 24 men with a mean age of 46 years and a mean baseline BMI of 25.4. In the beginning of the trial, mean TC and LDL-C levels were 6.34 and 4.55 mmol/l, respectively. After an initial 3-week low-fibre diet, subjects were assigned to one of the three fibre diets for 4 weeks each in a crosso-ver design. The oat diet significantly lowered TC by 5.6 % compared to the wheat diet and by 3.8 % compared to the rice diet. LDL-C was lowered by 6.6 % compared to the wheat diet and by 5.5 % compared to the rice diet.

Table 2. Characteristics of studies with bread, muffins and cookies.

Study	Product (source of β-glucan)	Dose of β-glucan (g/d)	MW of β-glucan (kDa)	Study design	Subjects [1]	Change in blood lipids (%)
Robitaille *et al*. (2005)	Muffins (oat bran)	2.31		4 week randomized, controlled trial	n=34 y=38.3 Only women	HDL-C +11.2
Kerckhoffs *et al*. (2003)	Study 1: Bread, cookies (oat bran, OBC[3])	5.9	Frozen bread: 15 % >1000 30 % 250-1000 55 %<250 Frozen cookie: 40 % >1000 45 % 250-1000 15 %<250	4-week parallel randomized, controlled trial	n=48 y=51.5	No significant change
Pick *et al*. (1996)	Bread, buns, muffins (OBC[3])	9.0[2]		12-week cross-over	n=8 y=45.5 Type 2-diabetics Only men	TC -14 LDL-C -23
Liatis *et al*. (2010)	Bread (OBC[3])	3.0		3-week randomized, controlled, double-blind	n=41 y=63 Type 2-diabetics	TC -5 LDL-C -10
Kestin *et al*. (2012)	Bread, muffins (oat bran)	5.8[2]		4-week double blind, crossover trial	n=24 y=46.0 Only men	TC -5.6 LDL-C -6.6

[1] n: number of subjects receiving treatment or control, y: mean age, [2] soluble fibre, [3] OBC: oat bran concentrate

4 Discussion

Eight out of the selected eleven studies reported a significant decrease in TC and or LDL-C. In terms of dose of β-glucan and study subjects, the studies with RTE cereals had more similarities and achieved more consistent results compared to the studies with bread. Possible reasons for the observed differences are discussed in the following section.

4.1 No significant effect of treatment

Lovegrove *et al*. (2007), Kerckhoffs *et al*. (2003) and Robitaille *et al*. (2005) did not achieve any significant change in neither TC nor LDL-C after treatment. β-Glucan in the three studies was consumed through different products (oat bran cereal, bread and cookies, and muffins) and of different doses (3.0 g/d, 5.9 g/d and 2.31 g/d). Study design was similar in all three cases. Several factors can contribute to the absent reduction in cholesterol. The β-glucan was possibly not soluble enough, or did not disperse in the intestine. The dose and molecular weight were perhaps too low, or negatively affected by processing and storage (Wolever *et al.*, 2010). It has also been suggested that a lack of effect is connected to the frequency of ingestion of beta-glucan. Repeated intake of beta-glucan during the day possibly increase hepatic bile acid production in greater occurrence than a single larger intake (Othman *et al.*, 2011).

Detailed information about the products and β-glucan used in these three studies are lacking. Only Kerckhoffs *et al*. (2003) reported molecular weight of β-glucan. In bread, 55 % was less than 250 kDa, which contributed with the highest total consumed amount, and 45 % between 250-1000 kDa in cookies. In a separate part of the study (study 2), the effects of β-glucan incorporated to a drink was examined and unlike study 1, a decrease in LDL-C was observed. The source of β-glucan was the same in both studies, and the molecular weight distribution was similar. Therefore, these two factors are suggested to not be the reason for lack of effect. Instead is the baking of bread believed to be the cause, since studies on β-glucan in bread have observed varying results of cholesterol reduction (Kerckhoffs *et al.*, 2003). No information about the ingredients in the bread is provided in the report but if yeast and wheat flour was mixed into the dough, the outcome of the study was probably affected. During the time of fermentation, MW decreases and solubility increases, due to the activity of β-glucanases from flour (Andersson *et al.*, 2008). Tosh *et al.* (2008) found a drop in solubility in response to MW lower than 400 kDa. Since the β-glucan MW distribution in bread in Kerckhoffs *et al*. (2003) by 55 % was constituted of ≤ 250 kDa, it is plausible that solubility was also decreased. The cookies and bread had been frozen before consumption, which as well have been reported to reduce solubility of β-glucan (Wang & Ellis, 2014). Possibly, the aggregative effect is both lowered MW and solubility to the extent of loss of cholesterol reducing properties of β-glucan.

