DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

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**Food Chemistry and Food Development**
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Food Chemistry and Food Development
Department of Life Technologies
University of Turku, Finland

Supervised by
Professor Kaisa Linderborg, Ph.D.
Department of Life Technologies
University of Turku
Turku, Finland

Postdoctoral researcher
Lotta Nylund, Ph.D.
Department of Life Technologies
University of Turku
Turku, Finland

Professor Baoru Yang, Ph.D.
Department of Life Technologies
University of Turku
Turku, Finland

Reviewed by
Professor emeritus Hannu Salovaara, Ph.D.
Department of Food and Environmental Sciences
University of Helsinki
Helsinki, Finland

Adjunct Professor Sijo Joseph Thandapilly, Ph.D.
Department of Human Nutritional Sciences
University of Manitoba
Winnipeg, Canada

Opponent
Senior Lecturer Lieselotte Cloetens, Ph.D.
Department of Pure and Applied Biochemistry
Lund University
Lund, Sweden

Research director
Professor Baoru Yang, Ph.D.
Department of Life Technologies
University of Turku
Turku, Finland



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*To those suffering from gut symptoms*

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# ABSTRACT

Recently, the nutritional advantages of oats have been acknowledged worldwide and several novel oat-based products have entered the market. Health claims on the cholesterol- and postprandial glycemia-lowering effects of oat β-glucan have been accepted in the EU, and oats are known to contain antioxidative phenolic compounds, such as avenanthramides. However, the development of novel moisture-rich oat products, such as drinks and puddings, has required processing techniques that aim for reduced viscosity. There are indications that the reduced viscosity and lowered molecular weight (MW) affect the health-promoting properties of β-glucan, but this is not acknowledged by the current health claims. Moreover, the effects of oat consumption on gut well-being and microbiota in individuals with gastrointestinal disorders associated with an unbalanced microbiota, such as those with celiac disease (CeD), non-celiac gluten sensitivity (NCGS) and irritable bowel syndrome (IBS), have not been substantiated.

In this thesis, in Study I, the fecal gut microbiota profiles and the production of selected microbial metabolites as well as food diaries and perceived gut well-being of oat-consuming healthy adult subjects (n = 14) and adult subjects with CeD (n = 19) or NCGS (n = 10) were evaluated. No microbiota dysbiosis was detected in the subjects with CeD or NCGS. However, the healthy subjects had a higher abundance of bifidobacteria compared to CeD and NCGS subjects and a higher proportion of fecal acetate compared to NCGS subjects. Yet, the subjects with NCGS perceived more gut symptoms and received more energy from fat and less from carbohydrates than healthy and CeD subjects.

In Study II, the effect of processing with enzyme treatment on the β-glucan behavior in the gastrointestinal tract was examined in healthy subjects (n = 14). The subjects consumed three meals containing either high, medium or low MW β-glucan, and the fecal excretion of bile acids, urine excretion of phenolic compounds and gastrointestinal pressure with an ingestible capsule were followed. It was observed that the high MW β-glucan induced the highest excretion of fecal bile acids and the highest pressure in the duodenum, while the low MW β-glucan resulted in the highest urine excretion of phenolic compounds. The medium MW β-glucan induced excretion of fecal bile acids and urine phenolic compounds, which resembled that of high MW β-glucan but resulted in a lower pressure in the duodenum similar to the low MW β-glucan. The perceived gut well-being was not linked to the MW of β-glucan but did differ between the sexes.

In Study III, the effect of oats, when ingested together with symptom-triggering compounds, on gut well-being was investigated in healthy subjects (n = 21) and in subjects with self-reported sensitivity to pulses or IBS (n = 21). The subjects consumed a pulse-based study meal with either rice or oat flour and the

breath gases and perceived gut well-being were followed. The oat flour meal induced higher breath hydrogen levels compared to the rice flour meal in both the healthy and sensitive subjects, which correlated to an increased perception of very mild to mild flatulence in both groups. The sensitive subjects perceived more symptoms after both meals, which was not explained by the hydrogen levels.

These results demonstrated that oats are well tolerated among the individuals with gastrointestinal disorders such as CeD, NCGS, IBS or self-reported sensitivity. Results suggest that oats are beneficial for the gut microbiota of the studied groups including healthy controls. It was also observed that the β-glucan viscosity and MW have an effect on β-glucan's health-promoting properties. A high MW results in a higher excretion of fecal bile acids, which indicates higher bile acid binding capacity and thus a maintained cholesterol-lowering effect, while a low MW is linked to higher urine excretion of beneficial phenolic compounds and their increased bioavailability. These results can be applied to the evaluation of the health effects of moisture-rich oat products.

# SUOMENKIELINEN TIIVISTELMÄ

Kauran ravitsemuksellisten etujen tunnettuus on viime vuosina lisääntynyt maailmanlaajuisesti ja markkinoille on tullut lukuisia uusia kaurapohjaisia tuotteita. EU on hyväksynyt kauran β-glukaanille terveysväitteet, joiden mukaan β-glukaani alentaa veren kolesterolitasoa ja tasaa aterian jälkeistä verensokerin nousua. Lisäksi kaura sisältää antioksidanttisia fenolisia yhdisteitä, kuten avenantramideja. Uusien kosteiden kauratuotteiden, kuten juomien ja vanukkaiden, kehitys on kuitenkin vaatinut tuotteen viskositeettia vähentävää prosessointia. Alhaisemman viskositeetin ja molekyylipainon on havaittu vaikuttavan mainittuihin β-glukaanin terveysvaikutuksiin, mutta tätä ei ole huomioitu nykyisten terveysväitteiden muotoilussa. Myöskään kauran käytön yhteyttä vatsan hyvinvointiin ja suolistomikrobistoon ei ole toistaiseksi virallisesti tunnustettu. Erityisesti ruuansulatuskanavan sairauksiin, kuten keliakiaan, gluteeniyliherkkyyteen ja ärtyvän suolen oireyhtymään tiedetään liittyvän mahdollinen suolistomikrobiston epätasapaino.

Tämän väitöskirjan ensimmäisessä osatutkimuksessa (I) analysoitiin kauraa käyttävien keliaakikkojen (n=19), gluteeniyliherkkien (n=10) ja terveiden kontrollien (n=14) suolistomikrobiston koostumus ja mikrobien aineenvaihduntatuotteita ulostenäytteistä sekä ruoka- ja vatsaoirepäiväkirjat. Keliaakikoilla ja gluteeniyliherkillä ei havaittu merkkejä mikrobiston epätasapainosta. Terveillä kontrolleilla havaittiin kuitenkin enemmän bifidobakteereita verrattuna keliaakikkoihin ja gluteeniyliherkkiin sekä suurempi ulosteen asetaattipitoisuus verrattuna gluteeniyliherkkiin. Gluteeniyliherkät raportoivat enemmän vatsaoireita sekä saivat enemmän energiaa rasvasta ja vähemmän hiilihydraateista kuin keliaakikot ja terveet kontrollit.

Toisessa osatutkimuksessa (II) tutkittiin entsyymikäsittelyn vaikutusta β-glukaanin käyttäytymiseen ruuansulatuskanavassa terveillä vapaaehtoisilla (n=14). Vapaaehtoiset nauttivat kolme tutkimusateriaa, joiden β-glukaanin molekyylipaino oli joko korkea, keskitasoa tai matala. Tutkimuksessa mitattiin ulosteen sappihappojen ja virtsan fenolisten yhdisteiden pitoisuus sekä ruuansulatuskanavan paine nielaistavan kapselin avulla. Tutkimuksessa havaittiin, että korkean molekyylipainon β-glukaani oli yhteydessä runsaimpaan ulosteen sappihappopitoisuuteen ja korkeimpaan paineeseen duodenumissa, kun taas matalan molekyylipainon β-glukaani oli yhteydessä runsaimpaan fenolisten yhdisteiden erittymiseen virtsaan. Keskitason molekyylipainon β-glukaani muistutti muutoin ominaisuuksiltaan korkean molekyylipainon β-glukaania, mutta aiheutti matalamman painevasteen duodenumissa, kuten matalan molekyylipainon β-glukaani. Molekyylipaino ei vaikuttanut koettuun vatsan hyvinvointiin, mutta vatsaoireiden kokeminen erosi sukupuolten välillä.

Kolmannessa osatutkimuksessa seurattiin kauran vaikutusta vatsan hyvinvointiin terveillä vapaaehtoisilla (n=21) ja vapaaehtoisilla (n=21), jotka kokivat saavansa vatsaoireita palkokasveista tai joilla oli ärtyvän suolen oireyhtymä (herkät). Vapaaehtoiset nauttivat palkokasvipitoisen tutkimusaterian joko riisi- tai kaurajauhon kanssa, jonka jälkeen mitattiin hengityskaasuja ja seurattiin koettua vatsan hyvinvointia. Sekä terveet että herkät vapaaehtoiset raportoivat enemmän hyvin lieviä tai lieviä ilmavaivoja kaurajauhoa sisältävän aterian jälkeen verrattuna riisijauhoa sisältävään ateriaan, mikä korreloi korkeamman uloshengitysilman vetypitoisyyden kanssa molemmilla ryhmillä. Herkät vapaaehtoiset raportoivat enemmän oireita kummankin aterian jälkeen verrattuna terveisiin, mikä kuitenkaan ei ollut yhteydessä uloshengitysilman vetypitoisuuteen.

Tulokset osoittivat, että kaura on hyvin siedettyä keliakiaa, gluteeniyliherkkyyttä, ärtyvän suolen oireyhtymää tai itse koettua herkkyyttä sairastavilla. Kauran havaittiin myös olevan eduksi suolistomikrobistolle niin kyseisillä ryhmillä kuin terveillä. Lisäksi havaittiin, että β-glukaanin viskositeetti ja molekyylipaino vaikuttavat sen terveyttä edistäviin ominaisuuksiin. Korkea molekyylipaino on yhteydessä runsaampaan sappihappojen erityksen ulosteeseen, mikä kertoo paremmasta sappihappojen sitomiskapasiteetista ja siten paremmasta kolesterolia alentavasta vaikutuksesta. Matala molekyylipaino on puolestaan yhteydessä runsaampaan fenolisten yhdisteiden erittymiseen virtsaan ja niiden parempaan biosaatavuuteen. Näitä tuloksia voidaan hyödyntää kosteiden kauratuotteiden terveysvaikutusten arvioinnissa.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AUC | Area under curve |
| AVA | Avenanthramide |
| CeD | Celiac disease |
| CA | Cholic acid |
| CDCA | Chenodeoxycholic acid |
| CTRL | Control (Healthy subject) |
| DCA | Deoxycholic acid |
| ESI | Electrospray ionization |
| FODMAP | Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols |
| GC | Gas chromatography |
| GFD | Gluten-free diet |
| GI | Gastrointestinal |
| HDL | High-density lipoprotein |
| HPLC | High-performance liquid chromatography |
| iAUC | Incremental area under curve |
| IBS | Irritable bowel syndrome |
| kDa | Kilodalton |
| LC | Liquid chromatography |
| LDL | Low-density lipoprotein |
| LCA | Lithocholic acid |
| MRM | Multiple reaction monitoring |
| MS | Mass spectrometer |
| MS/MS | Tandem mass spectrometer |
| MW | Molecular weight |
| NCGS | Non-celiac gluten sensitivity |
| QTOF | Quadrupole time of flight |
| RT | Room temperature |
| SCFA | Short-chain fatty acid |
| SPME | Solid phase microextraction |
| SIBO | Small intestinal bacterial overgrowth |
| UHPLC | Ultra-high-performance liquid chromatography |
| UDCA | Ursodeoxycholic acid |

# LIST OF ORIGINAL PUBLICATIONS

I. Nylund, L.; Hakkola*, S.; Lahti, L.; Salminen, S.; Kalliomäki, M.; Yang, B.; Linderborg, K. M. Diet, perceived intestinal well-being and compositions of fecal microbiota in oat-using subjects with celiac disease or gluten sensitivity. *Nutrients.* **2020**, *12, 2570*.

II. Hakkola*, S., Nylund, L.; Rosa-Sibakov, N.; Yang, B.; Nordlund, E.; Pahikkala, T.; Kalliomäki, M.; Aura, A-M.; Linderborg, K.M. Effect of oat β-glucan of different molecular weights on fecal bile acids, urine metabolites and pressure in the digestive tract – A human cross over trial. *Food Chemistry*. **2021** *342, 128219*

III. Laito, S.; Valkonen, N.; Laaksonen, O.; Kalliomäki, M.; Tuure, T.; Linderborg, K.M. Effect of oat or rice flour on pulse-induced gastrointestinal symptoms and breath hydrogen in subjects sensitive to pulses and controls – a randomized cross-over trial with two parallel groups. *Submitted*.

*Previous surname (Salla Laito, née Hakkola).

# 1 INTRODUCTION

During the past few years, a common interest towards oats as a food ingredient has risen worldwide, and several novel oat-based products have entered the market. This phenomenon has been even called the "oat boom." In Finland, the food consumption of oats per capita (kg/year) raised continuously from 2016 (6.0 kg/year) to 2019 (9.4 kg/year) and was still relatively high in 2020 compared to past years (8.5 kg/year) (Luke Natural Resources Institute Finland). The reasons for this increased interest may lie in the suitability of oats for special diets, such as gluten-free and low-FODMAP diets, the raising interest towards plant-based food solutions and the association of oats with maintained gut well-being.

Oats are known to have excellent nutritional properties. Health claims on oat β-glucan have been approved by the EU Commission on both the reduction of blood cholesterol and the glycemic response. These effects are known to be linked to the formation of viscous β-glucan in the gastrointestinal tract. The increased viscosity of the digesta delays the absorption of glucose and thus attenuates postprandial glycemia, while the cholesterol-lowering effect is linked to the bile acid binding of β-glucan. Another approved health claim states that oat fibers increase the fecal bulk. In addition, oats contain several bioactive phenolic compounds, such as avenanthramides (hereafter AVAs) and phenolic acids (Sang and Chu 2017, Zeng, Lazarova and Bordonaro 2014). Human studies have shown that AVAs have antioxidant (Chen et al. 2007) and anti-inflammatory activity (Koenig et al. 2014) and that they might even play a role in the cholesterol-lowering effect of oats (Soycan et al. 2020).

The recent development of novel oat-based drinks and spoonable products has required processing techniques that enable a reduced viscosity. However, while the viscosity can be easily modified with enzyme treatment, along with the viscosity, also other properties of β-glucan are altered. The viscosity is known to correlate to the MW of β-glucan (Rosa-Sibakov et al. 2020), which has been widely used in studies as a parameter reflecting the β-glucan properties as recently reviewed by Nishinari and Fang (2021). Furthermore, there are indications that the MW of β-glucan is linked to its health effects (Tosh et al. 2010). However, the current health claims do not specify requirements for the state of β-glucan in the final product.

Dietary fibers in oat support the growth of gut-beneficial microbes and increase the production of microbial metabolites, such as short-chain fatty acids (Kundi et al., 2020). In contrast, an unbalanced microbiota has been associated with certain disorders, such as celiac disease (CeD), non-celiac gluten sensitivity (NCGS) and irritable bowel syndrome (IBS) (Jeffery et al. 2012, Rajilic-Stojanovic et al. 2011, De Palma et al. 2009). These disorders are treated with a diet excluding gluten-containing cereals such as wheat, rye and barley, which are

common sources of dietary fiber. Similar alterations in the gut microbiota have also been reported on healthy subjects following a gluten-free diet (De Palma et al. 2009, Marasco et al. 2016). As the microbiota composition is known to change within the dietary habits, such as by the type and amount of carbohydrates consumed (Wu et al. 2011), it cannot be ruled out that the changes could be caused by the diet as such instead of the disorder. Yet, since oats do not contain gluten or FODMAP compounds (Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols), they may serve as an important source of dietary fiber for the individuals following a gluten-free or a low-FODMAP diet.

The research of this thesis focuses on the effects of the processing by enzyme treatment on the health-promoting properties of oats studied with biochemical measurements (Study II), the effects of daily oat consumption on the gut microbiota composition and metabolites and perceived gut well-being on subjects with CeD or NCGS (Study I) and the effects of oats ingested simultaneously with potentially gut symptom-inducing compounds, FODMAPs, on microbial fermentation-related gas production and perceived gut well-being on subjects with self-reported dietary sensitivity or IBS (Study III). The review of literature introduces oat phenolic compounds and oat β-glucan and discusses their health-promoting properties in relation to processing. Further, the role of oats on the gut microbiota and on diets used in the treatment of intestinal conditions is reviewed.

# 2 REVIEW OF THE LITERATURE

## 2.1 Oat phenolic compounds

### 2.1.1 Structures and functions in the plant

Phenolic compounds are secondary metabolites in plants characterized by at least one aromatic ring with one or more hydroxyl groups (Harborne 1989). Quideau et al. (2011) have suggested a definition that plant phenolics should refer to secondary natural metabolites that are derived *via* the shikimate phenylpropanoid pathway or the polyketide acetate/malonate pathway that produces simple phenols. To date, tens of thousands of plant phenolics have been identified and their diverse structures have been classified into numerous subclasses from simple phenolic acids to more complex polyphenols, such as flavonoids and tannins (Quideau et al. 2011). Bioactive secondary metabolites are called phytochemicals (Sang and Chu 2017). In oats, the phenolic acids as well as AVAs, which are unique to oats, are the most abundant phenolic compounds (Figure 1) (Dimberg, Theander, & Lingnert, 1993). Also unique saponins, avenacosides (Sang and Chu 2017), certain flavonoids (Wenzig et al. 2005) and tocols (Shewry et al. 2008) are present in oats. Tocols consider tocopherols and tocotrienols, of which, α-tocopherol is also known as vitamin E (Shewry et al. 2008).

AVAs consist of anthranilic acid linked to a hydroxycinnamic acid *via* an amide bond (Collins 1989). They were first found by Collins (1989) who identified over 40 different forms of them and named them alphabetically. Later, Dimberg and coworkers created another systematic nomenclature to name AVAs with numbers 1–4 to present the anthranilic acid moiety and letters a, c, f and p to present the cinnamic acid moiety. The numbers are equal to anthranilic acid (1), 5-hydroxyanthranilic acid (2), 5-hydroxy-4-methoxyanthranilic acid (3) or 4-hydroxyanthranilic acid (4), while the letters mark cinnamic acid (a), caffeic acid (c), ferulic acid (f), *p*-coumaric acid (p) or sinapic acid (s) (Bratt et al. 2003). Named as such, AVAs 2c, 2p and 2f are the most abundant ones (Figure 1). Considering them as structural blocks of AVAs, it is logical that the most abundant phenolic acids of oats are ferulic and *p*-coumaric acids followed by caffeic, sinapic, syringic, cinnamic and vanillic acids (Multari et al. 2018, Bei et al. 2017, Antonini et al. 2016)(Figure 1).

Both AVAs and phenolic acids are located in the outer layers of the oat grain with AVAs in the bran (Collins 1989) and phenolic acids in the cell walls of the bran with the endosperm covalently bound to the cell wall macromolecules (Liu 2007). In total, 75% of the phenolic acids are bound, primarily through ether

linkages to lignin or through ester bonds, to the cell wall proteins and polysaccharides (Antonini et al. 2016). The unbound phenolic acids are present in the vacuoles of the outer layer of the bran being the pericarp (Grundy et al. 2018) (Figure 2). This storage-like accumulation in the outer layers of the grain suggests that they have a role in the plant defense against herbivores and pathogens as well as UV radiation and oxidation. More specifically, AVAs are classified as phytoalexins, which are antimicrobial molecules of low-molecular weight and are synthesized and accumulated in plants after exposure to microorganisms (Lattanzio, Lattanzio and Cardinali 2006, Cheynier et al. 2013). The antifungal properties of AVAs were, for the first time, demonstrated in the *in vitro* study of Mayama et al. (1981) where they inhibited the growth of *Puccinia coronate avenae*. Moreover, *in vitro* studies have demonstrated antimicrobial activity of caffeic, ferulic, 2,4-dihydroxybenzoic, vanillic, syringic, cinnamic and *p*-coumaric acids against Gram-positive and Gram-negative bacterial species (Alves et al. 2013). Moreover, the phenolic ring structure is known to be responsible of the radical scavenging activity (Serbinova et al. 1991). In addition, the secondary metabolites are essential as signaling agents, metal chelators and for the growth of the plant (Lattanzio et al. 2006).

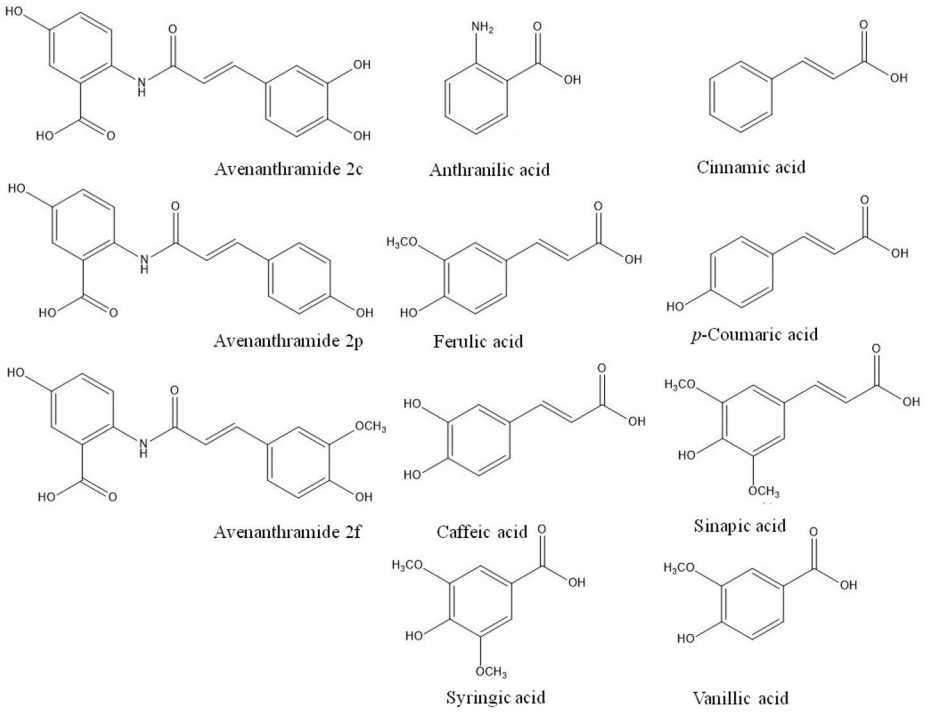


**Fig 1.** The most abundant avenanthramides and phenolic acids of oats.

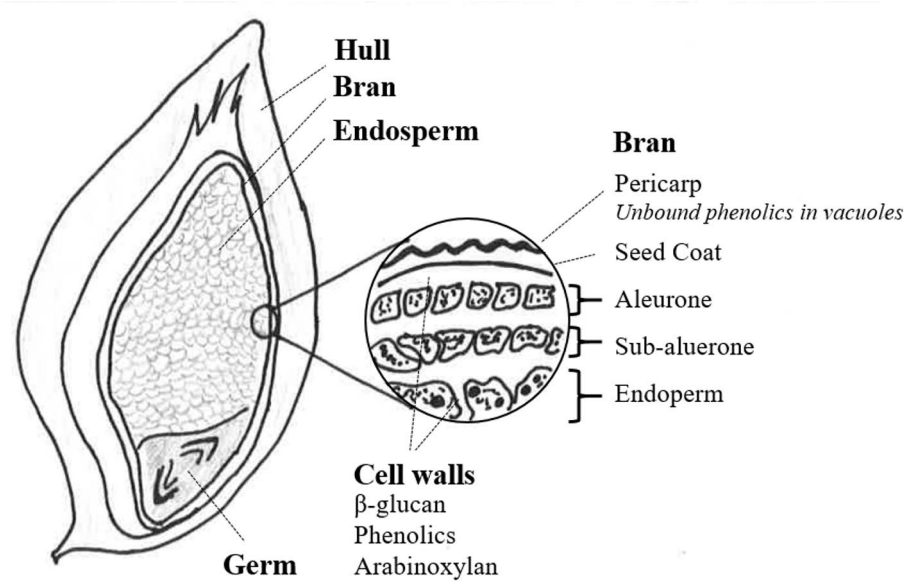


**Fig 2.** The structure of oat grain (Grundy et al. 2018, redrawn and modified).

### 2.1.2 Bioactive properties, bioavailability and metabolism of oat phenolic compounds

#### 2.1.2.1 *In vitro* studies measuring bioactive properties

The antioxidant capacity of the oat phenolic compounds was first measured *in vitro* from the extracts of oat hulls and groats (Emmons and Peterson 1999). They reported that the antioxidant capacity measured by the inhibition of the coupled auto-oxidation of linoleic acid and beta-carotene correlated to the total phenolic content determined with Folin and Ciocalteau's phenol reagent. The groats had significantly higher antioxidant activity than the hull, and the groats were particularly rich in AVAs and caffeic acid, while the hull contained more of other phenolics such as ferulic and vanillic acids, vanillin and p-coumaric acid (Emmons and Peterson 1999, Emmons, Peterson and Paul 1999). Later, it was reported that the bound phenolics of oat grain endosperm germ and bran fractions contributed 73–82% of the total phenolic content and 59–75% to the antioxidant capacity measured by 2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2-diphenyl-1-picrylhydrazyl (DDPH) and the ferric reducing ability of plasma assay (FRAP) methods *in vitro* (Martinez-Miguel et al. 2018). Of oat phenolics, AVA 2c is reported to have the strongest antioxidant activity measured by DPPH, FRAP and UHPLC–online ABTS coupled with LC-QTOF followed by caffeic acid, quercetin ‑ 3,4′-O-di-beta-glucopyranoside derivative and 6-hydroxykaempferol-3,5,7,4′-tetramethyl ether 6-rhamnoside.

Among AVAs, 2c with the highest antioxidant activity is followed by 2f and 2p (Rao et al. 2019). The antioxidant activity of oat phenolics is associated with the phenolic ring structure with attached hydroxyl groups (Heleno et al. 2015).

An anticancer activity of AVAs was reported by an *in vitro* study that measured the inhibition of COX enzyme activity and prostaglandin E2 (PGE2) production in lipopolysaccharide-stimulated mouse peritoneal macrophages (Guo et al. 2010). An anticancer activity of cinnamic and p-coumaric acids was detected in a study measuring their inhibition of cell line growth (Vaz et al. 2012, Heleno et al. 2014). Later, it was demonstrated *in vitro* that 24-hour treatment of AVAs attenuated inflammation and muscle atrophy in tumor necrosis factor (TNF)-alpha-treated cells. Muscle atrophy stands for a possible cause of tumors, muscle immobilization, denervation or starvation (Yeo et al. 2019).

#### 2.1.2.2 Animal studies measuring bioactive properties

The antioxidant activity of oat phenolic compounds is shown to be linked to the reduction of oxidative stress in mouse organs (Table 1). The treatment with oat extract was shown to reduce the markers of liver damage, such as aspartate transaminase, alanine transaminase and glutathione reductase in mice with liver damage induced with ethanol (Mir et al. 2018) or in mice with liver damage induced with lipopolysaccharide (Debnath et al. 2019). Instead, the levels of enzymes associated with antioxidant defense, such as glutathione S-transferase, catalase and superoxide dismutase, were increased (Mir et al. 2018; Debnath et. al. 2019). The protective effect of oat extract against oxidative stress was also observed to reduce estrogen deficiency-linked kidney damage in mice (Ltaif et al. 2020). Moreover, AVAs were shown to reduce the exercise-induced oxidative stress in rats (Ji et al. 2003) as well as tumors caused by oxidative stress and thus their growth in mice (Damazo-Lima et al. 2020, Aldubayan et al. 2019). Another study using mice reported that AVAs have an anti-itch effect possibly due to a structural similarity to the anti-histamine drug called Tranilast (Sur et al. 2008). Furthermore, AVAs were shown to reduce the body weight gain in mice, which was possibly related to a decreased relative abundance of Posteobacteria, which is a potentially harmful species (Zhang et al. 2020b).

#### 2.1.2.3 Human studies measuring bioactive properties

The antioxidant properties of oat phenolics have also been demonstrated in humans. Daily consumption of oat porridge during 4 weeks reduced the inflammatory markers such as hsCRP (high-sensitivity C-reactive protein), interleukin-6, interleukin-8 and TNF-alpha (tumor necrosis factor) of hypercholesterolemic adults compared to rice porridge consumption (Pavadhgul

et al. 2019). Consumption of AVA-enriched skim milk was shown to increase the plasma glutathione levels in healthy adults (Chen et al. 2007). AVA consumption was also demonstrated to reduce exercise-induced inflammation in young men and women (Zhang et al. 2020a) as well as in postmenopausal women (Koenig et al. 2014).

Besides β-glucan, also AVAs may play a role in the cholesterol-lowering effect, as a 1-month consumption of AVA-enriched capsules was shown to decrease the total cholesterol, triglycerides and LDL cholesterol levels and elevate HDL cholesterol levels in healthy adults (Liu et al. 2011). It was suggested that the effect may be linked to the prevention of lipid oxidation by AVAs. In another study, AVAs and oat phenolic acids combined lowered LDL and total cholesterol levels and decreased day time and night time systolic and diastolic blood pressure in hypertensive adults after 4 weeks daily consumption of oat phenolics-enriched oatmeal or cake (Soycan et al. 2020).

The anti-itch effect of oat extract was demonstrated in patients with chronically irritanting hand eczema. The patients received a 2-week treatment with fluocinolone followed by a 4-week treatment with either a colloidal oatmeal 1% cream or a base cream (Sobhan et al. 2020). Studies examining the bioactive properties of phenolic compounds in oats are summarized in Table 1.

#### 2.1.2.4 Bioavailability and metabolism

The bioavailability of AVAs and phenolic acids is substantiated in animal and human studies. Their absorption into the plasma and tissues in humans is known to be rapid as they have been detected in plasma already after 15 minutes of ingestion (Chen et al. 2007, Anson et al. 2011). AVAs are mainly broken down to phenolic acids in the intestine and colon (Schar et al. 2018, Wang et al. 2013). Only a minor part of them is directly conjugated to sulfates, glucuronides or methylated forms mainly in the intestine, kidney and liver (Chen et al. 2007). Also phenolic acids are rapidly conjugated mainly to glucuronides and sulfates (Nardini et al. 2009). The purpose of the conjugation is to detoxify the compounds and to increase their solubility in order to ease their excretion. As a result, their biological activity is typically diminished. However, studies have shown that the bioactive properties of certain phenolic acids are even stronger in their conjugated form. For example, caffeic and ferulic acid glucuronides have demonstrated stronger antioxidant activity *in vitro,* while their sulphate derivatives had lower activity compared to the acglycone form (Ohta et al. 1997, Piazzon et al. 2012). *p*-Coumaric and cinnamic acid glucuronide and methylated derivatives have demonstrated stronger anticancer activity compared to the acglycone form *in vitro* (Heleno et al. 2014). The authors suggested that since the 4'-hydroxyl group in the aromatic ring is the determinant for the antioxidant

activity, by remaining free, this may explain the antioxidant properties of the conjugated form. However, they did not find an explanation for the increased anticancer activity after conjugation (Heleno et al. 2015).

The extent of conjugation is likely linked to the activity and specificity of glucuronosyltransferase and sulfotransferase enzymes towards the molecular structure of the phenolic acid. It was observed that after 60 minutes of ingestion of beer, caffeic acid circulated in plasma exclusively in its conjugated form, while coumaric acid and other monohydroxy derivatives were more likely present in their free forms (Nardini et al, 2009). The degree of conjugation of ferulic and vanillic acids with 3-methoxy-4-hydroxy substitution was between these. The high activity of intestine sulfotransferase SULT1A3 on the 3,4-dihydroxy moiety of caffeic acid, a catechol group, has been also reported earlier in the review by Scalbert and Williamson (2000). Interestingly, the structures of the three most abundant AVAs differ in a similar manner than the phenolic acids discussed as AVA 2c is substituted with 3,4-dihydroxy, AVA 2f with 3-methoxy-4-hydroxy and AVA 2p with a monohydroxy group. To date, the structure-dependent conjugation rate of AVAs has not been studied in humans. Koenig et al. followed the AVA metabolism and conjugation in rats after administration of synthetic AVAs by oral gavage. They reported that after 60 minutes, 94, 72 and 91 percent of the AVAs 2c, 2f and 2p, respectively, were conjugated in plasma (Koenig et al. 2011). However, the metabolism of AVAs (free + conjugated) is reported to differ between humans and rats as discussed later on, which may also explain the difference in the AVA conjugation rate (Koenig et al. 2011, Chen et al. 2007). Moreover, since the main metabolism pathway of AVAs is through the breakdown to phenolic acids, their structure-dependent conjugation rate is less relevant than that of phenolic acids.

Conjugation taken aside, both the human study and the rat study (Chen et al. 2007, Koenig et al. 2011) agreed on the rank order of the maximum plasma concentration of the AVAs being 2p >> 2c > 2f. However, they noted that while the AVA 2f was the slowest to be eliminated in humans, it was the quickest in rats (Chen et al. 2007, Koenig et al. 2011). It was supposed that in humans, the less hydrophilic structure of 2p is less readily eliminated to urine than the others and thus the concentration can reach a higher level (Chen et al. 2007, Koenig et al. 2011). In addition to following the AVA circulation in plasma, the rat study measured their concentration in liver, heart and skeletal muscle tissues during 12 hours after ingestion (Koenig et al. 2011). In contrast to plasma, the concentration of AVA 2f was higher in liver and heart compared to 2p and 2c after 60 minutes of ingestion. In the gastrocnemius, the concentration of 2c was the highest after 60 minutes of ingestion. It was noted that AVAs are first absorbed from the gut *via* the hepatic portal vein to the liver and later they reach the heart and liver a second time *via* the hepatic artery. In the skeletal muscle

tissue, the concentration of AVAs remained lower during the 12 hours than in the liver and heart, but it must be noted that skeletal muscle composes up to 40% of body weight and could thus serve as an AVA storage site (Koenig et al. 2011). In humans, this circulation of AVAs and phenolic acids is known to be rapid, as they are mainly excreted to urine between 0–2 hours and 4–8 hours after ingestion (Schar et al. 2018). They are metabolized directly in the intestines and by colon microbiota (Wang et al. 2015) through breaking down to their structural blocks (Figure 3) (Schar et al. 2018).

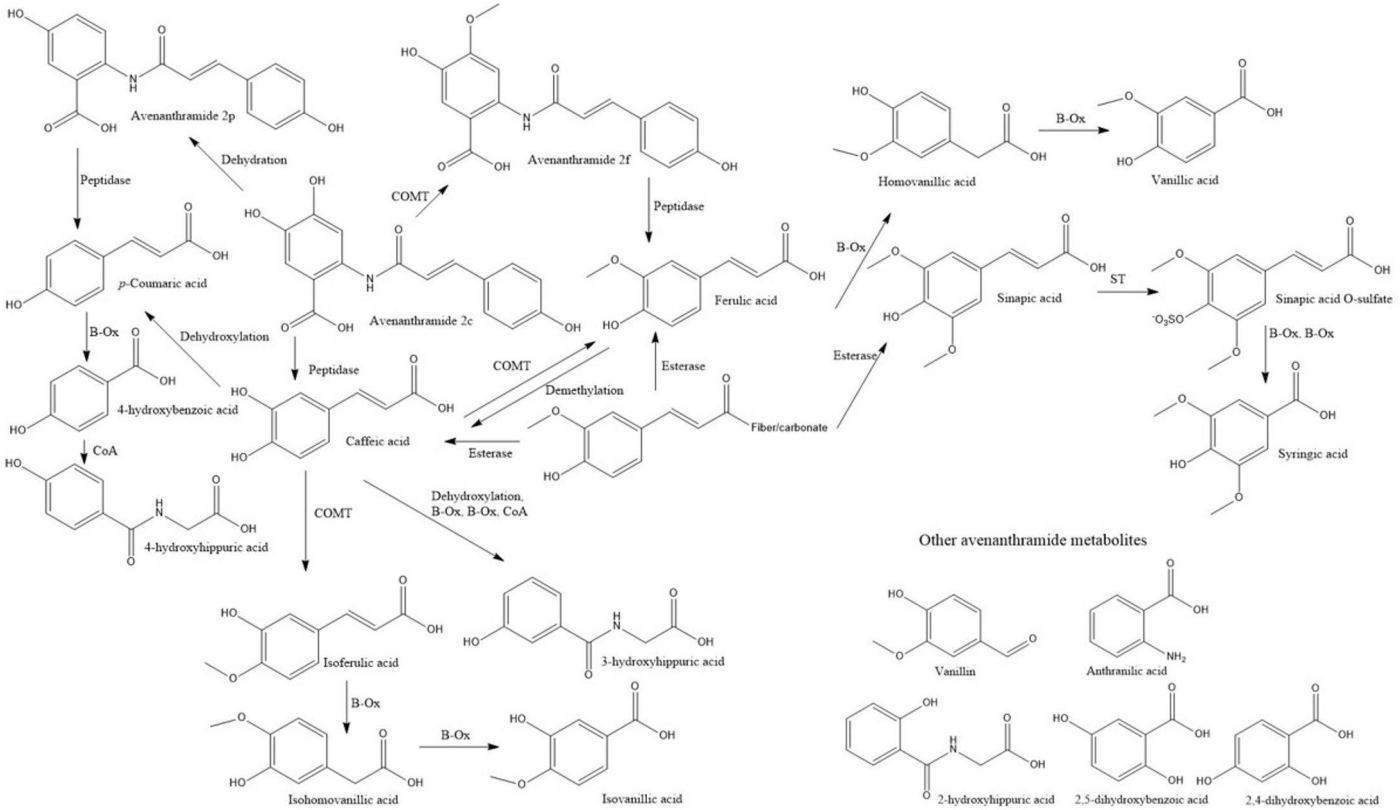


**Fig 3**. Suggested metabolic pathways of avenanthramides. B-Ox = β-oxidase, COMT = cathecol-O-methyl-transferase, CoA = coenzyme A, ST = sulfate transferase. Redrawn from Schar et al.

### 2.1.3 Effect of processing on the bioaccessibility and bioavailability of oat phenolic compounds

The recent development of novel spoonable and liquid oat products has required processing in order to achieve the desired physical properties. Often enzyme treatment is used to reduce the viscosity of the product. Viscosity is dependent on the β-glucan properties (see Section 2.2.2), and the enzymes with cell wall activity reduce the length of β-glucan chains bound in the cell walls. If the enzymes also have activity towards phenolic acids, bound phenolics are released as a side effect from the cell wall (Anson et al. 2011).

Earlier, the release of phenolic acids from oat bran after carbohydrase treatment has been demonstrated *in vitro*, and cellulase and α-amylase were reported as the most efficient enzymes (Alrahmany, Avis and Tsopmo 2013). A release of bound phenolics has also been reported in studies of enzyme-treated rye (Lappi et al. 2013) and enzyme- and yeast-treated wheat (Anson et al. 2011). In all these studies, the most remarkable increase was observed in the concentration of free ferulic acid, which is known to be the major phenolic acid in whole grains (Maillard and Berset 1995, Sosulski, Krygier and Hogge 1982).

Besides the enzyme treatment, also other industrial processes involving heat, pressure or mechanical treatment may cause changes in the composition of bound and free phenolic compounds. These are, for example, through milling, grinding, steaming, autoclaving, drum drying and extrusion cooking (Wang, He and Chen 2014). During the milling and grinding processes, the particle size of the bran is decreased. Some studies have reported that this increases the antioxidant activity, but it has been hypothesized that it was caused by the increased surface and better exposition of the phenolic acids and not through their release from the bound form. Still, the release of phenolic acids may occur if the process is extensive enough to break the covalent bonds of the cell walls (Wang et al. 2014). Processes involving heating seem to also make changes to the composition of phenolics in the bran. It was reported that steaming and autoclaving increased the concentration of free ferulic acid and vanillin but decreased the concentration of caffeic acid and AVAs, while drum drying decreased the concentration of all phenolic acids (Bryngelsson, Dimberg and Kamal-Eldin 2002). Extrusion cooking combines high temperature, high pressure and high shearing conditions, which causes the degradation of heat-sensible compounds (Altan, McCarthy and Maskan 2009) but also damages the cell walls causing a release of the compounds (Awika et al. 2003). The result depends on the dominating reaction and varies among different cereals and processes. A study of oat extrusion reported a decrease in the content of total phenolics in their free form but an increase of bound phenolics most likely due to the polymerization of phenolic acids (Zeng et al. 2016).

## 2.2 Oat β-glucan

### 2.2.1 Structure and physicochemical properties

β-glucans are polysaccharides composed of β-D-glucose units. They are found in the cell walls of cereals, algae, bacteria, yeast and fungi. Among whole cereal grains, oats (3.6–6.7%), barley (2.2–11.0%) and wheat (0.38–0.84%) contain β-glucan (Lazaridou et al. 2004, Jokinen et al. 2021). In oats, the cell walls of the bran and endosperm are particularly rich in β-glucan (Grundy et al. 2018). β-glucans of algae, bacteria, yeast and fungi consist entirely of β-(1→3) linked units forming thus an insoluble fiber (Zekovic et al. 2005). Cereal β-glucans consist of blocks of (1→4)-linked β-D-glucose residues, which form cellulose-like hydrogen bonds between the polymer chains. These blocks are separated by single β-(1→3) linkages that prevent the chains from packing close to each other and thus keeping the polysaccharide conformation irregular (Figure 4). This irregularity is proposed to be responsible for the solubility of the cereal β-glucans (Woodward, Phillips and Fincher 1988).

The number of β-(1→4) and β-(1→3) linkages and the level of organization of the polymer vary among the cereal β-glucans and determine their physical properties, such as their solubility and gelation properties (Lazaridou and Biliaderis 2007). It has been suggested that a higher amount of trisaccharide units or cellulose-like sequences consisting of more than 3 D-glucopyranosyl units would increase the regularity of the β-glucan conformation and lead to a higher degree of organization in the solution, which decreases its solubility (Izydorczyk, Jacobs and Dexter 2003, Woodward et al. 1988). On the contrary, increased regularity in the conformation has been associated with a higher gelling ability (Lazaridou and Biliaderis 2007). Compared to other cereals, oat β-glucan has less trisaccharide units (55.8%) when compared to wheat (67.1%) and barley (62.0–63.3%). Oat also has a lower molar ratio of trisaccharide units when compared to tetrasaccharide units (2.1%) compared to wheat (3.7%) and barley, (2.8–3.0%), while the number of cellulose-like oligomers is similar to other cereals (8.7–9.7%). Indeed, studies have shown that oat β-glucan has a higher solubility and lower gelling ability compared to other cereal β-glucans (reviewed by Lazaridou et al. 2004).

The water solubility of fiber is the key property for viscosity formation in an aqueous solution. In addition, the concentration and MW of β-glucan have been shown to increase viscosity (Skendi et al. 2003). When the MW of β-glucan is decreased, a higher concentration is required for the viscosity formation, i.e., a higher amount of molecules is required to build a network (Skendi et al. 2003). Nevertheless, the solubility of the β-glucan is increased, when the MW is

decreased, which facilitates the formation of a gel structure (Marasca, Boulos and Nystrom 2020).

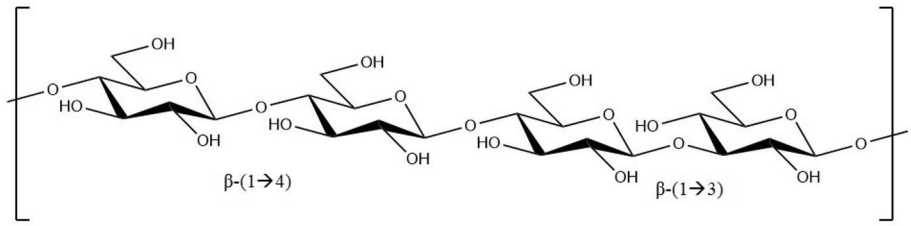


**Fig 4.** The structure of oat β-glucan.

### 2.2.2 The viscous moiety of β-glucan and its relation to health-promoting properties

The ability of oat β-glucan to form viscosity in aqueous solutions is strongly correlated with its physiological functionality. In the small intestine, β-glucan has two primary physiological effects as it increases the viscosity of the digesta and traps nutrients and thus decelerates their absorption rate (Eastwood and Morris 1992). Viscosity formation is suggested to be the key mechanism behind the substantiated health effects of oat β-glucan through decreasing postprandial glycemia and the blood cholesterol levels (Jenkins et al., 1978, Wood et al., 1994, Gunness and Gidley 2010).

#### 2.2.2.1 Glycemia attenuation and increase of satiety

The viscosity of soluble dietary fiber was first linked to the reduction of postprandial glycemia by Jenkins et al. (1978). They demonstrated that the glycemia-attenuating effect of the guar gum was lost after acid hydrolysis. In addition, they concluded that the increased viscosity of the digesta delays the absorption of glucose and thus prevents the fast rise of postprandial blood glucose concentration. Later, a drink model by Wood et al. showed that the relationship between β-glucan viscosity and plasma glucose and insulin responses accounted for 79–96% of the changes of the subjects' maximum and minimum peak values, the range between these and the 2-hour AUC after 50 grams of ingested glucose (1994). After that, the relationship between the oat β-glucan consumption and glycemic index has been established in multiple clinical trials both in diabetic subjects (Pick et al. 1996, Tappy, Gugolz and Wursch 1996, Wursch and PiSunyer 1997, Jenkins et al. 2002, Braaten et al. 1994) and in healthy subjects (Battilana et al. 2001, Juntunen et al. 2002, Behall, Scholfield and Hallfrisch 2005, Braaten et al. 1994, Granfeldt, Nyberg and Bjorck 2008). The effect of β-glucan on the reduction of the glucose rise after a meal has been

substantiated by the EU Commission (EFSA Panel on Dietetic Products, 2011) (Table 2).