In the study by Robitaille *et al*. (2005), muffins were prepared by the study objects at home according to instructions. The control group did not receive any control product, while control groups in the other studies consumed a corresponding wheat product. These two factors decrease the level of control over the subjects' actions and have possibly contributed to the outcome. Subjects were only women and had considerable lower baseline TC and LDL-C values compared to the previous two studies, which might have had an effect. However, EFSA's scientific opinion concerns adults with normal as well as mildly elevated blood cholesterol (EFSA, 2009).

A change could also have been undetected due to major variability in TC and LDL-C concentrations after treatment (Robitaille *et al.*, 2005). Robitaille *et al.* (2005) reported an increase in HDL-C, while Lovegrove *et al*. (2007) measured a decrease. Robitaille *et al*. (2005) suggests that the observed increase in their study was enhanced due to the low-dietary fat run-in period, which decreased HDL-C concentrations before the following oat bran-diet. Lovegrove *et al*. (2007) point out that the observed undesired decrease in HDL-C is statistically significant, although this reduction is quantitatively smaller than that of TC and LDL-C. It is further discussed that the lack of response could possibly be explained by a too low dosage of β-glucan or too few subjects for statistical significance. However, the viscosity, which was slightly more than six times higher than in the control, is not believed to be the cause.

4.2 Molecular weight and effects of processing

Beside Kerckhoffs *et al*. (2003), two of the selected studies, Charlton *et al.* (2012) and Wolever *et al.* (2010), contained information about MW of the β-glucan. The extruded RTE oat flakes in the study by Charlton *et al.* (2012) provided 1.45 g β-glucan/day, with a 90.6 % solubility and MW of 760 kDa. This was in terms of

MW most similar to extruded RTE cereal 4M (850 kDa), and in regard to solubility most similar to 3M (93.3 %), in the study by Wolever *et al.* (2010). Compared to these two and despite the low dose of 1.45 g β-glucan/day, a greater reduction in LDL-C was observed by Charlton *et al.* (2012). Both studies discuss that $M_p \times C$ (molecular weight of β-glucan in solution and daily dose of soluble β-glucan) is a favourable measure of β-glucan bioactivity and it is suggested that as the $M_p \times C$ increases, so does viscosity (Wolever *et al.*, 2010). Both 3M and 4M have a higher $M_p \times C$, 1500 and 2700, respectively, than the oat flakes in Charlton *et al.* (2012) which has a value of 1000. Thus, more factors presumably affected the outcome and contributed to the considerable decrease in LDL-C. The two studies had similar design, but Charlton *et al.* (2012) had a two-week longer treatment period and a background diet with a low fat intake.

Regarding the effect of processing in both cereals and bread when examining earlier published literature, extruded RTE cereals with β-glucan often seem proposed to be more effective in lowering cholesterol, or maintaining normal cholesterol concentrations, in comparison with bread (Andersson *et al.*, 2014). The retained MW and even dispersion of β-glucan in that type of cereal product (Tosh *et al.*, 2010), without any need for fermentation and mixing with wheat flour for longer periods of time as in bread, is possibly the key to success in this case. However, comparison of these factors and effects in bread and cereals are difficult since only eleven studies were selected for comparison in this literature review. The majority lack information about viscosity and for those that do provide it, is it important to remember that differences in MW and solubility can vary between different methods of analysis and conditions during measurement (Andersson *et al.*, 2014).