Recent studies have examined further the adequate dose of β-glucan to reach postprandial glycemia reduction. Moreover, the possibilities to introduce the β-glucan enrichment to food matrixes of different cultural cooking traditions have been considered. For example, a significant reduction in glycemic index and in glycemic load was reached with a 1 percent (w/w) dose of β-glucan in white-wheat-flour-containing bread in seven healthy subjects (Mohebbi et al. 2019). Another study concluded that even an addition of partially degraded β-glucan to white wheat bread lowered significantly the postprandial glycemia compared to the wheat control in 15 healthy subjects (Rieder et al. 2019). A study noted that compared to a rice meal, oats lowered the postprandial glycemia despite being served as whole grain kernels, pearled oats or combined with rice. Whole grain kernels and pearled oats also maintained the effect after cooking them either under normal or high pressure (Zhu et al. 2020). The glycemia-reducing effect of β-glucan also remained when oatmeal was prepared by soaking oats to milk overnight, both with and without seeds, nuts and sugars compared to rice cooked with cream and as measured in 40 healthy subjects (Wolever et al. 2019). Currently, the condition of the accepted health claim states that to reach the glycemia-reducing health effect, 4 grams of β-glucan from oats for each 30 grams of available carbohydrates should be consumed per meal (Pick et al. 1996, Tappy et al. 1996, Wursch and PiSunyer 1997, Jenkins et al. 2002, Braaten et al. 1994, Battilana et al. 2001, Juntunen et al. 2002, Behall et al. 2005, Granfeldt et al. 2008).

Lately, it has been discussed whether the increased viscosity of the digesta also affects gastrointestinal transit time and prolonged perception of satiety (Rebello, O'Neil and Greenway 2016). However, a health claim on a satiety increasing effect has not been accepted for oat β-glucan in the EU. Hunger and satiety are subjective perceptions that are not only biological phenomena but also affected by several factors, such as the surrounding environment, learned eating behavior, emotional state and individual metabolism, which all set a challenge to the measurement of this phenomena (Blundell et al. 1996). Rebello et al. described the phenomena of the eating-derived reduction of hunger by two terms, satiation and satiety. Satiation develops during eating and eventually causes the meal termination, while satiety is the state where further eating is inhibited (Rebello et al. 2016). To date, several mechanisms for the satiety-increasing effect of β-glucan have been presented. First, fiber-rich foods require longer mastication, which prolongs the oral phase and allows time for the satiety sensation mediating signals. The duration of the oral phase is suggested to have a positive impact on the sensation of fullness (Wijlens et al. 2012). Studies have also indicated that consumption of a fiber-rich meal prolongs the gastric phase

(Hlebowicz et al. 2007) but shortens the colonial transit time (Timm et al. 2011). Moreover, it has been suggested that the production of short-chain fatty acids from dietary fiber by the colon microbes (discussed further at Section 2.3) not only provides energy but also maintains the satiety sensation by activating G-protein coupled receptors in the colon and thus mediates the release of appetite-regulating peptides (Samuel et al. 2008, Cherbut et al. 1998). It was reported that acetate reaches the brain *via* blood circulation and reduces acute food intake by affecting the hypothalamic control of appetite in mice (Frost et al. 2014). However, the main mechanism behind the β-glucan-driven satiety increase might be the viscosity, which is associated with sensation of fullness and delayed gastric emptying (Section 2.2.3) (Rebello et al. 2016).

In an appetite study of three different panels of 41 participants, oats were considered as potentially more satisfying and had more impact on eating behavior compared to wheat (Berti et al. 2005). In a breakfast study of 30 healthy women who consumed 4 different meals with a varying amount of β-glucan (0, 4 and 8 grams), the gastric and intestinal viscosity of the meals were measured with an *in vitro* digestion tool consisting of a rheometer. It was observed that the viscosity increased with the amount of β-glucan and correlated positively with the sensations of satiety, fullness and a desire to eat (Pentikäinen et al. 2014). A similar result was also reached in a breakfast study in overweight subjects (n = 36) that compared the weekly appetite ratings after 3 different meals were consumed daily for 4 weeks being oatmeal, frosted cornflakes or a plain glass of water as a control. The oatmeal group reported across the intervention a higher fullness and a lower hunger compared to the control group (Geliebter et al. 2014). Another breakfast study of 33 healthy volunteers compared the postprandial glycemia and appetite ratings after meals of cornflakes and milk with or without added β-glucan in a randomized order. It was observed that β-glucan increased the satiety and lowered the glycemic response (Zaremba et al. 2018).

#### 2.2.2.2 Cholesterol lowering *via* bile acid binding

Elimination of blood cholesterol is almost fully (99%) carried out by the fecal route with two-thirds are excreted as cholesterol and one-third as bile acids (Hofmann 1999). The synthesis of bile acids in the liver uses approximately 500 mg of cholesterol daily (Figure 5), (Russell 2003). Two alternative routes are presented for the bile acid synthesis being the neutral (“classic”) and acidic (“alternative”) pathway. The 7α-hydroxylation of cholesterol by CYP7A1 is the rate-limiting enzyme of the neutral pathway, and it is the most important pathway of bile acid formation in humans. In the acidic pathway, cholesterol is first converted to hydroxycholesterols, and after that, further metabolized to bile acids

(Figure 5). Finally, the water solubility and polarity of the bile acids are increased by conjugation with taurine and glycine (Beilke et al. 2009).

Bile acids play an important role in cholesterol elimination, stimulation of bile flow and stimulation of biliary phospholipid secretion. They facilitate the absorption of lipids, cholesterol and fat-soluble vitamins from the small intestine. After being carried through the small intestine with the digesta, part of the primary bile acids undergoes structural modification by bacterial enzymes in the colon and is converted to secondary bile acids. The majority (95%) of all the bile acids are reabsorbed from the intestine, delivered back to liver and re-excreted 4–12 times a day depending on the meals consumed (Lu et al. 2010). The circulation from the intestines to the liver and the re-release to intestines is called the enterohepatic cycle. In humans, cholic acid (CA) and chenodeoxycholic acid (CDCA) are the main primary bile acids. CA is further metabolized to deoxycholic acid (DCA), which circulates with primary bile acids and is the the dominant biliary acid in some adults. The main secondary bile acids derived from CDCA in humans are ursodeoxycholic acid (UDCA) and lithocholic acid (LCA), which are formed *via* further 7-β-dehydroxylation (Figure 5) (Hofmann, 1999; Russell, 2003).

The mechanism of the cholesterol-lowering effect of β-glucan is linked to bile acid reabsorption. β-glucan distracts the reabsorption of the bile acids in the intestine, which accelerates the synthesis of bile acids in the liver. This increases the intake of cholesterol as a source material and lowers the blood cholesterol level (Ellegard and Andersson 2007, Gunness et al. 2016). It has been suggested that β-glucan forms a viscous layer in the small intestine, thus preventing the delivery of bile acid micelles into absorptive cells and/or captures the bile acids between the β-glucan chains. Also the molecular level association between the bile acids and β-glucan has been suggested (Figure 6) (Gunness and Gidley 2010). However, as discussed in Section 2.2.3, since the effect is clearly linked to the viscosity, the molecular level association is less relevant.

The reducing effect of oat β-glucan on LDL and total cholesterol has been substantiated in multiple clinical trials over the years including healthy subjects (Naumann et al. 2006) and subjects with hypercholesterolemia (Onning et al. 1999, Berg et al. 2003, Kerckhoffs, Hornstra and Mensink 2003, Karmally et al. 2005, Queenan et al. 2007, Theuwissen and Mensink 2007, Van Horn et al. 2008, Amundsen, Haugum and Andersson 2003), overweight subjects (Saltzman et al. 2001, Davy et al. 2002) or subjects with diabetes (Pick et al. 1996, Wursch and PiSunyer 1997). The cholesterol-lowering health claim for oat β-glucan has been accepted by the EU Commission. The latest studies have confirmed the result with an oat-based, β-glucan-free placebo (Cicero et al. 2020) and in different populations, such as Mediterranean (Cicero et al. 2020) and Asian populations (Gulati, Misra and Pandey 2017).

Some studies have aimed for an expanded cholesterol-lowering effect by combining oat β-glucan with another bioactive nutritional compound. It was observed that β-glucan combined with arabinoxylan improved the lipid metabolism of mice consuming a high-fat background diet compared to a single arabinoxylan diet (Chen et al. 2021). Another study reported that β-glucan combined with phytosterols lowered both total cholesterol and LDL cholesterol more efficiently than β-glucan or phytosterols alone in hypercholesterolemic individuals (Ferguson et al. 2020).

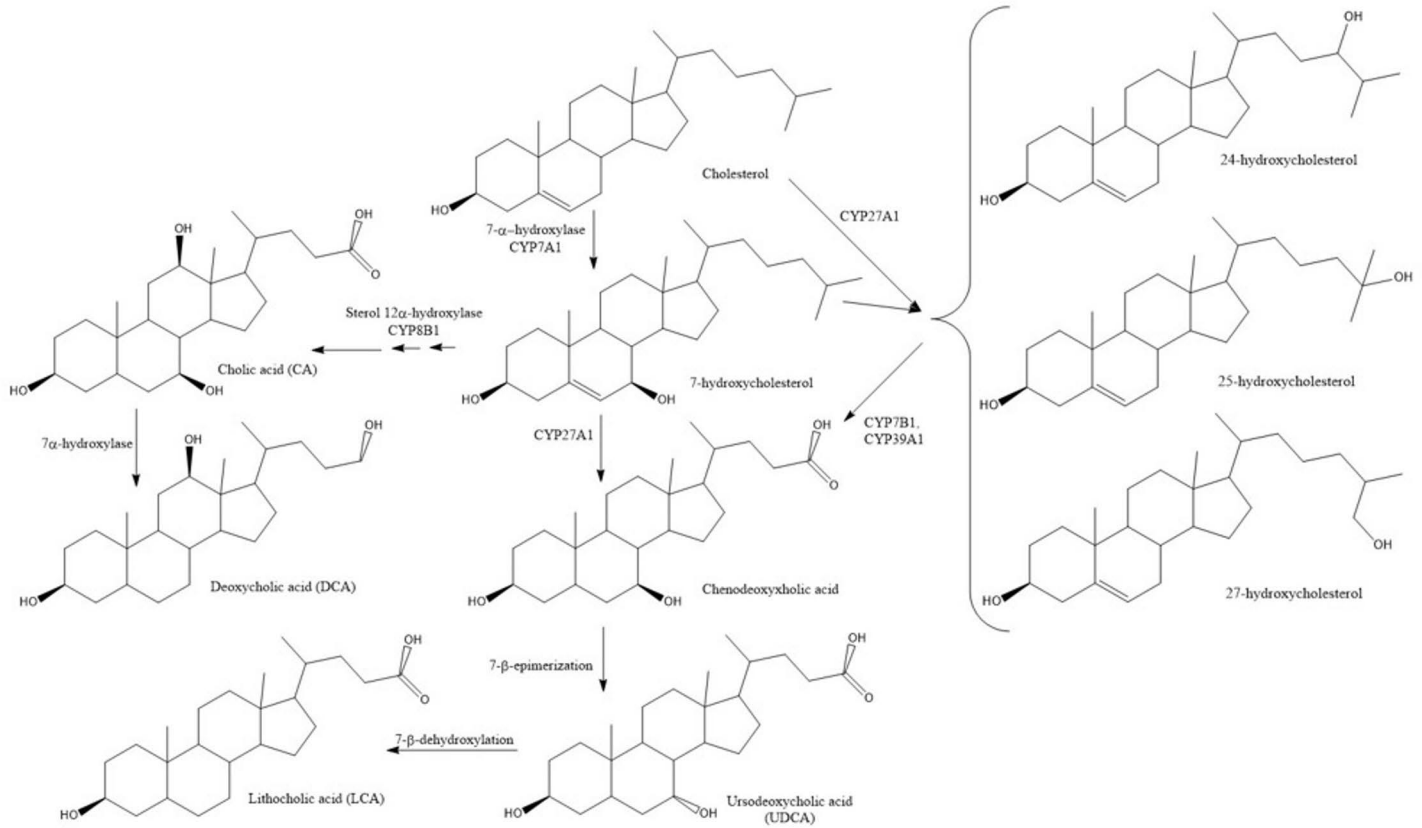


**Fig 5.** The synthesis of the major bile acids and cholesterol derivatives in humans (Beilke et al. 2009, Hofmann 1999).

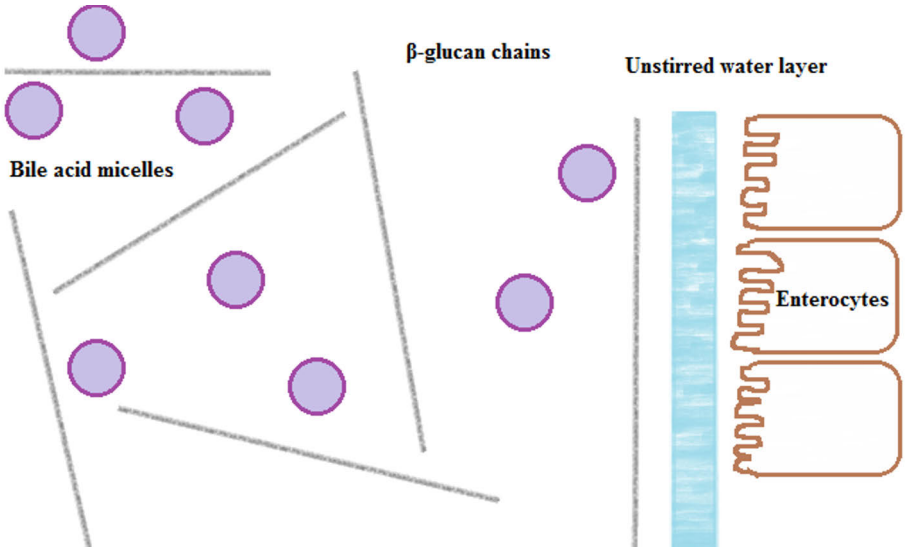


**Fig 6.** Illustration of the suggestive mechanisms of β-glucan prevention of bile acids absorption. The proposed mechanisms are that β-glucan forms a viscous layer between the absorptive cells and the digesta and captures the bile acid micelles between the chains or associates with the micelles at a molecular level (Gunness and Gidley 2010).

### 2.2.3 The effect of viscosity and molecular weight on the health-promoting properties of β-glucan

The development of oat-based drinks and spoonable products has required processing techniques which aim to achieve a reduced viscosity. Even though the desired state of viscosity is easy to design with enzyme treatment, along with the viscosity, also other properties of β-glucan are altered. The MW of β-glucan is known to correlate with the viscosity (Tosh et al. 2010), and multiple studies have found a relation between the viscosity, MW and the bioactivity of β-glucan (Table 3).

The bile acid binding capacity known to be related to the cholesterol-lowering effect of β-glucan has been reported to correlate with the MW *in vitro* (Rosa-Sibakov et al. 2020, Marasca et al. 2020). The bile acid binding capacity of oat bran with varying β-glucan MW was measured *in vitro* with a small intestine model (Rosa-Sibakov et al. 2020). It was observed that, while the native oat bran formed the highest viscosity and had the highest bile acid binding capacity, also the moderately processed oat bran had detectable viscosity and bile acid binding properties. The most processed bran had lost these properties (Rosa-Sibakov et al. 2020). Another recent study used a dialysis model to determine the bile acid binding capacity of oxidized or hydrolyzed oat β-glucan compared to native β-glucan *in vitro* (Marasca et al. 2020). It was observed that the bile acid binding is highly dependent on the viscosity of the β-glucan, which is reduced during the processing (Marasca et al. 2020). However, this has been clarified only recently. Earlier *in vitro* studies have stated that reducing the MW of β-glucan has no effect or even increases the bile acid binding capacity of the β-glucan (Sayar, Jannink and White 2011, Kim and White 2010). As it was noted by Marasca et al. (2020), the centrifugation technique used in these studies is not optimal for soluble fiber, since it overlooks the viscosity effect. Within this technique, the sample is centrifuged to separate the solid phase containing the bile acids bound into the fiber from the supernatant containing the unbound bile acids. In a successful centrifugation method, solubilizing the fiber into supernatant is avoided or at least the ratio of the solid phase and the supernatant is maintained constant. Still, the solubilization of the fiber cannot be completely avoided during the process. Since the low MW fiber is more soluble than native, it forms a faster viscosity and thus binds more bile acids during the time frame of the method (Marasca et al. 2020). In addition, several of these studies have only compared the β-glucans with a relatively low MW (Table 3).

The correlation between the β-glucan MW, the bile acid binding and cholesterol-lowering effect has also been demonstrated *in vivo*. The blood cholesterol and postprandial glycemia were examined in diabetic mice, which were fed with β-glucans with MW of 172 kDa, 635 kDa and 743 kDa. It was observed that the higher the MW of fed β-glucan was, the more intense were the

observed decreases in blood cholesterol and postprandial glycemia (Zhao et al. 2014). A clinical study followed healthy subjects receiving meals with varying β-glucan MWs of 2210 kDa, 530 kDa or 210 kDa twice a day during 4 weeks (Wolever et al. 2010). It was observed that the LDL cholesterol levels were lowered similarly after consumption of the 2210 kDa or 530 kDa β-glucan meals, but the efficacy was reduced by 50% after the 210 kDa meal. Another human study with 30 mildly hypercholesterolemic subjects observed that β-glucan with a high MW (1349 kDa) lowered the cholesterol more efficiently than β-glucan with a lower MW (290 kDa) during a 5-week intervention period. Moreover, it was found that the cholesterol-lowering effect of high MW β-glucan correlated to a genotype with the individuals carrying the CYP7A1 SNP rs3808607-G allele were more responsive to the cholesterol-lowering effect of high MW β-glucan than CYP7A1 SNP rs3808607-T allele carriers. Instead, no interaction between the diet and ApoE polymorphism was observed in their study (Wang et al. 2016), although the Apo E polymorphism is known to influence individual cholesterol metabolism (Marais 2019). For this reason, the complexity of the mechanisms behind the β-glucan cholesterol-lowering effect may explain why certain studies on mice (Immerstrand et al. 2010) and on humans (Frank et al. 2004) have not observed a correlation between the MW and cholesterol-lowering effect of β-glucan.

The correlation between β-glucan MW and glycemia attenuation was first observed when the MWs of the β-glucans used in the early clinical trials were analyzed (Wood, Beer and Butler 2000). It was seen that the reduction in the postprandial glycemia was reached already with a low MW β-glucan. A study of 89 healthy subjects reported that an oat beverage containing 5 grams of β-glucan with a MW of 70 kDa reduced the postprandial glycemia compared to a beverage prepared with a rice starch. In contrast, neither a 5-gram nor a 10-gram dose of barley β-glucan with a MW of 40 kDa had an effect on glycemia (Björklund et al. 2005). It has been also studied if the increased viscosity and thus more intense glycemia-reducing effect could be achieved simply by decreasing the volume of the β-glucan dose and thus increasing the concentration besides increasing the MW. Kwong et al. measured (2013) the postprandial glycemia of 15 healthy subjects who received a beverage with 50-gram glucose dose and either high MW β-glucan (580 kDa), low MW β-glucan (145 kDa) or a beverage without β-glucan. The beverage volume was either 250 mL or 600 mL. It was observed that the beverage with 580 kDa β-glucan reduced the postprandial glycemia more than the 145 kDa β-glucan, but the glycemia response of the beverage with smaller volume and thus higher viscosity did not differ from the one of larger volume (Kwong et al. 2013).

A correlation between the β-glucan MW and glycemia attenuation was observed in a study of 12 healthy subjects (Regand et al, 2011). The subjects

consumed 8 different study meals with either a dose of 6.2 grams of β-glucan with a MW of 2133, 435 or 57 kDa or a wheat control with a minimum of 0.6 grams of β-glucan each ingested with either 40 or 60 grams of starch. It was observed that the iAUC value of postprandial glycemia was significantly higher after all the 60-gram starch dose formulations compared to the 40-gram starch dose formulations. The 2133 kDa β-glucan lowered the glycemia more efficiently with both the 40-gram and 60-gram dose formulations compared to the 57 kDa β-glucan or to the control, while the effect was significant with the 435 kDa β-glucan compared to the 57 kDa β-glucan and control meal only with the 40-gram starch dose formulation (Regand et al. 2011). Recently, the effect of MW on the glycemia-reducing properties of β-glucan was clearly demonstrated in a study of 16 healthy adults (Wolever, 2020). It was observed that when a 4-gram dose of β-glucan was taken before consumption of a white wheat bread breakfast, a lower Log MW and Log viscosity values were associated with a higher incremental AUC value of postprandial glucose from 0 to 45 minutes after the meal and with a shorter time to reach the peak value of postprandial glucose (Wolever et al. 2020a). Yet, since the conclusion was based on post hoc calculations, they confirmed the result with another breakfast study. In that study, 28 healthy adults consumed a breakfast meal consisting of instant oatmeal with either 3 grams of oat bran (having 2 grams of β-glucan), 10 grams of oat bran (having 4 grams of β-glucan) or 10 grams of oat bran with added *β*-glucanase for the reason to reduce the β-glucan MW or white bread and milk with hot rice as a control. It was observed that not only did the AUC values and the peak rise of both glucose and insulin, but both were significantly less after all oatmeal groups compared to the rice control. Also, they were significantly less after 4 grams of β-glucan meal compared to the meal with 2 grams of β-glucan and the meal treated with β-glucanase (Wolever et al. 2020b).

The effect of β-glucan MW on the perceived satiety has also been studied. A study of 48 healthy volunteers compared the effects of 3 isocaloric breakfasts being instant oatmeal, traditional oatmeal and an oat-based, ready-to-eat breakfast cereal. Instant oatmeal and traditional oatmeal had a higher β-glucan MW than cereal (at 389 and 378 kDa compared to 221 kDa). The higher viscosity of the meals was measured by an *in vitro* digestion procedure, and it correlated to the MW and to the hunger perceptions of the volunteers. Instant oatmeal increased fullness, suppressed the desire to eat and reduced prospective intake more than the ready-to-eat breakfast cereal over four hours, and traditional oatmeal reduced prospective intake more than the ready-to-eat breakfast cereal (Rebello et al. 2014). A similar study compared the postprandial glycemia and appetite ratings and gastric emptying measured with a MRI scan of healthy volunteers after 2 oatmeals prepared with oat flakes or oat flour. It was seen that the flake oatmeal induced a lower glucose response compared to flour oatmeal

and a similar trend was also seen in appetite and gastric emptying (Mackie et al. 2017). Another study examined the satiety perception of 29 healthy volunteers after 6 different beverages with a varying amount and viscosity of β-glucan and varying energy content. The exact MW was not determined. It was concluded that the satiety increased in relation to the amount and viscosity of β-glucan, but the energy content did not have an effect (Lyly et al. 2010). A postprandial study followed 14 healthy volunteers consuming 5 cereal and milk bowls with different doses of β-glucan in a randomized order. It was observed that β-glucan decreased insulin secretion for 2 hours in a dose-dependent manner and a dose of 2.2 g of β-glucan increased perceived satiety and a dose of 5 grams reduced the subsequent meal energy intake by 400 kJ (Beck et al. 2009). Also, Wolever et al. followed the appetite of the subjects in their recent glycemia study of 28 volunteers as discussed earlier (2020). The gastric emptying of the volunteers was measured with a 13C breath test. While no differences were detected in the prospective lunch intake or subject appetite between the meals of different viscosities, it was observed that 4 grams of a β-glucan meal delayed the half-time of gastric emptying compared to the control (Wolever et al. 2020b). In contrast, a study of 20 healthy volunteers reported greater satiety perception after consumption of a beverage with β-glucan with lowered viscosity compared to a beverage with native β-glucan (Juvonen et al. 2009). However, the gastric emptying was faster after the beverage with β-glucan with lowered viscosity. The exact MW of β-glucan was not determined. It was concluded that β-glucan is likely to be able to increase the sensation of satiety even with reduced viscosity. Furthermore, it was discussed if the increase of the viscosity can be delayed and was not properly detected during the measurement time (Juvonen et al. 2009, Lyly et al. 2010).

Overall, the studies have shown a clear correlation between the viscosity and MW and the health-promoting effect of β-glucan (Table 3). There is not one critical MW to maintain the bioactive properties, but a MW range can be estimated in order to reach the maximum health benefit. Roughly, β-glucan with a higher MW (based on the MWs chosen to the studies discussed that are above 500 kDa) is more likely to reach the postdrandial glycemia and cholesterol level-maintaining effects, while lower MWs are associated with a release of beneficial phenolic compounds (2.1.3). This MW-health effect relation can be balanced and used in future food solutions.

## 2.3 Oat fibers and gut microbiota

### 2.3.1 Microbiota composition and microbial metabolites

The human gut microbiota comprises of large variety of microorganisms of which number is estimated to exceed $1 \times 10^{14}$ (Thursby and Juge 2017). The microbial community serves a great benefit to its host by providing energy both for the host and directly for the intestinal epithelium cells and by protecting the host against pathogens and improving immunity (Thursby and Juge 2017). The online database of the Human Microbiome Project listed 2172 microbila species from human beings (Hugon et al. 2015). These species were classified mostly as from the Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes phyla (Hugon et al. 2015). The microbiota composition varies largely among individuals and is affected by several factors, such as nutrition and dietary habits, the living environment, antibiotic treatments and diseases (Thursby and Juge 2017).

Previous studies have outlined the impact of dietary fiber on the gut microbiota ( reviewed by Zeng et al. 2014, Cronin et al. 2021). As dietary fibers are not digested in the small intestine, they proceed to the colon and are fermented by microbes. Fermentation of dietary fibers produces beneficial compounds for both the microbiota and the host and promotes the growth of the microbiota (Cronin et al. 2021). Instead, a lack of dietary fiber in the diet leads to a less diverse microbial community. Additionally, the decreased colonialization of beneficial bacteria leaves more space to possibly harmful microbes (Koc et al. 2020).

Short-chain fatty acids (SCFAs) are beneficial compounds produced by microbes as a result of dietary fiber fermentation. Three major SCFAs are acetate, propionate and butyrate. SCFAs serve as an important energy source for the colon enterocyte cells. They play a role in crucial processes such as glucose and lipid metabolism and in satiety regulation (den Besten et al. 2013). In addition, they are associated with several other health-promoting effects including anti-pathogenic effects (O'Keefe 2016, Louis et al. 2014). The production of SCFAs is dependent on the microbiota composition and the type and amount of carbohydrates consumed (Cronin et al. 2021). Another example of a dietary fiber-related microbial compound is β-glucuronidase, a microbial enzyme that mediates the reactivation of metabolites that are inactivated by conjugation in liver. Such metabolites are, for example, toxic carcinogens whose increased activities in the colon have been associated with a higher incidence of gastrointestinal diseases such as colon cancer, Crohn’s disease and colitis as well as to high-fat diets (Pellock and Redinbo 2017). High-fiber diets are associated with a decreased activity of β-glucuronidase (Shen et al. 2012, McIntosh et al.

2003). Dietary fiber has been also shown to decrease the production of ammonia, a toxic compound produced in the deamination of amino acids (McIntosh et al. 2003, Lupton and Marchant 1989, Conlon et al. 2012).

The prebiotic effect of oat fibers on the gut microbiota has been studied in vitro (Kedia et al. 2009). The fermentation rates of oat bran, glucose and fructo-oligosaccharide in human fecal microbiota fermentation were compared in vitro. It was reported that fermentation of oat bran and fructo-oligosaccharide decreased culturable anaerobes and clostridia and increased bifidobacteria and lactobacilli populations compared to glucose. Oat bran and fructo-oligosaccharide also induced higher butyrate production compared to glucose, while the oat bran-induced production of propionate was higher compared to both glucose and fructo-oligosaccharide (Kedia et al. 2009). In another study. the effect of the fecal sample donor on the microbial fermentation of oat, rye, corn and wheat was demonstrated in vitro (Brahma et al. 2017). The donor group 1 had a habitual diet high in beneficial nutrients, such as dietary fiber, plant protein, thiamin, riboflavin, folate, iron, magnesium and zinc, while the donor group 2 had a nutritionally poorer habitual diet. It was found that the microbiota of group 1 had higher carbohydrate utilization and butyrate production compared to group 2. The fermentation of the cereals only differed in group 1, in which fermentation of rye resulted in significantly higher butyrate production compared to other cereals and the fermentation of oats and rye resulted in lower ammonia production compared to other cereals (Brahma et al. 2017).

The impact of oat consumption on the gut microbiota has also been studied *in vivo* (Berger et al. 2014). Increases of lactobacilli and of butyrate and propionate production after an oat fiber-containing diet compared to a high-fat diet has been illustrated in a mice study (Berger et al. 2014). Recently, the impact of oats and rye consumption on gut microbiota and microbial metabolites was demonstrated on mice fed with a western diet (Kundi et al., 2020). Both oat and rye fibers were beneficial for the microbiota compared to the western diet. At the phylum level, the proportion of Bacteroides was increased and the Bacteroides:Firmicutes ratio was greater after consumption of oats or rye compared to the western diet. At the genus level, oat consumption increased the amount of *Lactobacillus* spp, while rye consumption led to a significant increase in *Bifidobacterium* spp. Both oat and rye also increased the production of SCFAs (Kundi et al., 2020). In addition, similar changes were also observed in a study comparing the microbiota and SCFA production in hamsters after an oat- and buckwheat-containing diet when compared to a high-fat diet (Sun et al. 2019). The production of acetate, propionate, butyrate and the total concentration of SCFAs was higher after the oat- and buckwheat-containing diet compared to the high-fat diet. Yet, the results of the microbiota composition were controversial to the results of Kundi et al. The hamsters fed with an oat-buckwheat diet had a lower abundance in

Bacteroidetes and the Bacteroidetes/Firmicutes ratio was lower compared to the high-fat diet. However, they reported rather large variation in the gut bacteria compositions (Sun et al. 2019).

Furthermore, the effect of oat β-glucan on the gut microbiota has been scarcely studied in clinical trials of healthy subjects. Valeur et al. (2016) measured the breath hydrogen produced following lactulose ingestion, fecal excretion of SCFA, fecal levels of urease and β-galactosidase and rectal levels of Prostaglandin E2 ($PGE_2$) as a host inflammation marker in 10 healthy subjects after 1-week daily consumption of oat porridge. After such a short intervention, a decrease in fecal urease and β-galactosidase was observed as well as a non-significant decrease in the inflammation marker $PGE_2$ (Valeur et al. 2016). Yet, the microbiota composition was not determined. Another study measured the fecal microbiota of 32 individuals with an increased risk for metabolic syndrome after a 6-week daily consumption of either whole-grain oat granola or non-whole-grain cereal. Significant increases in the relative abundance of fecal bifidobacteria, lactobacilli and total bacterial count were observed after the oat intervention period compared to non-whole grain period (Connolly et al. 2016).

Recently, a scientific interest towards the relation of viscosity and MW on the prebiotic effect of β-glucan has been raised. A study compared *in vitro* human fecal fermentation of steamed β-glucan with a higher MW and microwaved β-glucan with a lower MW (Dong et al. 2020). It was reported that the microwaved β-glucan with a lower MW induced a higher promoting effect on *Lactobacillus spp* and *Bifidobacterium spp* and also exhibited higher butyrate and overall SCFA production compared to steamed β-glucan (Dong et al. 2020). It must be noted though that the fecal samples of only three healthy donors were analyzed, so further studies are needed. Earlier, a study compared the effects of oat flakes of two different sizes, oligofructose and cellulose, on human fecal microbiota *in vitro.* Both oat flakes had an impact on the microbiota composition as the smaller size increased the amount of *Prevotella* spp and the larger size oat flake increased *Bifidobacterium* spp. Moreover, the larger-sized flake resulted in significant increases in propionate and butyrate concentrations after 24 hours of fermentation (Connolly, Lovegrove and Tuohy 2010). The effect of β-glucan MW on SCFA production was also compared in a mice study (Immerstrand et al. 2010). While no differences were observed in the total SCFA production, they noted a significant positive correlation between the MW of β-glucan (2348, 1311, 241, 56, 21 or >10 kDa) and a ratio of (propionic acid + butyric acid)/acetic acid (Immerstrand et al. 2010).

#### 2.3.1.1 Breath gas measurements

Microbial carbohydrate fermentation also produces intestinal gas. A healthy human has on average 100 mL of intestinal gas ranging from 30 to 200 mL, which consist of hydrogen ($H_2$), carbon dioxide ($CO_2$) and methane ($CH_4$), and of lesser amounts of oxygen ($O_2$), nitrogen ($N_2$), hydrogen sulfide ($H_2S$), indole, skatole and ammonia ($NH_3$). Of these, $H_2$ and $CH_4$ are exclusively products of microbial carbohydrate fermentation (Rezaie et al. 2017). Their production varies individually and depends on the microbiota composition and activity. $CH_4$-producers have more methatogens in their microbiota that convert $H_2$ to $CH_4$. (Ong et al. 2010). A small portion of the intestinal gas passes to the blood circulation and is exhaled through the lungs. Therefore, the measurement of breath gases can be used to indicate intestinal gas production (Gibson et al. 1990).

$CO_2$ has been used in the measurement of gastric emptying (Steinert, Meyer-Gerspach and Beglinger 2012, Wolnerhanssen et al., Wolever et al. 2020b). It was first observed that the rate of gastric emptying correlated with the recovery of $^{14}CO_2$ from the breath (Ghoos et al. 1993, Maes et al. 1998). Later, the isotope $^{14}C$ in the labelled test meals was replaced with $^{13}C$, as it was more stable and safe to use also with children and pregnant women (Choi et al. 1997). Yet, the measurement is not direct, since the substrate has to be digested, absorbed and metabolized before the excretion. Thus, the time of gastric emptying was modeled with a mathematical formula (Ghoos et al. 1993), which is still used in recent studies (Wolever et al. 2020b).

It was noted already in early studies that the consumption of oats produces higher breath hydrogen responses compared to glucose in healthy subjects (Behall et al. 1998, Hallfrisch and Behall 2003). Also, a positive correlation between AUC values of hydrogen production and the amount of ingested dietary fiber has been proposed. A study observed higher breath hydrogen production in adults with metabolic syndrome (n = 15) after consumption of semolina porridge with added arabinoxylan, rye kernels or both compared to plain semolina porridge (Hartvigsen et al. 2014). Another postprandial cross-over study of healthy men (n = 12) reported that whole grain rye kernel bread or boiled rye kernels as an evening meal induced higher breath hydrogen at the following morning compared to white wheat bread (Ibrugger et al. 2014). The breath hydrogen production in healthy adults (n = 21) was also compared after consumption of 6 rye porridge breakfasts containing varying amount of fiber and protein. They concluded as well that the amount of breath hydrogen increased proportionally to the dietary fiber content (Lee et al. 2016a). Moreover, it was suggested that the breath hydrogen concentration may be related to the production of SCFAs. The concentration of SCFAs in plasma (Hartvigsen et al. 2014) and in feces (Ibrugger et al. 2014) was observed to raise simultaneously with elevated breath hydrogen, when soluble fiber was consumed.

## 2.4 Oats and gastrointestinal diseases

### 2.4.1 Gluten-related diseases

Gluten-related disorders refers to a number of disorders related to a gluten ingestion. Most importantly, this includes celiac disease (CeD) and non-celiac gluten sensitivity (NCGS). During the past decades, the prevalence of CeD and NCGS has increased worldwide (1–1.5% and 0.6–6% of the world population, respectively) probably partly due to involvement of improved screening methods (Caio et al. 2020, Caio et al. 2019). The increased prevalence of gluten-related diseases is also seen in the raising demand of gluten-free products. According to a report by Satafood (2018), the consumer demand of gluten-free products is currently rising by 15 to 30% per year (Laurila and Saarinen 2018). Celiac disease is a chronic, systemic autoimmune disorder, which occurs in genetically susceptible individuals (Marietta, David and Murray 2011). Exposure to gluten proteins damages their intestinal mucosa, which distracts the nutrient absorption and causes gastrointestinal and extra-intestinal symptoms. Individuals with NCGS perceive similar symptoms after exposition to gluten, but they are not related to intestinal mucosa impairment. The etiology of the NCGS is unknown (Caio et al. 2020).

Currently, the only treatment to these disorders is the avoidance of gluten found in wheat, rye and barley. Therefore, a fiber source to the diet must be found elsewhere. Gluten-free oats may serve an important source of fiber in the gluten-free diet (GFD). In Finland, the consumption of pure oats has been allowed for adult CeD subjects since 1997 and for pediatric patients since 2000, and, currently, most of the Finnish CeD subjects consume pure oats as a part of their diet (Aaltonen et al. 2017, Alakoski et al. 2020). Pure oats are grown, milled, handled and stored without contamination of any other cereals. The understanding of pure-oat manufacturing is still rather new, since only a decade ago, gluten cross-contamination was common in the oat supply chains in Europe, the United States and Canada (Hernando et al. 2008). Therefore, it is explicable why a singular counterargument against the safety of oats in a GFD is risen every now and then. Still, study after study states that oats are well tolerated among CeD subjects (Aaltonen et al. 2017), and it has been suggested that the studies questioning the safety of oats to the CeD subjects may have used contaminated oats instead of pure (Fritz and Chen 2018).

Celiac disease and obeying a GFD diet have been linked to a suboptimal microbiota composition. The reported harmful alterations of CeD microbiota have been increased abundances of Gram-negative bacteria, such as Proteobacteria and Bacteroidetes, and reduced abundances of *Bifidobacterium* spp. and *Lactobacillus* spp. (Lin and Zhang 2017). Similar alterations were also

observed in the microbiota of healthy subjects after 1 month on GFD (De Palma et al. 2009, Marasco et al. 2016) and on CeD subjects in remission and on GFD (Viitasalo et al. 2018, Wacklin et al. 2014, Collado et al. 2009). However, of all these studies reporting a harmful microbiota alteration among CeD subjects and subjects obeying GFD and of the studies reviewed by Marasco et al., only Wacklin et al. stated that the subjects used oats as a part of their diet.

### 2.4.2 Intestinal inflammation

Inflammation is a part of the defense mechanism of the body's immune system against pathogens (Ferrero-Miliani et al. 2007). In some disorders, the inflammatory response becomes continuous, and a chronic inflammatory disease may develop. Recently, the role of oat β-glucan in the prevention and treatment of intestinal inflammation occurring in disorders such as ulcerative colitis, inflammatory bowel disease and celiac disease has been discussed (Nie, Lin and Luo 2017). β-glucan has been shown to reduce the intestinal inflammation in rat studies (Zyla et al. 2019, Blaszczyk et al. 2015, Suchecka et al. 2016). Specifically, a viscous layer formed by β-glucan with a high MW protects the inflamed intestinal tissue from irritation, while β-glucan with a low MW modulates the immune cell function at a molecular level (Zyla et al. 2019, Blaszczyk et al. 2015, Suchecka et al. 2016). In a study of 22 patients with ulcerative colitis, it was observed that a 60-gram daily consumption of oat bran for 4 weeks decreased the abdominal pain and reflux compared to their habitual diet (Hallert et al. 2003). Moreover, no disease relapse or gastrointestinal complaints was reported during the trial. The authors suggested that butyrate may be able to modulate intestinal epithelial cell transcription factors, such as NF-KB, known to be increased in inflammatory bowel disease and thus possess anti-inflammatory activity. These results indicate that oats may be feasible in alleviation of symptoms of inflammatory bowel diseases (Hallert et al. 2003).

### 2.4.3 Irritable bowel syndrome

Irritable bowel syndrome (IBS) is the most commonly diagnosed gastrointestinal condition worldwide with a prevalence varying from 7% of the population in Southeast Asia to 21% in South America (Chey, Kurlander and Eswaran 2015). The exact pathological cause of the disorder is unknown, and the diagnosis is based on symptoms and exclusion of other diseases. The IBS patients can be distributed to three subtypes being IBS with diarrhea, IBS with constipation and IBS with a mixed type of bowel pattern. In addition, the symptoms may be related to the gut-brain function and psychosocial distress, such as a harmful alteration of gut microbiota or anxiety and depression, respectively. The

spectrum of symptoms varies greatly among patients (Chey et al. 2015). Moreover, IBS has been associated with small intestinal bacterial overgrowth (SIBO) (Quigley 2014).

Studies have shown that certain carbohydrates may trigger the symptoms of IBS patients (Bohn et al. 2015, McIntosh et al. 2017). Those have been determined as "fermentable oligo-, di-, mono-saccharides and polyols" or FODMAPs for short (Figure 7). They are commonly found in pulses and certain vegetables, e.g., cabbages and onions, some fruits, such as apples and pears, and cereals such as rye, barley and wheat. Also lactose, from milk products; fructose; and polyols used as sweeteners, such as sorbitol, are known to trigger the symptoms (McIntosh et al. 2017). The mechanism of the FODMAP symptom triggering is unclear, but a few theories have been presented. The large bowel hypothesis suggests that FODMAPs are not digested in the small intestine, and therefore they end up in the colon and are fermented by the colon microbes. The rapid fermentation process produces intestinal gas, which has been proposed to induce the intestinal symptoms, such as bloating, flatulence and pain (Major et al. 2017). The higher production of intestinal gas can be detected from breath (2.3.2), and earlier studies supporting the large bowel hypothesis have reported higher hydrogen production among the IBS patients compared to healthy subjects after consumption of foods both high and low in FODMAP compounds (Ong et al. 2010, Huang, Jia and Liu 2019). Yet, as also the colonial fermentation of non-FODMAP carbohydrates produces gas, the large bowel hypothesis only partly explains the mechanism of FODMAP symptom triggering.

The small bowel hypothesis claims that non-digested carbohydrates are osmotically active and draw water into the small intestine, which causes bloating and discomfort. A higher water content also accelerates the oro-cecal transit and reduces small-intestinal absorption (Major et al. 2017). The symptoms of the IBS patients could then be explained by visceral hypersensitivity, i.e., the IBS patients would be more sensitive to intestinal distension caused by gas or water (Major et al. 2017, Spiller 2017, Mavroudis et al. 2020). According to a review, the visceral hypersensitivity plays a role in several functional gut disorders but cannot still explain the entire clinical syndrome (Azpiroz 2002).

The longer polymers, such as oat β-glucan, develop a viscous mass instead of drawing water in the intestine (Spiller 2017, Makela et al. 2020). The viscous mass binds intestinal water, which soothes the bulk in the colon and thus lowers the rate of colon extension (Atzler et al. 2021). Furthermore, the fermentation of the long chains of β-glucan occurs in a less rapid manner than FODMAP compounds, such as FOS and GOS with small molecular structures. Therefore, as longer polymers are fermented over a longer time period, the colonial extension may be less sudden and the polymers, such as β-glucan, well-tolerated.

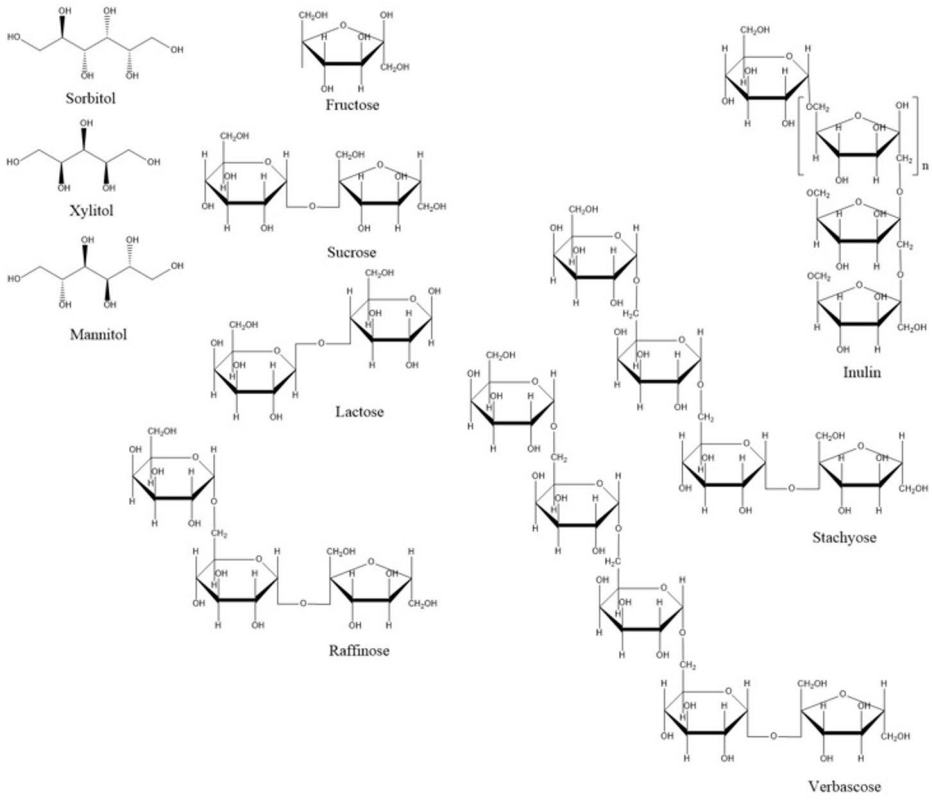


**Fig 7**. Structures of some FODMAP compounds.

Studies have also presented that the gut microbiota of the IBS patients may differ from that of healthy subjects (Rajilic-Stojanovic et al. 2011, Jeffery et al. 2012). However, since the microbiota composition is known to change within the dietary habits, such as the type and amount of carbohydrates consumed (Wu et al. 2011), it cannot be ruled out that the changes could be caused by the studied low-FODMAP diet rather than the IBS as such. In fact, the observed changes in the microbiota of the IBS patients are similar to those observed in celiac disease patients and healthy subjects following a gluten-free diet, where wheat, rye and barley are excluded. In both IBS and celiac disease patients avoiding the common sources of whole grains, an increased abundance of *Bacteroides* spp. and decreased abundance of *Bifidobacterium* spp. have been reported (Jeffery et al. 2012, Rajilic-Stojanovic et al. 2011, Lin and Zhang 2017, De Palma et al. 2009). This noted, oats may serve as an important source of fiber with a prebiotic effect for the individuals following a diet excluding FODMAPs, which are known to stimulate the growth of the beneficial microbes (Lim, Ferguson and Tannock 2005).

### 2.4.4 Perceived gut well-being

Gut well-being has a great influence on general everyday welfare. Perceived symptoms may be a sign of stress or food intolerance but also an indicator of intestinal problems. The diagnosis of certain intestinal disorders, e.g., NCGS and IBS, relies completely on perceived gut symptoms. Still, the subjective experiences are rather challenging to measure and to put into a scientific context. Firstly, the perceptions vary greatly among individuals. A few studies have indicated that the perceptions differ between the genders (Mayer et al. 1999, Mavroudis et al. 2020, Camilleri 2020b). A review by Mayer et al. reported that women were more eager to report gastrointestinal symptoms and pain in general. It was also suggested that the menstrual cycle may affect female symptom perception (Mayer et al. 1999). A recent review summarized that the differences in sex hormones, serotonergic mechanisms, pain pathways and psychosocial issues between male and women are reflected in symptom perception of IBS patients mainly as greater pain perception of females. This should be taken into account in the diagnosis and treatment of IBS. Moreover, the premenopausal compared to postmenopausal state in women should be taken into account in the further studies of IBS (Camilleri 2020b).