4.3 Significant effect of treatment

The greatest reduction in TC and LDL-C of the selected studies was reported by Pick *et al.* (1996) where 9.0 g/d of soluble fibre, with β-glucan originating from OBC provided through bread, buns and muffins, was the highest dose used in all studies. Study subjects were men with type 2-diabetes. Two studies were similar to this study, regarding the diabetic subjects in Liatis *et al.* (2010) and in terms of being a cross-over with male subjects and a high soluble fibre content in Kestin *et al.* (2012). Although significant changes in cholesterol levels in these two studies, the decrease was two or three times larger in Pick *et al.* (1996). The high dose of soluble fibre and long treatment period are presumably the underlying causes to this. However, conflicting conclusions about the importance of these factors have been reported. A 6-week study investigating the blood cholesterol-lowering effect of oatmeal and oat bran detected a dose-dependent decrease (Davidson *et al.*, 1991). On the contrary, a meta-analysis of 28 randomized clinical trials found no significance of neither duration nor dose, when over 2 weeks and 3 g β-glucan/day. This meta-analysis included both above mentioned studies with diabetics, as well as one other, and concluded that the cholesterol-lowering effect is greater in diabetic subjects. This effect is purposed to be linked to an insulin-dependent mechanism (Whitehead *et al.,* 2014).

Maki *et al*. (2010), Karmally *et al*. (2005) and Johnston *et al*. (1998) all used the commercially available RTE oat cereal Cheerios as intervention product. A very similar change in blood cholesterol concentration was observed in the three studies. Maki *et al*. (2010) reported the greatest decrease, which possibly is explained by the intentional weight loss and the twice as long treatment period compared to the other two studies. Weight loss have earlier been associated with reduced blood lipids and lipoproteins in overweight individuals (Dattilo & Kris-Etherton, 1992). All studies included in this review, except for Johnston *et al*. (1998), reported the mean BMI of subjects to be over 25, which is considered to be the limit of defining as overweight (Risérus *et al.*, 2013). Amongst the six studies on β-glucan incorporated in RTE cereals, body weight was maintained stable during the treatment period in order to not affect cholesterol concentrations other than by the assigned intervention product. In contrast to this, Maki *et al*. (2010) aimed for weight loss. The treatment and control group in this study achieved similar weight loss. However, a greater decrease in waist circumference was observed in the treatment group, which is linked to lowered risk for metabolic complications (Wirfält & Andersson, 2013). This positive outcome is possibly in response to the higher dose of β-glucan, which was the only factor differing between the two groups.

4.4 Health claims

Increasing total- and LDL-cholesterol levels are risk factors for cardiovascular disease. An approved health claim for a food product can, apart from being favourable for the manufacturer, increase the costumers' awareness. Since EFSA's scientific opinion do not specify the meaning of non-processed or minimally processed oat β-glucan, studies with seemingly similar products report quite varying results. As shown in Table 1 and Table 2, the required dose of 3.0 g β-glucan per day do not per se imply a favourable outcome. Increased knowledge of the cholesterol lowering mechanism and effects of processing oat β-glucan could possibly adapt the use of the health claim and result in a more effective control over blood cholesterol concentrations. However, it is probably possible for the public to achieve that positive effect at todays 3.0 g limit anyways, considering the wide range of products that can be enriched with β-glucan in the form of either rolled oats, oat bran or oat bran concentrate.

4.5 Conclusion

Along the lines with earlier observations, consumption of RTE cereals with oat β-glucan seem to result in a greater cholesterol reduction than of bread, due to the more beneficial effects of processing on molecular weight and solubility of the β-glucan. To increase the certainty of this conclusion, more studies that include information about the properties of the β-glucan would be desirable.