Recently, an ingestible pH, temperature and pressure measuring capsule, Smartpill®, was introduced to nutritional intervention trials to link the perceived symptoms to the physiological environment of the intestinal tract (Nuora et al. 2018, Pirkola et al. 2018) among others (Timm et al. 2011, Willis et al. 2011). The temperature is used to follow the capsule ingestion and exiting, while the pH is an indicator of the part of the gastrointestinal tract. Intestinal pressure is used to track and calculate the peristaltics of the GI tract. Linking the pressure to the gut perceptions would provide valuable information, especially in disorders where liquid or gas accumulates in the intestine. However, researchers have not been able to link the timepoints of intense pressure to the timepoint of symptoms perceived. Would such a correlation exist, the measured pressure during perception of symptoms could serve as a physiological marker in the diagnosis of certain intestinal conditions and food intolerances besides a symptom-based criteria. Therefore, further studies are still needed.

If the etiology of the disorder is unknown, the diagnosis has to lean to the subjective symptom perception. Consensus of the criteria for IBS was reached in 1989 and named the Rome criteria. The criteria were first based on four symptoms frequently occurring in IBS patients being bloating, pain relief with bowel action and more frequent and looser stools with the onset of pain. Later, the criteria has been redefined several times, and the Rome I, II, III and IV criteria have minor differences in the spectrum and frequency of the symptoms, (Camilleri, 2020a). Despite the attempts to link the symptoms to gut microbiota

alterations, intestinal gas production or visceral hypersensitivity, no unambiguous biomarker has been found so far (2.4.2).

Also, the diagnosis of non-celiac gluten sensitivity (NCGS) is based mainly on symptom perception. Normally, the disorder is diagnosed by excluding the possibility of celiac disease and then with the follow-up of the Salerno criteria (Catassi et al. 2015). However, serum zonulin, a protein involved in the regulation of the paracellular permeability in the intestine, has recently been proposed as a biomarker of NCGS (Barbaro et al. 2020). Yet, thus far, following the Salerno intervention involves 6 weeks on a gluten-containing diet followed by 6 weeks of a GFD and a further 1 week of a test period containing GFD supplemented with either gluten test meals or placebo, a 1-week washout and another test period in a cross-over manner. Symptoms are reported during the whole intervention and a 30% variation of symptoms between the GFD and gluten-containing diet periods is required for the NCGS status (Catassi et al. 2015). The method is currently recommended even to child patients (Devulapalli, 2020). Still, despite a strict GFD, subjects with NCGS seem to report a high number of gastrointestinal symptoms according to recent studies. In study of Tovoli et al. (2019), 66% of NCGS subjects diagnosed according to the Salerno criteria reported symptoms even years after a GFD. Of the CeD subjects following the same diet, the corresponding percent was 33%. A similar observation was made in the study of Skodje et al. (2019), in which self-diagnosed NCGS subjects reported a high number gastrointestinal symptoms.

Thus, it seems that despite a carefully composed criteria, the link between symptoms and gluten avoidance in NCGS is not that straightforward. Recently, it has been proposed that instead of gluten, other wheat components such as fructans or amylase–trypsin inhibitors may cause the symptoms (Skodje et al. 2018, Reig-Otero, Manes and Manyes 2018) and even the term NCGS has been proposed to be changed to non-celiac wheat sensitivity (Dale, Biesiekierski and Lied 2019). Furthermore, while the physiological reason behind the symptoms of NCGS is not known, also the composition of GFD and the reasons for following the diet are somewhat unclear to self-diagnosers in general. In a recent survey study, 24% of responded Australians avoided gluten completely or partially, while 14% had self-reported non-celiac wheat sensitivity and 1% had celiac disease (Potter et al. 2020). Others avoided gluten for “general health” or as a treatment of abdominal pain without being diagnosed with CeD or NCGS. The authors supposed that the general gluten avoidance may be linked to the current well-being trend and NCGS may overlap with other gastrointestinal diseases (Potter et al. 2020). Thus, it seems that gluten avoidance is seen as a trendy solution to maintain generally a better health status.

**Table 1** The bioactive properties of avenanthramides and phenolic acids.

| ***Study design*** | ***n*** | ***Impact of AVAs / phenolics*** | ***Conclusions*** | ***Ref.*** |
|---|---|---|---|---|
| ***In vitro studies*** | | | | |
| *In vitro* antioxidant properties of oat phenolic acids and AVAs. | - | Caffeic acid, ferulic acid derivatives, vanillic acid, vanillin and AVAs alone and in combinations with other phenolics had the highest antioxidant activity of oats phenolics. | Caffeic acid, ferulic acid derivatives, vanillic acid, vanillin and AVAs have antioxidant activity. | (Emmons et al. 1999) |
| *In vitro* $Cu^{2+}$-induced oxidation of human LDL in presence of oat bran-derived phenolics and vitamin C. | - | The lag time of LDL oxidation ↑. | Oat phenolics combined with vitamin C enhance the resistance against LDL oxidation. | (Chen et al. 2004) |
| *In vitro* human cells with TNF-α-induced inflammation and muscle atrophy were treated with AVAs. | - | AVAs reduced proinflammatory cytokine and reactive oxygen species production. | AVAs reduce the inflammation and muscle atrophy. | (Yeo et al. 2019) |
| *In vitro* human colon cancer cell lines were treated with AVAs. | - | AVAs inhibited inflammation-associated proliferation of colonic cancer cells. | AVAs have activity against colon cancer risk. | (Guo et al. 2010) |
| *In vitro* lung cancer cell lines were treated with phenolic acids containing ethanol extract of mushroom *Clitocybe alexandri*. | - | Cinnamic acid was identified as the most potent growth inhibitor. | Cinnamic acid has anticancer activity. | (Vaz et al. 2012) |
| *In vitro* lung, colon and hepatocellular cell lines were treated with *p*-coumaric and cinnamic acids and their derivatives. | - | Organic acids showed weak activity against the cell lines, but their glucuronide and methylated derivatives had moderate activity. | p-coumaric and cinnamic acid glucuronide and methylated derivatives have anticancer activity. | (Heleno et al. 2014) |
| *In vitro* antimicrobial effect of phenolic acids was evaluated. | - | Caffeic, ferulic, 2,4-dihydroxybenzoic, vanillic, syringic, cinnamic and *p*-coumaric acids showed antimicrobial activity against Gram-positive and Gram-negative bacteria species. | Caffeic, ferulic, 2,4-dihydroxybenzoic, vanillic, syringic, cinnamic and *p*-coumaric acids have antimicrobial activity. | (Alves et al. 2013) |

| *Study design* | *n* | *Impact of AVAs / phenolics* | *Conclusions* | *Ref.* |
|---|---|---|---|---|
| ***Animal studies*** | | | | |
| Mice with $CCl_4$-induced chronic liver injury were were treated with vanillic or syringic acid by intravenous administration twice a week for 4 weeks. | - | Syringic and vanillic acids suppressed the hepatic inflammation, collagen accumulation and liver fibrogenesis. | Syringic and vanillic acids have hepatoprotective effect. | (Itoh et al. 2010) |
| Mice with lipopolysaccharide-induced liver inflammation were fed with oat bran extracts for 20 days. | 16 | MDA, aspartate transaminase and alanine transaminase ↓<br>SOD, CAT and GPX enzymes ↑ | Oat extracts have anti-inflammatory, antioxidant and hepatoprotective effect. | (Debnath et al. 2019) |
| Mice with ethanol-induced liver injury were treated intragastially with oat extract for 12 days. | - | Aspartate transaminase and alanine transaminase ↓<br>SOD, CAT, and GSH ↑ | Oat extracts have anti-inflammatory, antioxidant and hepatoprotective effect. | (Mir et al. 2018) |
| Mice with stress-induced kidney damage resulting from an estrogen deficiency were fed with a diet enriched with oat grains for 60 days. | - | Weight gain ↓<br>SOD, CAT, GPX and GSH ↑ | Oats have antioxidant effect and may prevent obesity and kidney oxidative damage during menopause. | (Ltaif et al. 2020) |
| Rats were fed either AVA-supplemented diet or control diet for 50 days and divided to exercised or rested groups. | 48 | SOD activity in the deep portion of DVL, liver and kidney ↑<br>GPX in the heart and DVL↑ | AVAs demonstrated antioxidant function against exercise-induced oxidative stress. | (Ji et al. 2003) |
| Mice with Ehrlich solid tumors were fed by oral gavage with AVAs for 2 weeks. | 75 | Tumor volume ↓ MDA, aspartate transaminase and alanine transaminase, serum cholesterol and triglycerides ↓<br>SOD, CAT and GSH ↑ | AVAs have antitumor and antioxidant activity. | (Aldubayan et al. 2019) |
| Mice were fed with high-fat diet combined with AVAs for 8 weeks. | 84 | Weight gain ↓<br>LDL, total cholesterol, insulin, blood glucose ↓<br>SOD, CAT and GPX ↑<br>Harmful bacteria of microbiota ↓ | AVAs alter body weight by reducing oxidative stress and inflammation and by improving blood lipid profile and by regulating the gut microflora. | (Zhang et al. 2020b) |

| *Study design* | *n* | *Impact of AVAs / phenolics* | *Conclusions* | *Ref.* |
|---|---|---|---|---|
| Mice had an induced an ear edema or topical irritation to back skin and treated with AVAs; Human epidermal cells were treated with AVAs. | 20 | Mice: Inflammation and number of scratches ↓ Cells treated with AVAs regulate NF-κB signaling and IL-8 production. | AVAs demonstrate anti-inflammatory and anti-itch activity. | (Sur et al. 2008) |
| ***Human studies*** | | | | |
| Healthy adults consumed AVA-enriched skim milk for breakfast. | 6 | Plasma GSH ↑ | AVAs increase antioxidant capacity. | (Chen et al. 2007) |
| Healhty postmenopausal women consumed for 8 weeks daily an AVA-enriched cookie or a control cookie and walked downhill on a treadmill before and after dietary intervention. | 16 | Plasma total antioxidant capacity ↑ Erythrocyte SOD activity ↑ | AVA consumption attenuates exercise-induced inflammation measured post-exercise. | (Koenig et al. 2014) |
| Young adults consumed AVA-enriched cookies or control cookies daily for 8 weeks and ran downhill before and after dietary intervention. | 24 | Circulatory inflammatory cytokines ↓ Chemokines and cell adhesion molecules ↓ | AVA consumption attenuates exercise-induced inflammation measured post-exercise. | (Zhang et al. 2020a) |
| Healthy adults consumed AVA-containing capsules or control/placebo for 1 month. | 120 | SOD and GSH ↑ MDA ↓ Total cholesterol, triglycerides and LDL ↓ HDL ↑ | AVAs demonstrated antioxidant and cholesterol-lowering activity. | (Liu et al. 2011) |
| Hypercholesterolemic adults consumed oat porridge daily for 4 weeks. | - | CRP, interleukin-6, interleukin-8, and TNF-α↓ antioxidant capacity ↑ | Oat consumption decreased inflammation marker levels and increased antioxidant capacity. | (Pavadhgul et al. 2019) |

| *Study design* | *n* | *Impact of AVAs / phenolics* | *Conclusions* | *Ref.* |
|---|---|---|---|---|
| Adults with a risk of cardiovascular disease consumed oat phenolics-enriched oatmeal or cake daily for 4 weeks or a control. | 28 | Total cholesterol and LDL ↓<br>Day time and nighttime systolic and diastolic blood pressure ↓ compared to baseline | Oat phenolics affect on blood pressure and cholesterol levels. | (Soycan et al. 2020) |
| Adults with chronic irritant hand eczema were treated with colloidal oatmeal 1% cream or base cream for 4 weeks after 2 weeks treatment with fluocinolone. | 50 | Prevention in the return of the symptoms<br>Improvement in the Dermatology Life Quality Index compared to a control group | Oats have an ameliorative effect on symptoms in patients with chronic irritant hand eczema. | (Sobhan et al. 2020) |

AVA = avenanthramide, CAT = catalase, SOD = superoxide dismutase, GSH, reduced glutathione, GPX = glutathione peroxidase, CRP = C-reactive protein (inflammation marker), TNF = tumor necrosis factor, DVL = vastus lateralis muscle, MDA = malondialdehyde, LDL = low-density lipoprotein, HDL = high-density lipoprotein, NF-κB = nuclear factor controlling cell survival and cytokines, IL-8 = pro-inflammatory cytokine, ↑ = increased, ↓ = decreased

**Table 2**. Accepted health claims for oats by the EU and their conditions (EFSA Panel on Dietetic Products 2011).

| ***Oat health claim*** | ***Condition*** | ***Accepted*** |
|---|---|---|
| Oat beta-glucan has been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease. | Foods should provide at least 3 g of oat β-glucan per day. | 2009 |
| Beta-glucans contribute to the maintenance of normal blood cholesterol levels. | Foods should provide at least 3 g per day of β-glucans from oats, oat bran, barley, barley bran or from mixtures of non-processed or minimally processed β-glucans in one or more servings. | 2009 |
| Consumption of beta-glucans from oats or barley as part of a meal contributes to the reduction of the blood glucose rise after that meal. | 4 g of β-glucan from oats for each 30 g of available carbohydrates should be consumed per meal. | 2011 |
| Oat grain fiber contributes to an increase in fecal bulk. | Foods should be at least "high in fiber" from oats as per Annex to Regulation (EC) No 1924/2006 (i.e., a claim that a food is high in fiber may only be made where the product contains at least 6 g of fiber per 100 g or at least 3 g of fiber per 100 kcal). | 2011 |

**Table 3** Studies examining the effect of molecular weight and viscosity on the bioactive properties of β-glucan.

| ***A. The relation of molecular weight, viscosity and the bile acid binding capacity and cholesterol-lowering effect of β-glucan.*** | | | | | |
|---|---|---|---|---|---|
| ***Study design*** | ***n*** | ***Method*** | ***β-glucan MW / kDa*** | ***Results*** | ***Ref.*** |
| *In vitro* study of oxidized barley β-glucan | - | Colorimetric method | Highest 600-700<br>560-575<br>240-250<br>150-190<br>75-98<br>Lowest 37-55 | Oxidation → MW↓, BA-binding capacity ↓ | (Lee et al. 2016b) |
| *In vitro* study of sulfated oat β-glucan | - | Colorimetric method | High 130<br>Low 68 | Sulfation → MW↓, BA-binding capacity ↓ | (Chang et al. 2006) |
| *In vitro* study of muffins containing oat β-glucan | - | Centrifugation method | High 560<br>Medium 277<br>Low 157 | MW ↓ → BA-binding ↑ | (Sayar et al. 2011) |
| *In vitro* study of oat β-glucan | - | Centrifugation method | High 687<br>Low 156 | MW ↓ → BA-binding ↑ | (Kim and White 2011) |
| *In vitro* study of native and modified oat and barley β-glucan | - | Dialysis membrane model | High oat/barley 1584/1300<br>Modified oat/barley 6-275 | MW ↑ → Viscosity ↑ → BA-binding ↑ | (Marasca et al. 2020) |

| | | | | | |
|---|---|---|---|---|---|
| *In vitro* study of native and modified oat β-glucan | - | Gut digestion model | High 1000<br>Medium 200-500<br>Low <100 | MW ↑ → Viscosity ↑ → BA binding ↑ | (Rosa-Sibakov et al. 2020) |
| Mice on an atherogenic and a western diet mimicking diet were fed with oat β-glucan with varying MW for 4 weeks. | 110 | Blood cholesterol screening | 2348, 1311, 241, 56, 21, <10 | No correlation between the MW and the cholesterol-lowering effect | (Immerstrand et al. 2010) |
| Diabetic mice were fed with β-glucan with varying MWs. | 40 | Blood and liver cholesterol screening | 743, 635, 172 | MW ↑ → Cholesterol ↓ in serum and in liver | (Zhao et al. 2014) |
| Healthy adults consumed bread containing high or low MW oat β-glucan daily as a part of habitual diet for 3 weeks. | 14 | Blood cholesterol screening | High 797<br>Low 217 | No correlation between the MW and the cholesterol lowering effect. | (Frank et al. 2004) |
| Healthy adults consumed cereal containing oat β-glucan twice a day as a part of habitual diet for 4 weeks. | 345 | Blood cholesterol screening | High 2210<br>Medium 530/850<br>Low 210 | High and Medium MW → LDL ↓ | (Wolever et al. 2010) |
| Mildly hypercholesterolic adults consumed high or low MW barley β-glucan daily for 5 weeks as a part of controlled diet. | 30 | GC-MS, fecal samples | High 1359<br>Low 288 | High MW → fecal excretion of lithocholic acid ↑ | (Thandapilly et al. 2018) |

| | | | | | |
|---|---|---|---|---|---|
| Mildly hypercholesterolic adults consumed daily breakfast for 5 weeks containing barley β-glucan either 3 g high MW, 5 g low MW or 3 g low MW or a control diet. | 30 | Blood cholesterol, genotype analysis | 1349, 290 | High MW > Low MW → cholesterol ↓ the cholesterol-lowering effect was linked to the genotype. | (Wang et al. 2016) |
| ***B. The relation of molecular weight, viscosity and the glycemia attenuating and the satiety increasing effects of β-glucan.*** | | | | | |
| ***Study design*** | ***n*** | ***Method*** | ***β-glucan MW / kDa*** | ***Results*** | ***Ref.*** |
| Healthy volunteers consumed 6 beverages with varying energy content, β-glucan amount and viscosity in a randomized order. | 29 | Appetite ratings | Exact MWs not determined | Fiber amount ↑ → Viscosity ↑ → Satiety ↑<br>Energy content did not affect satiety. | (Lyly et al. 2010) |
| Healthy volunteers consumed 2 oat-bran enriched beverages with different viscosities for breakfast in a randomized order. | 20 | Appetite ratings, postprandial glycemia, gastric emptying by paracetamol absorption | Exact MWs not determined | Viscosity ↓ → Blood glucose and insulin ↑ → Satiety ↑<br>Viscosity ↓ → faster gastric emptying | (Juvonen et al. 2009) |
| Healthy volunteers consumed 8 meals with either a dose of 6.2 g of β-glucan with 3 different MWs or a wheat control with a minimum of 0.6 g of β-glucan with each combined with 40 or 60 g of starch. | 12 | iAUC of postprandial glycemia | 2133, 435, 57 | Starch amount ↑ → iAUC of postprandial glycemia ↑<br>MW↑ → iAUC of postprandial glycemia ↓ | (Regand et al. 2011) |

| | | | | | |
|---|---|---|---|---|---|
| Healthy volunteers consumed a beverage with 50 g of glucose and either of β-glucan with low MW, high MW or no of β-glucan and either a volume of 250 mL or 600 mL. | 15 | Postprandial glycemia | 145, 580 | MW ↑ → postprandial glycemia ↓ The volume had no effect on glycemia. | (Kwong et al. 2013) |
| Healthy volunteers consumed (1) instant oatmeal, (2) traditional oatmeal and (3) oat-based, ready-to-eat breakfast cereal for breakfast in randomized order. | 48 | Appetite ratings for following 4 h, meal viscosity and β-glucan characteristics | (1) 389, (2) 378 (3) 221 | (1) Increased the fullness, suppressed desire to eat and (1) and (2) reduced prospective intake compared to (3) | (Rebello et al. 2014) |
| Healthy volunteers consumed β-glucans with varying MWs or a glass of water before eating white wheat bread. | 16 | Postprandial blood glucose, posthoc calculations | 52, 76, 153, 393, 841, 1980 | Log(MW) ↑ → Log(Viscosity) ↑ → iAUC of postprandial glycemia ↓ time to reach the peak value of postprandial glucose ↑ | (Wolever et al. 2020a) |
| Healthy volunteers consumed instant oatmeals with (1) 3 g or (2) 10 g of oat bran or with (3) 10 g of oat bran with reduced viscosity or (4) a low-fiber control meal in a randomized order. | 28 | Appetite ratings, postprandial glycemia, gastric emptying, subsequent meal intake | (1) 1990 (2) 2260 (3) < 10 | MW↑ → postprandial glycemia ↓ gastric emptying half-time ↑ Appetite ratings and subsequent meal intake did not differ between the meals. | (Wolever et al. 2020b) |

MW = Molecular weight, iAUC = incremental area under curve, ↑ = increased, ↓ = decreased

# 3 AIMS OF THE STUDY

The aim of this study was to investigate the effect of dietary oats on markers of their intestinal behavior and the resulting perceived gut health in three separate clinical trials. The first trial was an observational study that followed the effect of habitual oat consumption on gut microbiota composition and function in subjects with gluten-related disorders and healthy controls. The second trial examined the effect of processing by enzyme treatment on postprandial gastrointestinal behavior of oats in healthy subjects. The third trial examined the postprandial microbial fermentation of oats combined with pulses in subjects with self-reported pulse-related sensitivity and healthy controls. All three studies compared the obtained physiological data on self-reported gastrointestinal symptoms perceived by the subjects.

The aims of the separate trials were:

I. To research the effect of habitual oat consumption on gut microbiota composition, SCFA production, markers of unhealthy gut and gastrointestinal symptoms in subjects with celiac disease or gluten sensitivity compared to healthy controls.

II. To study how the enzyme treatment of oats affects, postprandially, the excretion of bile acids and cholesterol derivatives to feces, the excretion of phenolic compounds to urine, intestinal pressure and gastrointestinal symptoms.

III. To investigate the effect of simultaneous ingestion of oats with pulses on pulse-induced gastrointestinal symptoms and on the fermentation rate of the meal (as detected from breath hydrogen and methane) in subjects with self-reported sensitivity to pulses compared to healthy controls.

# 4 MATERIALS AND METHODS

## 4.1 Study design and ethical considerations

Study I was an observational study in which subjects with celiac disease, non-celiac gluten sensitivity and healthy controls fulfilled gut symptom diaries for 30 days and a food diary for 4 days and donated a fecal sample for the microbiota, SCFAs, β-glucuronidase and ammoniacal nitrogen analyses.

Study II was a double-blind, randomized, cross-over intervention study that consisted of three 4-day intervention periods with ≥ 2 weeks washout period between them. Each period started with a 48-hour run-in period with a low-phenolics and a low-fiber diet, the study day with three meals (breakfast, lunch and dinner with study oat product) and a follow-up of 24 hours. A Smartpill® capsule was ingested together with the study breakfast. A 24-hour urine sample was collected before (the control sample) and after (the study sample) the study breakfast. In addition, subjects collected a fecal sample when the Smartpill® capsule exited. The subjects were asked to keep a gut symptom diary 5 days before and after the study day including the study day.

Study III was a double-blind, randomized, cross-over intervention study that consisted of two 4-day intervention periods with ≥ 2 weeks washout period between them. Subjects were asked to avoid pulses for two weeks before each intervention period. For the first two days of the intervention period, the diet was restricted to include only food ingredients causing a low intestinal gas production (a low-FODMAP diet) supplemented with a study product twice a day. For the third day, the low-FODMAP diet was restricted further to be low-phenolic, low-fiber and non-dairy including the study product twice a day. On the fourth day, the subjects had the 2 portions of the study product for breakfast and the breath hydrogen and methane were measured for 8 hours every 15 minutes. Lunch, consisting of rice and chicken or rice and eggs for a vegetarian choice, was served after 4 hours of the first breath gas measurement. Subjects kept a food diary for the first 3 days of the intervention period and gut symptom and defecation diaries during the 4-day intervention period.

The study plans were approved by the Ethics Committee of the Hospital District of Southwest Finland, and each of studies were registered at ClinicalTrial.gov with the identifiers being NCT02761785 (Study I), NCT02764931 (Study II) and NCT04273659 (Study III). The study subjects gave their written informed consent for the study, and each subject was free to discontinue their participation at any phase of the study without explanation.

## 4.2 Study subjects

For each study, study subjects were recruited from the Turku (Finland) area. The inclusion criteria were an adult age of 18–64 and a normal to overweight body mass index (BMI) between 18.5–30.

For Study I, healthy subjects and subjects with celiac disease in a remission state as being on a GFD at least 1 year or non-celiac gluten sensitivity were eligible. Additional exclusion criteria were antibiotic treatment within the previous or 6 months, blood donation or participation to other clinical study within 1 month or use of any medication with gastrointestinal interactions (e.g., laxatives or proton pump inhibitors). The subjects were ascertained to be in good health by means of self-reporting and through screening blood tests for normal liver, kidney and thyroid functions, cholesterol, blood count, fasting glucose, wheat allergy and celiac disease antibodies. For Study I, 74 subjects were recruited and, of which, 49 completed the study. Six subjects were excluded based on the food diaries and, thus, the final study groups were 19 subjects with celiac disease, 10 subjects with NCGS and 14 healthy subjects.

For Study II, only healthy adult subjects were recruited. Additional exclusion criteria were antibiotic treatment within the previous 3 months, blood donation or participation to other clinical study within 1 month or use of any medication with gastrointestinal interactions (e.g., laxatives or proton pump inhibitors) and any gastrointestinal disorder or surgery. The subjects were ascertained to be in good health by means of self-reporting and through screening blood tests for normal liver, kidney and thyroid functions, cholesterol, blood count, fasting glucose, wheat allergy and celiac disease antibodies. For Study II, 14 healthy subjects completed the study, of which, 10 ingested the Smartpill® capsule and 4 only participated in the other legs of the study due to personal preference.

For Study III, healthy subjects and subjects with IBS or self-reported sensitivity for pulses were eligible. Additional exclusion criteria were antibiotic treatment within the previous 3 months, blood donation or participation to other clinical study within 1 month or use of any medication with gastrointestinal interactions (e.g., laxatives or proton pump inhibitors). The subjects were ascertained to be in good health by means of self-reporting and through screening blood tests for normal liver, kidney and thyroid functions, cholesterol and celiac disease antibodies. For Study III, 61 subjects were recruited and, of which, 50 subjects were allocated to the interventions. Six subjects interrupted the trial due to personal reasons, and two were excluded based on analysis of the food diary (1) or outlying breath results (1). 21 subjects with self-reported sensitivity to pulse products and 21 healthy controls completed the study.

## 4.3 Study diets

In Studies I and III, the overall diet quality was assessed by a FFQ validated for evaluation of diet quality index (Leppala et al. 2010). The questionnaire contains 18 questions regarding the frequency and amount of consumption of food products during the preceding week. The quality of the diet was defined as poor when index points were less than 10 out of the maximum 15 points and good when points were 10 or more.

In Study I, all study subjects reported consumption of oat products on a daily basis. Their everyday dietary habits were followed through 4-day food diaries. The energy and macronutrients intakes were calculated with software (AivoDiet 2.0.2.3; Aivo, Turku, Finland), which utilized the food composition database provided by the Finnish National Institute for Health and Welfare.

In Study II, the study product consisted of untreated oat bran concentrate (control) or oat bran concentrate that was treated with 1 or 50 nkat/g dm of β-glucanase for 2 hours at 50°C and then heated to 90°C to inactivate the enzymes. The molecular weight of β-glucan in untreated product was >1000 kDa (high MW), 200–500 kDa in the 1 nkat/g dm treated product (medium MW) and >100 kDa in the 50 nkat/g dm treated product (low MW). The products were freeze-dried, ground and packed in servings consisting of 9.33 g dietary fiber and stored at -20°C until analyzed. On the morning of the test day, the study subjects ate the study breakfast, which included the study product mixed with 3 dL of lactose-free, fat-free yogurt. Lunch and a portion of the study meal were consumed six hours later and dinner with an additional study product meal 10–12 h after the breakfast. Thus, volunteers consumed a total amount of 28 g of oat fiber from the study meals during each study day. The low-phenolic study diet supplemented with the oat products was continued the following day until the capsule was exited and/or the fecal sample was collected.

In Study III, the study product was a spoonable pulse-based product supplemented with either rice flour mixed with rice protein or fiber-rich oat flour. The products were flavoured either with fruit or with berry jam. The participants were given one of both flavors daily. The pulse products were manufactured by Valio LtD for the study purposes only and frozen (-18°C) until provided to the subjects in identical packages. The products were matched for energy, fat, proteins and digestible carbohydrates content differing mainly in the amount of dietary fiber. The flour ingredients were purchased commercially. The nutrient compositions of product with fruit jam, the product with berry jam, rice mix and oat flour were analyzed separately. After homogenization of the sample, the moisture, fat and ash contents were analyzed with a gravimetric method according to SFS-EN ISO/IEC 17025:2017. The protein content was analyzed with the Kjeldahl method according to SFS-EN ISO/IEC 17025:2017. The total

carbohydrates and energy content were calculated based on the other nutrient content analyzes. The total dietary fiber and the amount of soluble and insoluble fiber were analyzed by an enzymatic-gravimetric method according to AOAC 991.43, ISO/IEC 17025:2005. The amount of vesbascose, raffinose and stachyose were analyzed by HPAEC-PAD based on the ISO-EN 22184 & IDF 244 and ICUMSA method GS4/8-19 (2005). Glucose, fructose, saccharose, maltose and galactose were analyzed by HPAEC-PAD according to ISO/IEC 17025:2017. The total amount of β-glucan was analyzed only from oat flour with enzymatic-spectrophotometric method. The study diet was continued until the participant finished the breath measurements of the fourth day. Food for the third day of the intervention was provided to the participants together with a list of allowed and avoided foods.

## 4.4 Methods

### 4.4.1 Microbiota and microbiota metabolites analysis (I)

Microbial DNA was extracted from fecal samples using the repeated bead beating with the KingFisher® method as described in detail previously (Nylund et al. 2010). The quality of the DNA was good in all samples, namely, an OD 260/280 ratio ≥ 1.8. For the NGS library preparation, the recommended protocol for preparing 16S ribosomal RNA gene amplicons for the Illumina MiSeq system was used (Illumina 2013), and the preparation was started from 12.5 ng of total DNA. The libraries were sequenced in a single 2x300 bp run with Illumina MiSeq instrument using v3 sequencing chemistry.

The amounts of fecal SCFAs were measured by solid-phase microextraction coupled to gas chromatography and mass spectrometry (SPME-GC-MS). Fecal samples (0.1 g) were weighted and suspended into 5 mL of deionized water by vortexing. 1.5 mL of fecal suspension was added into a 10 mL vial with 0.5 g of $NaH_2PO_4$ (Fiorini et al. 2015). Acetic acid, propanoic acid and butyric acid (Sigma-Aldrich, WGK Germany) were used as external standards in order to control the daily variation of instrument and sample preparation. The SPME fiber used was a 75-µm CAR/PDMS, Fused Silica (Supelco, Bellefonte, PA). The SPME-GC-MS analysis was carried out with Thermo Trace 1310 – TSQ 8000 Evo equipped with autosampler (Thermo Scientific, Wilmington, DE). Compounds were separated by a Supelco-fused silica capillary column SPB-624, (30 m x 0.25 mm x 1.4 µm) under a carrier gas (helium) 1 mL/min with a splitless mode. The oven temperature program was as follows: 40°C hold for 2 min and then 5°C/min rise until 200°C, hold for 10 min. A voltage of 70 eV was set in the EI. The system was operated using Xcalibur 4.0 (Thermo Scientific,

Wilmington, DE). Compounds were identified by the NIST library (accessed July and August 2018) and quantified by comparing to external standards.

An ammoniacal nitrogen assay was carried out by an indophenol blue method reported in detail elsewhere (Koroleff 1966). Briefly, 0.1 g of wet fecal sample was diluted with 5 mL of deionized water, shaken for 60 min and centrifuged at 3000 g for 3 minutes. The ammoniacal nitrogen concentration was measured from the supernatant based on absorbance measured at 630 nm (Hidex Sense microplate reader, Hidex Oy, Turku, Finland). The β-glucuronidase assay was carried out by the protocol of Shen (Shen et al. 2012). Briefly, 0.1 g of wet fecal sample was diluted with 5 mL of deionized water and shaken for 60 min. 0.1 ml of diluted sample was added into an Eppendorf tube® with 0.4 ml of 2 mM p-nitrophenyl-β-d glucuronide solution (Sigma Aldrich, WGK Germany). Suspensions were incubated in anaerobic conditions at 37°C for 60 minutes followed by addition of 0.5 mL of 0.5 M NaOH. This suspension was centrifuged at 3200 g for 10 min and absorbance 194 was measured on 405 nm (Hidex Sense microplate reader, Hidex Oy, Turku, Finland).

### 4.4.2 LC-MS analysis of fecal bile acids (II)

Fecal samples were freeze-dried and homogenized by mortar and pestle. 5 mg of dry fecal powder was extracted and sonicated with 1000 µL of methanol-containing internal standards. The sample was centrifuged for 10 min at 24 400 g and the supernatant was dried under nitrogen flow (John et al. 2014). Samples were redissolved in 200 µL of methanol for UHPLC-QTOF-MS analysis. The UHPLC-QTOF system consisted of Elute UHPLC paired with Impact II QTOF system (Bruker Daltonik GmbH, Bremen, Germany). Analytes were separated by an Accucore$^{TM}$ Polar Premium HPLC column (2.6 m, 150 mm × 2.1 mm i.d.) attached to Accucore$^{TM}$ Polar Premium defender guard column (Thermo Fischer Scientific Inc., Waltham, MA, USA) at 20°C with a flow rate of 300 µL/min. The mobile phase A was water and B methanol with both containing 0.2% formic acid and 10 mM ammonium acetate. The time and gradient percentage of mobile phase B was the following: 0 min, 60%; 2 min, 60%; 18 min, 95%; 22 min, 100%; 30 min, 100%; 33 min, 60% and 43 min, 60%. The injection volume for all samples was 2 µL. The ESI source was operated in positive mode with the following settings: ESI capillary voltage: 4500 V; ion source temperature: 250°C; and dry gas (nitrogen) 10 l/min. The system was controlled by Compass HyStar software (Bruker Daltonik GmbH, Bremen, Germany).

### 4.4.3 LC-MS/MS analysis of oat phenolic compounds (II)

To extract the AVAs, oat bran (2.5 g) was extracted twice with 17.5 mL of 80% methanol for 1 h at RT by using the Stuart roller mixer (Cole-Parmer, Staffordshire, UK). Samples were centrifuged for 10 min, 600 g at RT, and supernatants were combined and dried under reduced pressure at temperature not exceeding 40°C. Extracts were redissolved in 1.5 mL of methanol and filtered through a 0.2-µm PTFE filter and analyzed by UHPLC-MS/MS (Bryngelsson et al. 2002).

To extract phenolic acids, oat bran (0.1 g) was dissolved in 3 mL of 0.2 M HCL and extracted twice with 6 mL of ethyl acetate for 1 h using the Stuart roller mixer. Samples were centrifuged for 10 min at 600 x g. Supernatants were combined and left to stand over anhydrous $NaSO_4$ followed by filtering. Samples were dried under reduced pressure at temperature not exceeding 40°C. Extracts were redissolved to 1 mL of methanol and analyzed by UHPLC-MS/MS (Multari et al. 2018).

Urine samples were stored at -80°C until analyzed. Samples were acidified and hydrolyzed by using a β-glucuronidase and arylsulfatase enzyme mix (*Helix Pomatia)* for 16 h at 37°C (Vetrani et al. 2014) and extacted with OASIS® HLB 1 cc Cartridges (Waters Corp., Milford, MA, USA) with 1 mL methanol.

A LC-MS/MS method was developed on the basis of the method of Schar. The UHPLC-ESI-MS/MS system consisted of an Acquity UPLC (Waters) coupled with a Xevo TQ-S electrospray ionization mass spectrometer (Waters) operated by Masslynx software (V. 4.1, Waters Inc, USA). Compound separation was performed using an Aquity UPLC HSS T3 1.8-µm column (2.1 x 100 mm) attached to a VanGuard pre-column of the same material and pore size. The column oven temperature was set to 45°C and the flow rate was 0.65 mL/min with a sample injection volume being 2 µL. The mobile phase consisted of: A) 0.1/99.9 v/v formic acid in water and B) 0.1/99.9 v/v formic acid in acetonitrile. The gradient was the following: 1% B at 0 min, 1% B at 1 min, 30% B at 10 min, 95% B at 12 min, 95% B at 13 min, 1% B at 13.10 min and 1% B at 16 min. A scheduled multiple reaction monitoring (sMRM) method was created based on syringe infusion of 16 standards by using Masslynx Intellistart software (V. 4.1, Waters Inc, USA) to determine MRM transitions, collision energies and MRM modes (positive or negative ionization). The most intense MRM transition was chosen for the quantification for each analyte. Exact masses were determined in the UHPLC-QTOF system (Bruker Daltonik GmbH, Bremen, Germany) using the same LC conditions. The ESI source was operated in positive mode with the following settings: ESI capillary voltage: 4500 V; ion source temperature: 300°C and dry gas (nitrogen) at 12 L/min. The concentrations of phenolic compounds in control samples were subtracted from the concentrations of phenolic

compounds in the study samples in order to focus only on oat-derived compounds. Concentrations were proportioned by the concentration of urine creatinine.

### 4.4.4 Intestinal pressure and transit time (II)

The SmartPill® GI monitoring system (Given Imaging LTD., Yoqneam, Israel) consisted of an ingestible capsule (13 mm x 26 mm) and a data receiver that was instructed to be worn close to the body (Study II). The system recorded gastrointestinal pH, temperature and pressure. The temperature was used to follow the capsule ingestion and exiting. The pH was used to determine the timeframe of the parts of the gastrointestinal tract, and the pressure was an indicator of gut functions and gastrointestinal symptoms (Figure 8). Measurement data was uploaded to a computer with the help of a docking station and analyzed by the MotiliGI® program (Given Imaging LTD., Yoqneam, Israel) that provided the mean pressure, median pH and transit times based on changes in pH and temperature for the different parts of the gastrointestinal tract. In addition, pressure data provided by the MotiliGI® program was analyzed as areas under curve (AUC) and compared to the self-reported gut symptom data.

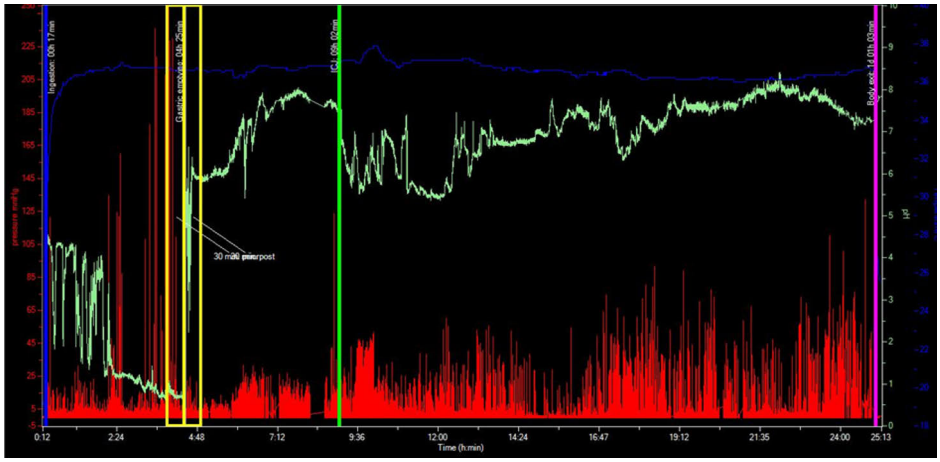


**Fig 8.** A typical SmartPill® data graph. Time of the activation of the capsule is shown on the X axis and the temperature, pH and pressure are shown on the Y axis. The blue largely horizontal line describes the temperature, the light green line the pH and the red bars the pressure. The blue vertical line represents the ingestion, the yellow lines are the transit from the stomach to the small intestine, the green line is the transit from the small intestine to the colon and the purple line is the exit from the digestive tract.

### 4.4.5 Breath gas measurements (III)

Breath hydrogen and methane were analyzed with the Gastrocheck gastrolyzer® device, Bedfont, GB, during the fourth day of each intervention (Study III). The device was calibrated monthly according to the instructions provided by the manufacturer. The participants exhaled to the mouthpiece of the device every 15 minutes for 8 hours. Time of occurrence and magnitude of peak gas production, the mean of the five highest gas values and the total area under the gas production curve for each subject were analyzed.

### 4.4.6 Symptom and defecation diaries

Volunteers reported gut symptoms in the Studies I, II and III. Perceived symptoms were reported for 1 month for Study I, 3 x 11 days for the Study II and 2 x 4 days for Study III. The symptoms listed in the diaries were upper abdominal pain, lower abdominal pain, cramping, bloating, flatulence, diarrhea, constipation, rumbling or another type of symptom. Volunteers were asked to fill the severity and the duration of the symptom. In Study III, the volunteers also filled the defecation diary during the 4-day interventions by reporting the time, date and stool form by Bristol scale (Heaton et al. 1992).

## 4.5 Statistical analyses

Statistical analyses of the data in all studies were carried out using IBM SPSS Statistics 25 software. Normal distribution of data was tested with Shapiro–Wilks test. In Study I, the differences between the study groups were compared with ANOVA (Analysis of variance) test with contrast. In Study II, the parameters were mostly not normally distributed and thus the differences between the meals were analyzed with non-parametric tests of related samples (Wilcoxon for multiple comparison, Friedman for paired comparisons). In Study III, the differences among the groups were compared with an ANOVA test and the parameters that were not normally distributed by the Mann-Whitney U test. The differences between the meals were analyzed by the Wilcoxon Rank test, and the correlations were made with the Spearman correlation test.

# 5 RESULTS AND DISCUSSION

## 5.1 Nutrient composition of the study meals and the diet quality and food consumption of the study subjects

The dietary quality, as assessed by the validated index of diet quality questionnaire in Studies I and III, was considered good in the study subjects with the average diet quality being close to 10 in both studies.

In Study I, based on food diaries, it was observed that subjects with NCGS received a higher proportion of their energy (E %) from fat and a lower proportion (E %) from carbohydrates when compared to healthy controls ($p = 0.025$ and $p = 0.045$, respectively). Additionally, subjects with NCGS tended to receive more energy than celiac disease patients when adjusted per body weight (kcal/kg of body weight, $p = 0.09$, data not shown). The mean intake of dietary fiber was at the lower end of the recommendation level in the three groups (CeD 25.5 ± 9.1, NCGS 27.6 ± 7.7, controls 26.0 ± 7.4 as g/day). This was in contrast to earlier studies in which the GFD has been observed to be associated with inadequate fiber and nutrient intake (Hallert et al. 2002, Thompson et al. 2005). The difference most likely resulted from the habitual oat consumption of the study subjects.

In Study II, the nutrient compositions of the study meals differed in the MW of β-glucan (High MW > 1000 kDa; MediumMW = 524 kDa and Low MW = 82 kDa), while in Study III, they differed in the amount of soluble fiber (meal with oat flour, 1.2%; meal with rice flour, 0.3%; w/w of soluble fiber in 125 g of study product, respectively).

## 5.2 Gluten-related conditions and gut microbiota (Study I)

In Study I, the gut microbiota composition and microbial metabolites of oat-using subjects with celiac disease and non-celiac gluten sensitivity were compared to healthy controls. Previous studies have reported of microbiota dysbiosis of CeD patients even in the remission phase of the disease (Marasco et al. 2016) as well as changes in bacteria abundances with an increase of Gram-negative bacteria, such as Proteobacteria and Bacteroidetes, and a decline of bifidobacteria and lactobacilli (Marasco et al. 2016). Similar changes have been reported after a 1-month consumption of GFD among healthy participants (Bonder et al. 2016, De Palma et al. 2009). However, the studies have mainly focused on pediatric CeD patients, while adult subjects with CeD or NCGS have received limited attention. Moreover, the oat consumption of the subjects has

scarcely been reported. In Study I, no signs of microbiota dysbiosis in CeD subjects or any major changes in NCGS subjects were detected (Figures 9, 10 ,12). The healthy controls tended to have a higher mean abundance of *Bifidobacterium* spp compared to CeD and NCGS subjects ($p = 0.067$) (Figure 11).

SCFAs are an important energy source for enterocytes and have been associated with several health-promoting effects (Louis et al. 2014, O'Keefe 2016). Their production varies among the individual microbiota compositions and by the type and amount of carbohydrates consumed (Pylkas, Juneja and Slavin 2005). The production of acetic acid was higher in the control group compared to the NCGS group ($p = 0.03$), which may be linked to the higher abundance of *Bifidobacterium* spp. in the control group compared to CeD and NCGS groups ($p = 0.067$). The production of propionic acid and butyric acid was comparable among the study groups (Table 4). Previously, the concentration of fecal SCFAs on pediatric CeD patients is reported to be lower compared to healthy subjects (Di Cagno et al. 2011). Yet, the SCFA production among adult patients with CeD or NCGS was analyzed the first time in this study.

The production of ammonia and the activity of β-glucuronidase were comparable among the study groups (Table 4). Ammonia is produced by the microbial breakdown of amino acids, and it is toxic in high amounts (Brahma et al. 2017, Lupton and Marchant 1989, McIntosh et al. 2003). β-glucuronidase is an enzyme produced by the gut microbes that reactivates the compounds that are inactivated by conjugation in liver. A high activity of the enzyme has been associated with gastrointestinal diseases (Pellock and Redinbo 2017). Previously, high consumption of fiber is reported to decrease the production of ammonia and β-glucuronidase (Shen et al. 2012, Freeman 1986, Brahma et al. 2017, Lupton and Marchant 1989, McIntosh et al. 2003).

Overall, the production of SCFAs, ammonia and β-glucuronidase was comparable to previous reports of healthy adults (Delgado et al. 2006, Garcia-Villalba et al. 2012, McOrist et al. 2011, Nemoto et al. 2012, Slavin and Feirtag 2011). No microbiota dysbiosis was detected among CeD or NCGS subjects, but the potential differences in the microbiota composition and function of subjects with CeD and NCGS should be studied further.

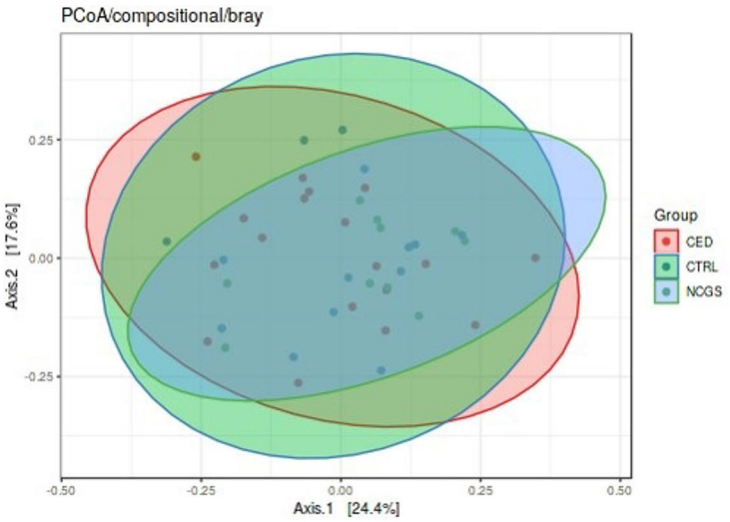


**Fig 9.** Total microbiota profiles did not differ among healthy subjects compared to subjects with CeD and NCGS as assessed by Principal Component Analysis (PCoA).