References

Andersson, A., Rüegg, N. & Åman, P. (2008). Molecular weight distribution and content of water-extractable β-glucan in rye crisp bread. *Journal of cereal science,* 47(3), pp. 399-406.
Andersson, A.A.M., Rakha, A., Andersson, R. & Åman, P. (2014). Rye Dietary Fiber. In: Poutanen, K. & Åman, P. (eds) *Rye and Health* AACC International, Inc. , pp. 27-28.
Andersson, R., Fransson, G., Tietjen, M. & Åman, P. (2009). Content and molecular-weight distribution of dietary fiber components in whole-grain rye flour and bread. *Journal of Agricultural and Food Chemistry,* 57(5), pp. 2004-2008.
Becker, W. (2013). Fetter. In: Abrahamsson, L., Andersson., A., Nilsson, G. (ed. *Näringslära för högskolan*6). Stockholm: Liber AB, pp. 61-75.
Beer, M.U., Wood, P.J., Weisz, J. & Fillion, N. (1997). Effect of cooking and storage on the amount and molecular weight of (1→ 3)(1→ 4)-β-d-glucan extracted from oat products by an in vitro digestion system 1. *Cereal Chemistry,* 74(6), pp. 705-709.
Charlton, K.E., Tapsell, L.C., Batterham, M.J., O'Shea, J., Thorne, R., Beck, E. & Tosh, S.M. (2012). Effect of 6 weeks' consumption of beta-glucan-rich oat products on cholesterol levels in mildly hypercholesterolaemic overweight adults. *British Journal of Nutrition,* 107(7), pp. 1037-1047.
Dattilo, A.M. & Kris-Etherton, P. (1992). Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. *American Journal of Clinical Nutrition,* 56(2), pp. 320-328.
Davidson, M.H., Dugan, L.D., Burns, J.H., Bova, J., Story, K. & Drennan, K.B. (1991). The hypocholesterolemic effects of β-glucan in oatmeal and oat bran: a dose-controlled study. *Jama,* 265(14), pp. 1833-1839.
De Groot, A., Luyken, R. & Pikaar, N. (1963). Cholesterol-lowering effect of rolled oats. *The Lancet,* 282(7302), pp. 303-304.
EFSA (2009). *Scientific Opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823) pursuant to Article 13(1) of Regulation (EC) No 1924/2006 on request from the European Commission*.
Ellegård, L. & Andersson, H. (2007). Oat bran rapidly increases bile acid excretion and bile acid synthesis: an ileostomy study. *European Journal of Clinical Nutrition,* 61(8), pp. 938-945.
FDA (1997). Health claims: oats and coronary disease. *Fed Reg,* 62, pp. 3583-3601.
Gunness, P. & Gidley, M.J. (2010). Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. *Food & Function,* 1(2), pp. 149-155.
Johnston, L., Reynolds, H.R., Patz, M., Hunninghake, D., Schultz, K. & Westereng, B. (1998). Cholesterol-lowering benefits of a whole grain oat ready-to-eat cereal. *Nutr Clin Care,* 1(1), pp. 6-12.
Karmally, W., Montez, M.G., Palmas, W., Martinez, W., Branstetter, A., Ramakrishnan, R., Holleran, S.F., Haffner, S.M. & Ginsberg, H.N. (2005). Cholesterol-lowering benefits of oat-containing cereal in Hispanic Americans. *Journal of the American Dietetic Association,* 105(6), pp. 967-970.

Kerckhoffs, D.A., Hornstra, G. & Mensink, R.P. (2003). Cholesterol-lowering effect of β-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. *Am J Clin Nutr,* 78(2), pp. 221-227.

Kestin, M., Moss, R., Clifton, P.M. & Nestel, P.J. (1990). Comparative effects of three cereal brans on plasma lipids, blood pressure, and glucose metabolism in mildly hypercholesterolemic men. *Am J Clin Nutr,* 52(4), pp. 661-666.

Kulp, K. (2000). *Handbook of Cereal Science and Technology, Second Edition, Revised and Expanded*: Taylor & Francis. Available from: https://books.google.se/books?id=gtqEWcA73BEC.

Levrat, M.-A., Favier, M.-L., Moundras, C., Rémésy, C., Demigné, C. & Morand, C. (1994). Role of dietary propionic acid and bile acid excretion in the hypocholesterolemic effects of oligosaccharides in rats. *Journal of Nutrition,* 124(4), pp. 531-538.

Lia, A., Andersson, H., Mekki, N., Juhel, C., Senft, M. & Lairon, D. (1997). Postprandial lipemia in relation to sterol and fat excretion in ileostomy subjects given oat-bran and wheat test meals. *Am J Clin Nutr,* 66(2), pp. 357-365.

Liatis, S., Tsapogas, P., Chala, E., Dimosthenopoulos, C., Kyriakopoulos, K., Kapantais, E. & Katsilambros, N. (2009). The consumption of bread enriched with betaglucan reduces LDL-cholesterol and improves insulin resistance in patients with type2 diabetes. *Diabetes & Metabolism,* 35(2), pp. 115-120.

Lovegrove, J.A., Clohessy, A., Milon, H. & Williams, C.M. (2000). Modest doses of β-glucan do not reduce concentrations of potentially atherogenic lipoproteins. *Am J Clin Nutr,* 72(1), pp. 49-55.