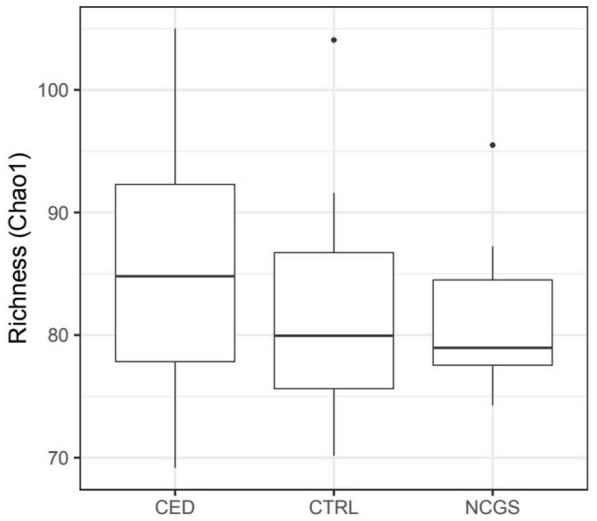


**Fig 10.** Microbiota richness did not differ among healthy subjects compared to subjects with CeD and NCGS. The box extends from 25$^{th}$ percentile to 75$^{th}$ percentile with a line at the median.

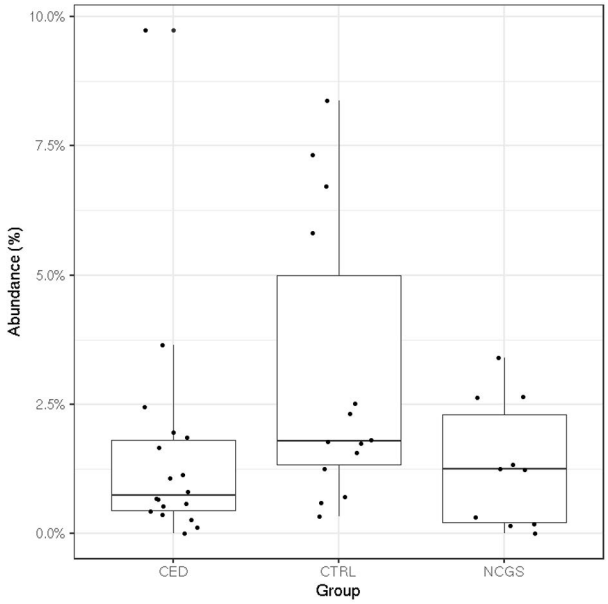


**Fig 11.** The mean relative abundance of *Bifidobacterium* in healthy subjects and subjects with Ced and NCGS. The difference between the three was borderline significant (p = 0.067, Kruskal–Wallis test).

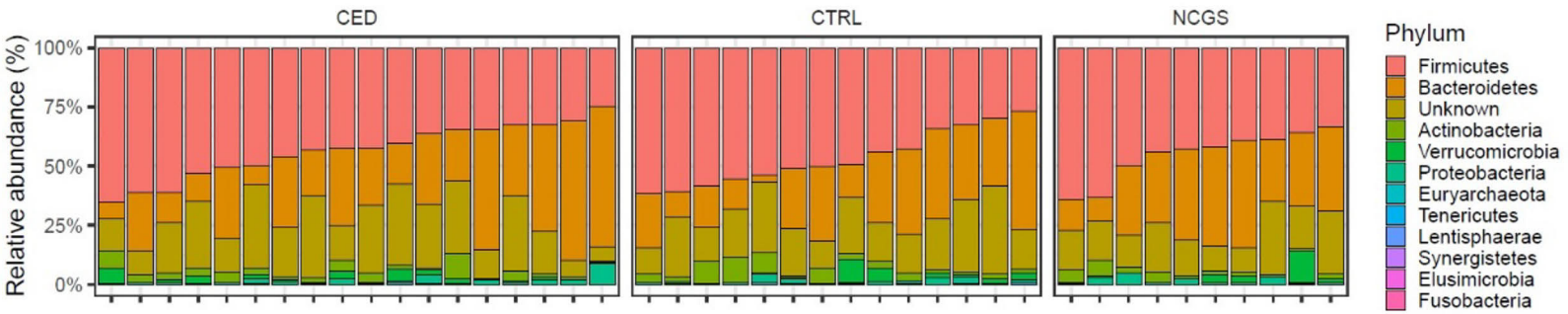


**Fig 12.** Relative abundances of bacterial phyla (% of total reads) in healthy subjects and subjects with CeD and NCGS. No statistically significant differences were observed among the study groups.

**Table 4**. Production of SCFAs, ammonia and the activity of β-glucuronidase in CeD and NCGS patients and healthy controls.

| | ***CeD*** | | ***NCGS*** | | ***CTRL*** | |
|---|---|---|---|---|---|---|
| | ***Concentration*** | ***% of total SCFA*** | ***Concentration*** | ***% of total SCFA*** | ***Concentration*** | ***% of total SCFA*** |
| Fecal acetic acid (µg) | 2144 (1228) | 63[ab] | 2149 (1205) | 59[a] | 2789 (1473) | 71[b] |
| Fecal propionic acid (µg) | 806 (607) | 23 | 948 (451) | 28 | 698 (521) | 19 |
| Fecal butyric acid (µg) | 337 (128) | 14 | 456 (258) | 13 | 424 (327) | 10 |
| Total SCFA (µg) | 3287 (1786) | | 3553 (1680) | | 3912 (2072) | |
| Fecal ammonia (µmol) | 18.0 (6.5) | | 18.5 (4.8) | | 15.7 (7.2) | |
| Fecal β-glucuronidase (U) | 30.0 (15.0) | | 25.9 (15.0) | | 29.9 (18.0) | |

Concentrations are presented per g of fecal wet weight. Values are presented as mean (SD). CeD = subjects with celiac disease, NCGS = subjects with non-celiac gluten sensitivity, CTRL = healthy controls and SCFA = short-chain fatty acids. Values with different letters differ from one another in each row.

## 5.3 Effect of bioprocessing on phenolic compounds in oat bran and the urine and fecal cholesterol derivatives (Study II)

### 5.3.1 Phenolic compounds

In Study II, the concentration of phenolic compounds of high, medium and low MW β-glucan oat bran concentrates were compared and the excretion of phenolics to urine was followed.

The concentrations of phenolic compounds were higher in the low MW β-glucan oat bran concentrate compared to the high MW and medium MW β-glucan bran concentrates that demonstrates the release of bound phenolic compounds by the enzyme treatment (Table 5). The most increased compound due to the release was ferulic acid, of which, the concentration was 2.08 ± 0.19, 0.38 ± 0.04 and 0.08 ± 0.02 μg/g in low MW, medium MW and high MW β-glucan bran concentrates, respectively. Besides ferulic acid, the most abundant compounds in oat bran concentrates were AVAs 2p, 2c and 2f. In addition, minor concentrations of *p*-coumaric acid, syringic acid, syringaldehyde, 2,4-dihydroxybenzoic acid and vanillin were detected from all the concentrates. The concentrations of each compound were comparable between the medium MW and high MW β-glucan oat bran concentrates (Table 5). Previously, a release of phenolic acids is reported in studies of bioprocessed rye and wheat (Lappi et al. 2013, Anson et al. 2009).

The release of bound ferulic acid was seen also in increased excretion in urine after ingestion of the low MW β-glucan meal compared to the medium MW and high MW β-glucan meals ($p < 0.001$ and $p < 0.001$, respectively) (Table 5). Ferulic acid was the most abundant oat-derived metabolite excreted in urine after ingestion of low MW and medium MW β-glucan meals but not after the high MW β-glucan meal. Beside ferulic acid, the most remarkable oat-derived metabolites in all urine samples were vanillic, homovanillic, syringic and 2-hydroxyhippuric acids. The concentration of AVA 2p was higher after consumption of the medium MW β-glucan meal compared to the low MW β-glucan meal ($p < 0.042$). The concentrations of other phenolic acids and AVAs were comparable after consumption of each oat extract. However, AVAs 2p and 2f were detected in very low concentrations while AVA 2c was not detected, which supports the theory that their main metabolic pathway is through their breakdown to phenolic acids (Schar et al. 2018, Wang et al. 2014, Liu 2007). It was observed that the urine metabolites vanillic, homovanillic, 2-hydroxyhippuric, anthranilic and isovanillic acids were absent from oat bran concentrates supporting the earlier findings that they are AVA derivatives (Schar

et al. 2018). In addition, the earlier finding was confirmed (Schar et al. 2018) that hippuric acid was the most common phenolic compound in urine, but its quantity was not increased in urine after ingestion of oat-based study meals indicating that it is not an oat metabolite (data not shown). These results demonstrated that the enzyme treatment of oats increases the bioavailability of phenolic compounds, which are known to have several beneficial bioactive properties (Table 1).

### 5.3.2 Fecal bile acid excretion

The concentrations of the two major bile acids, DCA and CDCA, were higher in feces after consumption of the high or medium MW β-glucan meal compared to the low MW β-glucan meal (Table 6). The concentration of DCA was higher after consumption of the high and medium MW β-glucan meal compared to the low MW β-glucan meal ($p < 0.02$ and $p < 0.03$, respectively). The concentration of CDCA was higher after consumption of the high MW meal and tended to differ after the medium MW β-glucan meal compared to the low MW β-glucan meal ($p < 0.02$ and $p < 0.06$, respectively). These results indicate that the bile acid binding capability of β-glucan is related to its MW. This is line with a result of a previous clinical study measuring LDL cholesterol after consumption of high MW, medium MW or low MW β-glucans with the LDL cholesterol of the participants being lower after consumption of high MW and medium MW β-glucans compared to the low MW β-glucan (Wolever et al. 2010).

The most abundant bile acid and cholesterol derivative compound excreted to feces was cholesterol followed by CDCA and DCA (Table 6). CA was only excreted in minor concentrations. In humans, CDCA and CA are the main primary bile acids, while DCA is a secondary bile acid derived from CA (Russell 2003, Hofmann 1999). DCA is known to circulate with primary bile acids and to be the dominant biliary acid in some adults (Hofmann 1999), which may explain its higher concentration over CA in these samples. The concentrations of cholesterol and cholesterol derivatives did not differ between the fecal samples, indicating that their excretion may be more dependent on the consumption of animal products (Table 6). However, the exclusion of animal products from a low-phenolics and low-fiber background was not reasonable. It is also worth noting that the genetic differences, e.g., an ApoE polymorphism, largely influence the proportion of ingested and excreted cholesterol coming from the diet (Marais 2019).

**Table 5.** Extractable phenolic compounds in oat bran concentrates (average of 3 parallel, no significances calculated) and their urine excretion (n = 14). Values with a different letter differ from one another within the urine samples of each row.

| *Compound* | *Oat bran concentrates µg × g$^{-1}$* | | | *Urine excretion ng × mL$^{-1}$ × creatinine$^{-1}$* | | |
|---|---|---|---|---|---|---|
| | *Low MW* | *Medium MW* | *High MW* | *Low MW* | *Medium MW* | *High MW* |
| Ferulic acid | 2.08 ± 0.19 | 0.38 ± 0.04 | 0.08 ± 0.02 | 35.47 ± 15.$^{64a}$ | 7.38 ± 4.11$^{b}$ | 2.37 ± 2.08$^{b}$ |
| Avenanthramide 2f | 0.71 ± 0 | 0.48 ± 0 | 0.48 ± 0.03 | 0.06 ± 0.03 | 0.10 ± 0.05 | 0.07 ± 0.04 |
| Avenanthramide 2c | 0.75 ± 0.001 | 0.48 ± 0 | 0.48± 0.03 | 0 | 0 | 0 |
| Avenanthramide 2p | 0.78 ± 0 | 0.54 ± 0 | 0.54 ± 0.03 | 0.20 ± 0.11$^{c}$ | 0.33 ± 0.11$^{d}$ | 0.23 ± 0.20$^{cd}$ |
| p-Coumaric acid | 0.15 ± 0.05 | 0.05± 0.01 | 0.03 ± 0 | 0.03 ± 0.04 | 0.06 ± 0.15 | 0.02 ± 0.04 |
| Syringic acid | 0.08 ± 0.03 | 0.04 ± 0.01 | 0.04 ± 0.006 | 3.87 ± 3.96 | 4.34 ± 2.78 | 3.20 ± 2.80 |
| Syringaldehyde | 0.02 ± 0.006 | 0.02 ± 0 | 0.02 ± 0 | 0.006 ± 0.01 | 0.02 ± 0.03 | 0.03 ± 0.04 |
| 2,5-dihydroxybenzoic acid | 0.02 ± 0 | 0 | 0 | 0.36 ± 0.67 | 0.19 ± 0.26 | 0.92 ± 2.80 |
| 2,4-dihydroxybenzoic acid | 0.05 ± 0.007 | 0.04 ± 0 | 0.04 ± 0 | 1.04 ± 2.61 | 1.35 ± 2.58 | 1.82 ± 3.21 |
| Anthranilic acid | 0 | 0 | 0 | 0.05 ± 0.12 | 0.14 ± 0.23 | 0.19 ± 0.32 |
| 2-hydroxyhippuric acid | 0 | 0 | 0 | 6.12 ± 5.80 | 6.65 ± 5.18 | 5.44 ± 6.93 |
| Vanillin | 0.15 ± 0.1 | 0.03 ± 0.007 | 0.04 ± 0 | 1.20 ± 6.01 | 0.03 ± 3.84 | 0.11 ± 11.1 |
| Homovanillic acid | 0 | 0 | 0 | 1.62 ± 3.57 | 2.63 ± 4.58 | 1.87 ± 4.68 |
| Isovanillic acid | 0 | 0 | 0 | 0.14 ± 0.15 | 0.10 ± 0.11 | 0.07 ± 0.10 |
| Vanillic acid | 0 | 0 | 0 | 5.55 ± 6.01 | 2.75 ± 3.84 | 3.86 ± 11.01 |
| Total concentration | 4.78 ± 0.39 | 2.05 ± 0.07 | 1.75 ± 0.11 | 55.7$^{e}$ ± 28.9 | 26.1$^{f}$ ± 16.4 | 20.2$^{f}$ ± 16.4 |

**Table 6.** Fecal excretion of bile acids and cholesterol derivatives µg × $mg^{-1}$ of dry weight (n = 14). Values with a different letter differ from one another in each row.

| | *Low MW* | | *Medium MW* | | *High MW* | |
|---|---|---|---|---|---|---|
| ***Compound*** | ***Average*** | ***Range*** | ***Average*** | ***Range*** | ***Average*** | ***Range*** |
| CDCA | 0.5[a] | 0.04–1.27 | 0.69[ab] | 0.24–1.20 | 0.78[b] | 0.33–2.24 |
| DCA | 1.49[c] | 0.22–3.28 | 1.95[d] | 0.88–3.58 | 2.3[d] | 1.03–6.01 |
| CA | 0.17 | 0.0–1.69 | 0.17 | 0.0–2.02 | 0.08 | 0.0–0.35 |
| Cholesterol | 5.96 | 1.22–19.06 | 7.54 | 1.45–18.41 | 6.33 | 1.31–17.17 |
| 7-hydroxycholesterol | 0.44 | 0.07–0.66 | 0.51 | 0.12–1.04 | 0.48 | 0.08–1.31 |
| Stigmasterol | 0.17 | 0.02–0.45 | 0.25 | 0.03–1.01 | 0.2 | 0.04–0.91 |
| Desmosterol | 0.014 | 0.0–0.08 | 0.025 | 0.0–0.09 | 0.016 | 0.0–0.09 |

### 5.3.3 Intestinal pH, pressure and transit time

In Study II, the intestinal pH, pressure and transit time were measured with an ingestible capsule, the Smartpill®. There were no statistical differences between the transit times of the whole gut or between the different parts of the gastrointestinal tract (Figure 13A). The median pH was comparable in all parts of the GI tract (Figure 13B). The mean pressure in the duodenum was higher after the high MW β-glucan meal compared to the medium MW β-glucan meal, $p < 0.041$, and compared to the low MW β-glucan meal, $p < 0.022$. Also, more contractions/min were measured in the duodenum after the high MW β-glucan meal compared to the medium MW β-glucan meal, $p < 0.013$, and compared to the low MW β-glucan meal, $p < 0.022$. A similar trend in mean pressure was observed in the small bowel, but the difference was not significant ($p = 0.154$) (Figure 13C). The mean pressures in the other parts of the GI tract and the contractions/min were comparable. The pressures calculated as areas under curve did not differ during the time that gut symptoms were perceived compared during the time without perceived symptoms (Figure 13D). In meals, a higher MW of β-glucan leads to increased viscosity (Du et al. 2019), which could explain the higher pressure measured in the duodenum. Previously, in the study of Timm (Timm et al. 2011), the gut transit times of ten healthy subjects were

compared after a 3-day consumption of high-fiber cereal or low-fiber cereal, and the colonial and whole gut transit times were significantly shorter after the consumption of high-fiber cereal. According to present results, the reduction of β-glucan MW did not have an effect on fiber behavior in the GI tract.

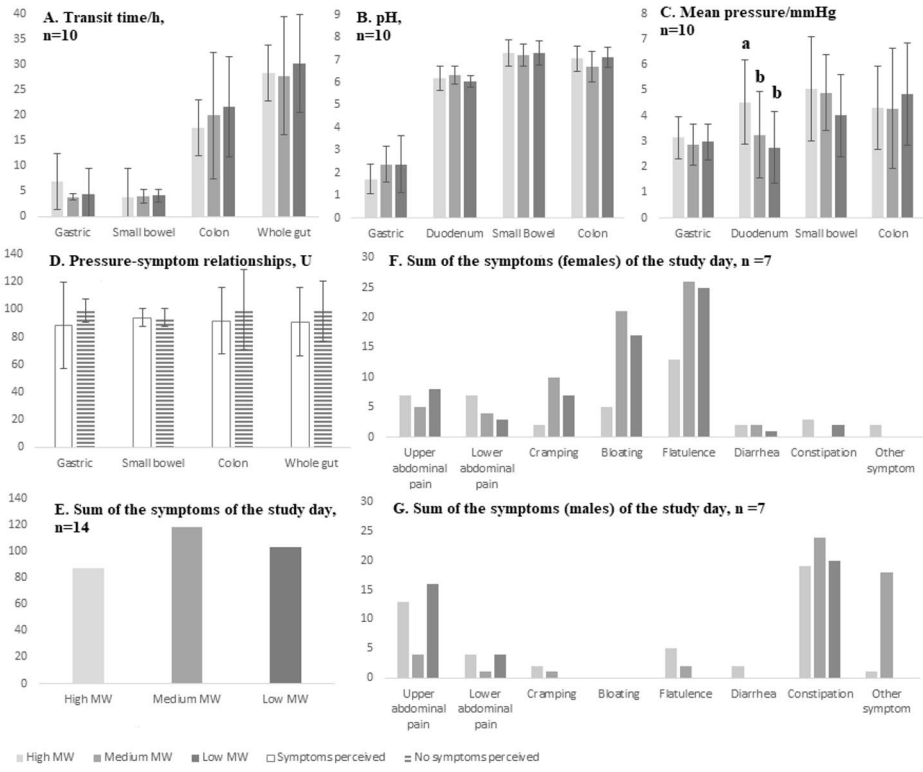


**Fig 13**. In Study II, the gastrointestinal transit time, pH and pressure were followed with an ingestible capsule. The measured values were compared to perceived gastrointestinal symptoms. **A**. Transit times/h, **B**. pH, **C**. Mean pressures / mmHg, **D**. The averages of the pressures as area under curve values of the time symptoms perceived compared to time of no symptoms perceived, **E**. Sum of the symptoms reported at the study day, **F**. Sum of the study day symptoms reported by females and **G**. Sum of the study day symptoms reported by males. Values with a different letter differ from each other.

## 5.4 Breath gas measurements (Study III)

The hydrogen production was higher after the oat flour meal compared to the rice flour meal in both the sensitive and control group (Figure 14). The measurement parameters were the area under curve (AUC) ($p = 0.001$ and $p = 0.001$ for the sensitive and control group, respectively), the highest value of the

hydrogen production ($p = 0.001$ and $p = 0.001$, respectively) and the average of five highest values ($p = 0.001$ and $p = 0.001$, respectively). The time of the hydrogen peak was on average between 5 to 6 hours after the zero point and did not differ between the groups or the meals. The methane production did not differ between the meals. No difference was observed in the hydrogen or methane production between the sensitive group and the control group after either of the study meals.

The higher hydrogen production induced by the oat flour meal was most likely induced by the colonial fermentation of the soluble fiber of the oat flour. Earlier studies of rye have also associated the elevated amount of breath hydrogen to the soluble fiber consumption (Hartvigsen et al. 2014, Ibrugger et al. 2014, Lee et al. 2016a). The authors of these studies have also suggested that the breath hydrogen concentration may be linked to the short-chain fatty acid (SCFA) production, since both were elevated after consumption of soluble fiber (Hartvigsen et al. 2014, Ibrugger et al. 2014). The results obtained in the thesis agree with the suggestion, since oat consumption is known to induce the colonial SCFA production (Pylkas et al. 2005). Yet, this matter should be further investigated.

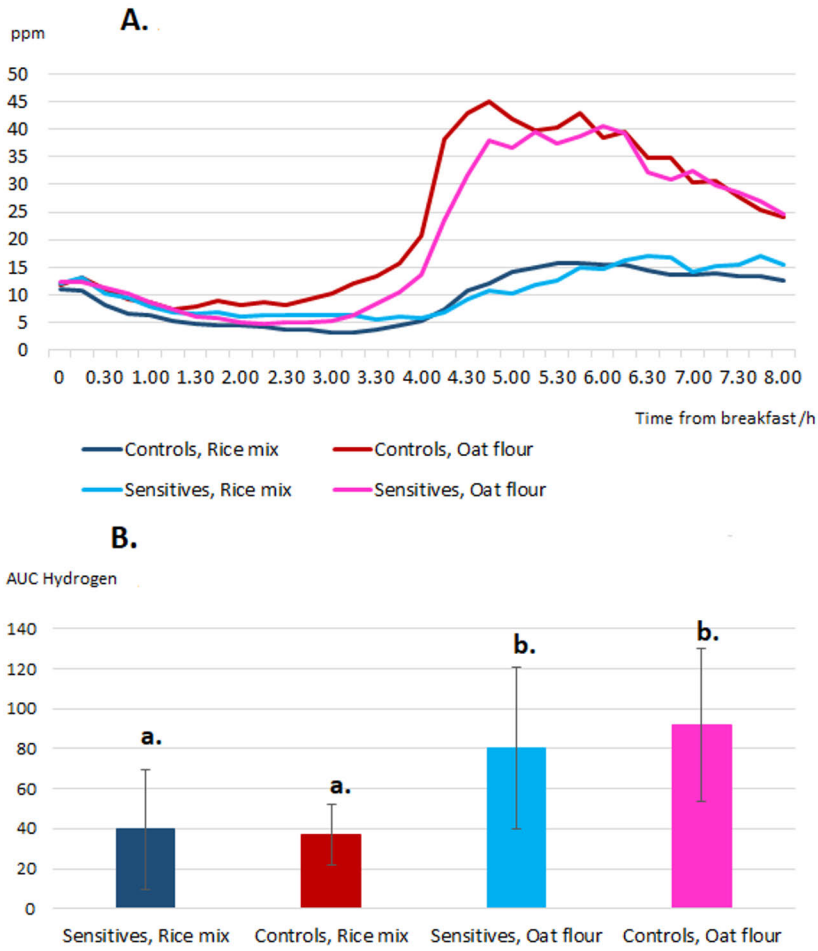


**Fig 14. A.** The average hydrogen production rate during the fourth study day (n = 42). Lunch was served at the time point of 4 hours. **B**. Area under curve values of hydrogen production. Data in a differs significantly from that in b ($p < 0.05$) (Wilcoxon Rank Test).