Maki, K.C., Beiseigel, J.M., Jonnalagadda, S.S., Gugger, C.K., Reeves, M.S., Farmer, M.V., Kaden, V.N. & Rains, T.M. (2010). Whole-grain ready-to-eat oat cereal, as part of a dietary program for weight loss, reduces low-density lipoprotein cholesterol in adults with overweight and obesity more than a dietary program including low-fiber control foods. *Journal of the American Dietetic Association,* 110(2), pp. 205-214.

NNR (2012). *Nordic Nutrition Recommendations 2012: Integrating Nutrition and Physical Activity*.

Othman, R.A., Moghadasian, M.H. & Jones, P.J. (2011). Cholesterol-lowering effects of oat β-glucan. *Nutrition reviews,* 69(6), pp. 299-309.

Pick, M.E., Hawrysh, Z.J., Gee, M.I., Toth, E., Garg, M.L. & Hardin, R.T. (1996). Oat bran concentrate bread products improve long-term control of diabetes: a pilot study. *Journal of the American Dietetic Association,* 96(12), pp. 1254-1261.

Rakha, A., Åman, P. & Andersson, R. (2010). Characterisation of dietary fibre components in rye products. *Food Chemistry,* 119(3), pp. 859-867.

Risérus, U., Andersson, A., Hambraeus, L. & Johansson, I. (2013). Kost och hälsa - kostrelaterade symtom och sjukdomar. In: Abrahamsson, L., Andersson., A., Nilsson, G. (ed. *Näringslära för högskolan*6). Stockholm: Liber AB, pp. 319-325.

Robitaille, J., Fontaine-Bisson, B., Couture, P., Tchernof, A. & Vohl, M.-C. (2005). Effect of an oat bran-rich supplement on the metabolic profile of overweight premenopausal women. *Annals of Nutrition and Metabolism,* 49(3), pp. 141-148.

Sonestedt, E. (2013). Kolhydrater. In: Abrahamsson, L., Andersson., A., Nilsson, G. (ed. *Näringslära för högskolan*6). Stockholm: Liber AB, p. 48.

Tosh, S.M., Brummer, Y., Miller, S.S., Regand, A., Defelice, C., Duss, R., Wolever, T.M. & Wood, P.J. (2010). Processing affects the physicochemical properties of β-glucan in oat bran cereal. *Journal of Agricultural and Food Chemistry,* 58(13), pp. 7723-7730.

Tosh, S.M., Brummer, Y., Wolever, T.M. & Wood, P.J. (2008). Glycemic response to oat bran muffins treated to vary molecular weight of β-glucan. *Cereal Chemistry,* 85(2), pp. 211-217.

Touminen, P. (2015). *Höga blodfetter*. http://www.1177.se/Uppsala-lan/Fakta-och-rad/Sjukdomar/Hoga-blodfetter/?ar=True.

Wang, Q. & Ellis, P.R. (2014). Oat β-glucan: physico-chemical characteristics in relation to its blood-glucose and cholesterol-lowering properties. *British Journal of Nutrition,* 112(S2), pp. S4-S13.

Whitehead, A., Beck, E.J., Tosh, S. & Wolever, T.M. (2014). Cholesterol-lowering effects of oat β-glucan: a meta-analysis of randomized controlled trials. *Am J Clin Nutr,* 100(6), pp. 1413-1421.

Wirfält, E. & Andersson, A. (2013). Nutritionsundersökningar. In: Abrahamsson, L., Andersson., A., Nilsson, G. (ed. *Näringslära för högskolan*6). Stockholm: Liber AB, p. 355.

Wolever, T.M., Tosh, S.M., Gibbs, A.L., Brand-Miller, J., Duncan, A.M., Hart, V., Lamarche, B., Thomson, B.A., Duss, R. & Wood, P.J. (2010). Physicochemical properties of oat beta-glucan influence its ability to reduce serum LDL cholesterol in humans: a randomized clinical trial. *Am J Clin Nutr,* 92(4), pp. 723-32.

Wood, P.J. (2001). Cereal β-Glucans: Structure, Properties and Health Claims. In: McCleary, B.V. & Prosky, L. (eds) *Advanced Dietary Fibre Technology* Blackwell Science Ltd, pp. 317-320.

Åman, P. & Bryngelsson, S. (2009). Grönt ljus för beta-glukan - men vad är "minimal processing"? *Nordisk nutrition,* 2(4), pp. 30-31.