## 5.5 Perceived symptoms and defecation patterns

In Study I, most gut symptoms per subjects were reported by the NCGS group (61.4%) when compared to CeD and healthy controls (39.1% and 19.7%, respectively, $p = 0.045$). Flatulence, bloating and lower abdominal pain were the most often reported symptoms in all groups. The results were in line with previous studies of gluten-sensitive and CeD subjects (Tovoli et al. 2019, Skodje et al. 2019). A study observed that a significant proportion of gluten-sensitive subjects (66%), which were diagnosed according to Salerno criteria, reported intestinal symptoms even years after the beginning of GFD. Compared to CeD

patients following the same diet, subjects with NCGS reported a higher amount of symptoms (33%) (Tovoli et al. 2019). Yet, the results of Study I did not support the hypothesis that microbiota dysbiosis would cause the symptoms, as no signs of dysbiosis were detected (5.3%).

In Study II, there were no statistical differences in perceived symptoms after consumption of the study meals ($p = 0.368$). Flatulence and constipation were the most commonly reported symptoms in each study period representing 23% and 22% of all the symptoms reported, respectively. However, the types of perceived gut symptoms varied significantly by gender. During the study day, female participants reported most commonly flatulence. In a total of three study days, it was reported 62 times, followed by bloating (43 times), while male participants reported flatulence in total 7 times and bloating once ($p < 0.001$, $p < 0.009$, respectively). In contrast, constipation was most frequently reported by males (63 times during the three study days), which tended to differ to that of females (5 times, $p < 0.057$, Figure 13 F, G). However, the low-phenolics and a low-fiber background diet caused gut symptoms to the participants with less symptoms reported 5 days before the study day (day -5) compared to the amount of the symptoms reported on the second day of the run-in period (day -1) on average for all the study periods, $p < 0.008$. Gender-related differences in self-reported gastrointestinal symptoms have been previously reported in irritable bowel syndrome studies indicating that females are more eager to report pain (Mayer et al. 1999). However, in this study, the symptoms differed in quality.

In Study III, the sensitive group, compared to the controls, perceived significantly more symptoms in total during the first 3 days of the study period ($p = 0.001$, $p = 0.001$, respectively) and during the fourth day, after both oat flour and rice flour meal ($p = 0.001$, $p = 0.001$, respectively). They also reported more symptoms at each intensity (i.e., very mild, mild moderate and severe) compared to the control group after both the meals with rice (during the first 3 study days, $p = 0.01$, $p = 0.001$, $p = 0.001$, $p = 0.008$, respectively), and during the fourth day, $p = 0.001$, $p = 0.001$, $p = 0.001$, $p = 0.038$, respectively) and the meal with oat flour (during the first 3 study days, $p = 0.038$, $p = 0.001$, $p = 0.001$, $p = 0.140$ [not significant], respectively), and during the fourth day, $p = 0.008$, $p = 0.001$, $p = 0.002$, $p = 0.019$, respectively). The sensitive group also reported each type of symptoms (i.e., upper abdomen pain, lower abdomen pain, cramping, bloating, flatulence, rumbling and other symptoms) more frequently than the control group after both the meals with rice (during the first 3 study days, $p = 0.213$ [not significant], $p = 0.015$, $p = 0.038$, $p = 0.002$, $p = 0.001$, $p = 0.001$, $p = 0.079$ [not significant], respectively, and during the fourth day, $p = 0.004$, $p = 0.035$, $p = 0.076$ [not significant], $p = 0.003$, $p = 0.002$, $p = 0.301$ [not significant], $p = 0.146$ [not significant], respectively) and the meal with oat flour (during the first 3 study days, $p = 0.039$, $p = 0.044$, $p = 0.307$ [no significant], $p = 0.023$, $p =$

0.020, p = 0.015, p = 0.124 [not significant], respectively, and during the fourth day, p = 0.038, p = 0.123 [not significant], p = 0.288 [not significant], p = 0.010, p = 0.001, p = 0.536 [not significant], p = 0.018, [Figure 15]). During the first 3 study days, the frequency, intensity and quality of the symptoms did not differ among the oat and rice meals (Figure 15). During the fourth day, both the sensitive and controls reported more flatulence after the oat flour meal compared to the rice meal (p = 0.006, p = 0.008, Figure 15). The control group also reported more rumbling after the oat flour meal (p = 0.025). As both groups also reported more mild or very mild symptoms during the fourth day after the oat flour meal compared to the rice flour meal (sensitives: very mild symptoms, p = 0.006, mild symptoms, p = 0.001; controls: very mild symptoms, p = 0.027, mild symptoms, p = 0.034), and no difference was seen in the total amount of moderate or severe symptoms with the increased flatulence and rumbling being most likely mainly mild.

The perceived flatulence of the fourth day correlated positively to elevated breath hydrogen values after the oat flour meal in both the sensitive group and the control group (p = 0.042, p = 0.003, respectively). After the rice flour meal, no such correlation was observed (p = 0.746, p = 0.560, respectively).

More defections from both extremity ends of the Bristol scale were reported by the sensitive group compared to controls (Figure 16). Statistically, after the rice flour meal, the sensitive group reported more stools of type 5 and type 7 compared to the controls (p = 0.045, p = 0.042, respectively), while after the oat flour meal, the control group reported more of type 4 and the sensitive more of type 5 stools (p = 0.016, p = 0.019, respectively). The frequency of the defecations did not statistically differ between the groups. The defecations did not differ between the meals in either the sensitive or the control group.

The symptoms and abnormal defecations perceived by the sensitive group are known to be typical for patients with irritable bowel syndrome (Camilleri 2020a). The results indicate that both colonial fermentation as well as visceral hypersensitivity play a role in the FODMAP compound symptom triggering in the sensitive subjects. The increased perception of flatulence correlated to elevated breath hydrogen after the oat flour meal in both the sensitive and control groups after the oat flour meal. This indicates that colonial fermentation is linked to the symptom triggering. Yet, the breath gas production did not differ between the groups, which signifies that the excessive gas production is not the only factor causing symptom, but visceral sensitivity, i.e., the sensitivity to intestinal distension induced by gas or water, also plays a role. It was observed that consumption of oats together with pulses did not reduce but did not worsen either the pulse-related symptoms of the sensitive subjects during the first 3 study days and only caused very mild to mild flatulence to both sensitive and controls during the fourth day. This implies that oats are a rather well-tolerated grain also for

individuals with sensitive stomach. This may be explained by the properties of the β-glucan. The long polymer chains of oat fibers, mostly β-glucan, increase the viscosity of the digesta in the small bowel instead of drawing water to the intestine (Makela, Brinck, & Sontag-Strohm, 2020). The increase of the viscosity of the digesta binds intestinal water, which soothes the bulk in the colon and thus lowers the rate of colon extension (Atzler, Sahin, Gallagher, Zannini, & Arendt, 2021). Furthermore, the slow rate of fermentation of oat fibers plays a key role in the tolerance of oats, since the gas is produced over a longer time period and the colonial extension is less sudden compared to the fermentation of FODMAP compounds with small molecular structures.

According to the results of Studies I, II and III, oats are well tolerated among healthy subjects and subjects with CeD, NCGS or IBS. They are also tolerated among the healthy subjects despite the state of processing. Thus, they serve an essential source of dietary fiber, phenolics and other nutrients in those with special diets to avoid wheat, rye and barley (Lim et al. 2005, Holm et al. 2006, Schar et al. 2018).

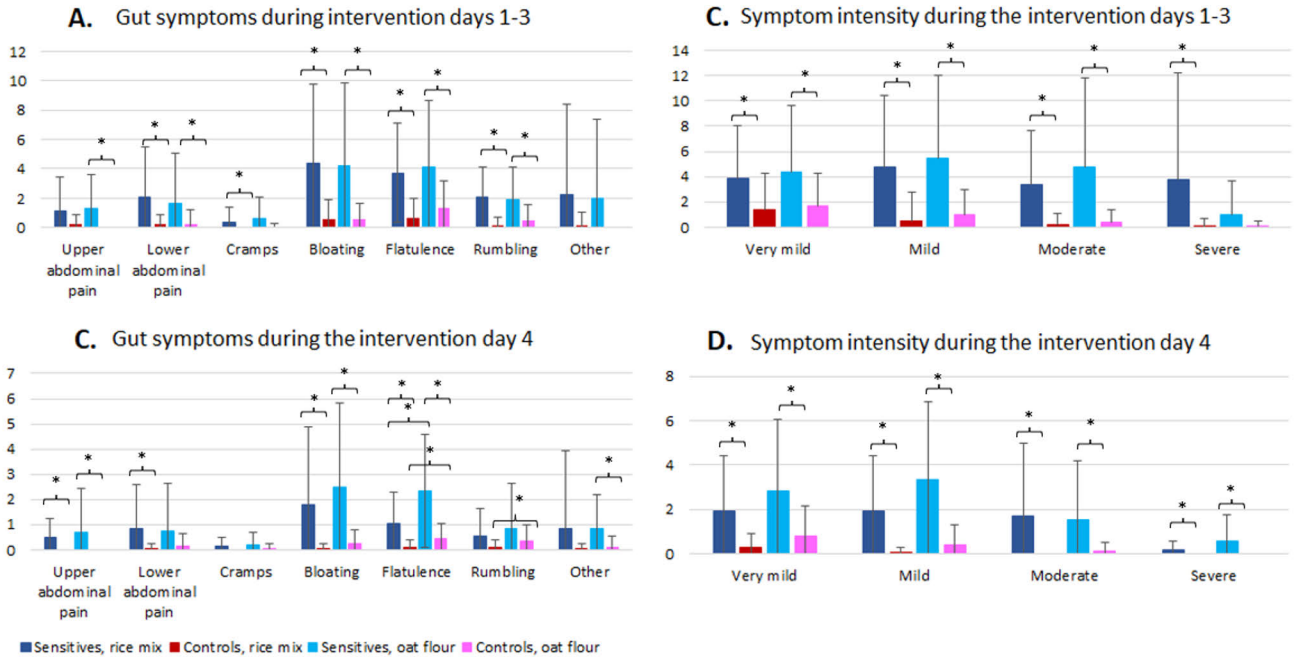


**Fig 15. A.**

The average number of reported gut symptoms during the intervention days 1–3. **B**. The average number of reported symptom intensities during the intervention days 1–3. **C**. The average number of reported gut symptoms during the intervention day 4. **D**. The average number of reported symptom intensities during the intervention day 4. Values are mean of 21 ± SD. Significant differences ($p < 0.05$) are marked with * (Mann-Whitney U-test, Wilcoxon Rank test).

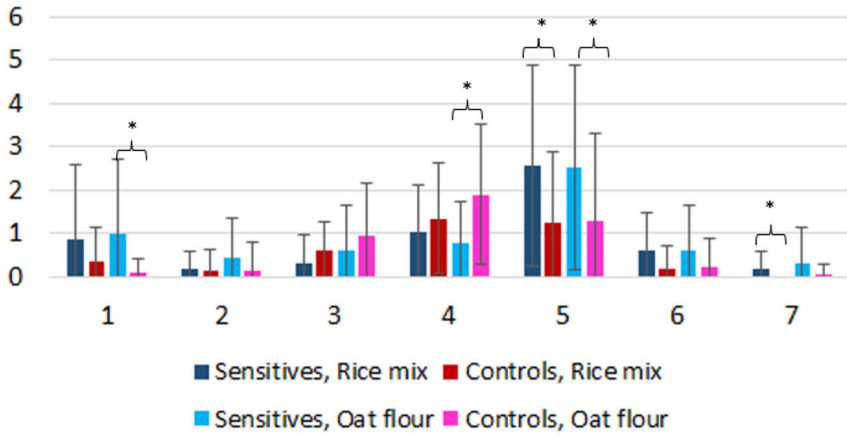


**Fig. 16**. The number of stools during the 4-day intervention period by the Bristol scale. Values are mean of 21 ± SD. Significant differences ($p < 0.05$) are marked with * (Mann-Whitney U test).

## 5.6 General discussion

### 5.6.1 Limitations and strengths of the studies

The strengths in Study I were the profound analyses of fecal microbiota composition and analyses of oat-consuming adult CeD and NCGS subjects of which there is only limited data available. The comparison of biological measurements to perceived symptoms and dietary patterns was also a strength of Study I. Originally, a study group of non-oat-consuming CeD subjects was planned, but their recruiting from the area of Turku, Finland was nearly impossible. In Finland, since 1997, oats are officially recommended as part of GFD for adult CeD patients and for pediatric patients since 2000 (Peraaho et al. 2004, Kaukinen et al. 2013), and most of the CeD subjects used oats. Thus, this was a limitation in Study I.

The strength in Study II was the linking of β-glucan MW to the excretion of fecal bile acids and urine phenolic compounds, which was not studied before. In addition, the comparison of β-glucan MW to intestinal pressure and perceived gut symptoms was presented for the first time. As a limitation, while the excretion of bile acids was dependent of the MW of β-glucan, this relationship was not detected in the fecal excretion of cholesterol and cholesterol metabolites indicating that they were more related to the consumption of animal products. Therefore, excluding animal products from the diet might have improved the visibility of the phenomenon. However, as the diet was already rather limited and excluding already all the vegetables and whole grains, excluding also the animal products was not plausible.

The strengths in Study III were in the analysis of the fermentation rate of oat fibers ingested together with symptom-triggering pulse products detected from breath hydrogen measurements and perceived gut symptoms of healthy subjects and subjects with self-reported sensitivity to pulses. Yet, correlating the breath hydrogen measurements to the analysis of plasma SCFAs would have brought additional information of the oat fermentation rate, and thus the lack of SCFA analysis can be considered as a limitation. However, the 8-hour continuous blood testing would have been a significant burden for the participants and required a presence of a study nurse.

### 5.6.2 Relevance and significance of the research

This thesis has highlighted the significance of oats as a whole grain and fiber source in special diets, such as GFD and low-FODMAP diets. The previous studies analyzing the microbiota CeD patients have mainly focused on pediatric

patients and have not considered the oat consumption by the CeD patients, and the microbiota composition of NCGS patients was not studied before (Lin and Zhang 2017, De Palma et al. 2009, Marasco et al. 2016, Viitasalo et al. 2018, Collado et al. 2009). Moreover, the fermentation rate of oats ingested together with pulses in subjects with pulse-related sensitivity was not studied before.

Furthermore, this thesis broadened the understanding of the effects of reducing the MW of β-glucan *via* enzyme treatment on the physiological behavior of oats. No clinical trial has examined the relation of β-glucan MW to gastrointestinal pressure and excretion of fecal bile acids and urine phenolic compounds before. The markets of moisture-rich oat products, such as drinks and many spoonable products are still rather new, and the knowledge of this research can be applied to the analysis of the physiological effects of such products. Moreover, the state of β-glucan is not considered in the current health claims of β-glucan.

### 5.6.3 Future prospects

New scientific questions were risen within the three clinical trials. The minor microbiota alterations of oat-consuming CeD, NCGS and IBS patients should be further examined. Furthermore, the effect of long-term oat consumption on microbiota composition and function and on subjects following GFD or low-FODMAP diets would broaden the understanding of oat's beneficial properties. Moreover, the symptom-triggering mechanisms in NCGS and IBS patients are still not fully understood and thus require scientific attention. These studies should underline the associations of diet and diet counseling to symptom perception. In addition, the sex as a factor in the symptom perception should be used in future studies.

The gastrointestinal behavior and microbial fermentation of oats could be also examined in more detail. For instance, the link between the production of breath hydrogen should be further compared to the production of SCFAs. Furthermore, as the Study II was the first clinical trial linking the β-glucan MW to fecal bile acid excretion, further studies are still needed on this matter. This future knowledge would enable designing the oat products for customers with special needs and finding the optimum balance between the maximum health benefits and preferable product design.

# 6 SUMMARY AND CONCLUSION

This thesis highlighted the influence of oats as a whole grain and fiber source on individuals following a gluten free or low-FODMAP diet. The consumption of oats was evaluated observationally in the microbiota composition and function in subjects with CeD and NCGS and postprandially when ingested with symptom-triggering compounds in subjects with self-reported sensitivity or IBS. Moreover, the thesis investigated the relation of processing with enzyme treatment to the physiological behavior of β-glucan and phenolic compounds of oats on healthy subjects.

In Study III, the microbial fermentation of oat fibers was demonstrated as higher breath hydrogen production compared to the meal with rice in both healthy subjects and subjects with self-reported sensitivity to pulses or IBS. In Study I, the effect of oat fiber on the gut microbiota was seen on subjects with CeD and NCGS as absent signs of microbiota dysbiosis typically associated with these disorders. However, the higher amount of *Bifidobacterium* spp. and higher concentration of fecal acetic acid in healthy subjects compared to subjects with CeD and NCGS implies that minor microbiota alterations of oat-consuming individuals with CeD and NCGS should be investigated in more detail.

In Study II, the focus was directed to the physiological behavior of oats with varying MWs. It was demonstrated that the MW and thus also viscosity of β-glucan affects the nutritional properties of oat bran and on the functional properties of the β-glucan in the human gastrointestinal tract. The consumption of high MW β-glucan induced the highest excretion of fecal bile acids, which is known to be linked to β-glucan bile acid binding capacity and a cholesterol-lowering effect. The high MW also induced the highest pressure in the duodenum. The consumption of low MW β-glucan was linked to the lowest excretion of fecal bile acids and the lowest pressure in the duodenum but induced the highest excretion of phenolic compounds in the urine. That indicated a release of bound phenolic compounds induced by the enzyme treatment. The behavior of medium MW β-glucan resembled that of the high MW β-glucan in terms of phenolics and bile acids excretion, but the induced pressure in the duodenum was lower and closer to that of the low MW β-glucan. These results could be applied to the evaluation of health effects of the oat-based products requiring enzyme treatment.

Finally, this thesis examined the perceived gut well-being related to oat consumption in each trial. It was observed that oats were well tolerated and beneficial to the individuals with CeD and NCGS in their daily consumption. When ingested together with pulses, oats induced postprandially very mild to mild flatulence in healthy subjects and subjects with self-reported sensitivity to pulses or IBS. Therefore, oats were rather well tolerated also among individuals

with self-reported sensitivity to pulses or IBS. Moreover, oats were demonstrated to be well tolerated among the healthy subjects despite of the state of processing and altered MW. However, future studies should focus on the mechanism of the symptom triggering in individuals with NCGS and IBS. In addition, further studies should acknowledge the sex as a factor in the symptom perception.

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Helsinki, January 2022

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Zhang, Y., T. H. Ni, D. W. Zhang, H. L. Liu, J. Wang & B. G. Sun (2020b) Consumption of avenanthramides extracted from oats reduces weight gain, oxidative stress, inflammation and regulates intestinal microflora in high fat diet-induced mice. *Journal of Functional Foods,* 65**,** 10.

Zhao, Q., X. Z. Hu, Q. B. Guo, S. W. Cui, Y. S. Xian, S. P. You, X. Y. Chen, C. Xu & X. Gao (2014) Physicochemical properties and regulatory effects on db/db diabetic mice of beta-glucans extracted from oat, wheat and barley. *Food Hydrocolloids,* 37**,** 60-68.

Zhu, R. X., Z. H. Fan, G. J. Li, Y. X. Wu, W. Q. Zhao, T. Ye & L. L. Wang (2020) A comparison between whole grain and pearled oats: acute postprandial glycaemic responses and in vitro carbohydrate digestion in healthy subjects. *European Journal of Nutrition,* 59**,** 2345-2355.

Zyla, E., K. Dziendzikowska, M. Gajewska, J. Wilczak, J. Harasym & J. Gromadzka-Ostrowska (2019) Beneficial Effects of Oat Beta-Glucan Dietary Supplementation in Colitis Depend on Its Molecular Weight. *Molecules,* 24.

# APPENDIX: ORIGINAL PUBLICATIONS

I Reprinted from Nutrients 2020, 12, 2570, with permission from MDPI.

II Reprinted from Food Chemistry 2021, 342, 128219, with permission from Elsevier.

III Submitted manuscript.

## DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

1. **REINO R. LINKO (1967)** Fatty acids and other components of Baltic herring flesh lipids. (Organic chemistry).
2. **HEIKKI KALLIO (1975)** Identification of volatile aroma compounds in arctic bramble, *Rubus arcticus* L. and their development during ripening of the berry, with special reference to *Rubus stellatus* SM.
3. **JUKKA KAITARANTA (1981)** Fish roe lipids and lipid hydrolysis in processed roe of certain *Salmonidae* fish as studied by novel chromatographic techniques.
4. **TIMO HIRVI (1983)** Aromas of some strawberry and blueberry species and varieties studied by gas liquid chromatographic and selected ion monitoring techniques.
5. **RAINER HUOPALAHTI (1985)** Composition and content of aroma compounds in the dill herb, *Anethum graveolens* L., affected by different factors.
6. **MARKKU HONKAVAARA (1989)** Effect of porcine stress on the development of PSE meat, its characteristics and influence on the economics of meat products manufacture.
7. **PÄIVI LAAKSO (1992)** Triacylglycerols – approaching the molecular composition of natural mixtures.
8. **MERJA LEINO (1993)** Application of the headspace gas chromatography complemented with sensory evaluation to analysis of various foods.
9. **KAISLI KERROLA (1994)** Essential oils from herbs and spices: isolation by carbon dioxide extraction and characterization by gas chromatography and sensory evaluation.
10. **ANJA LAPVETELÄINEN (1994)** Barley and oat protein products from wet processes: food use potential.
11. **RAIJA TAHVONEN (1995)** Contents of lead and cadmium in foods in Finland.
12. **MAIJA SAXELIN (1995)** Development of dietary probiotics: estimation of optimal *Lactobacillus* GG concentrations.
13. **PIRJO-LIISA PENTTILÄ (1995)** Estimation of food additive and pesticide intakes by means of a stepwise method.
14. **SIRKKA PLAAMI (1996)** Contents of dietary fiber and inositol phosphates in some foods consumed in Finland.
15. **SUSANNA EEROLA (1997)** Biologically active amines: analytics, occurrence and formation in dry sausages.
16. **PEKKA MANNINEN (1997)** Utilization of supercritical carbon dioxide in the analysis of triacylglycerols and isolation of berry oils.
17. **TUULA VESA (1997)** Symptoms of lactose intolerance: influence of milk composition, gastric emptying, and irritable bowel syndrome.
18. **EILA JÄRVENPÄÄ (1998)** Strategies for supercritical fluid extraction of analytes in trace amounts from food matrices.
19. **ELINA TUOMOLA (1999)** *In vitro* adhesion of probiotic lactic acid bacteria.
20. **ANU JOHANSSON (1999)** Availability of seed oils from Finnish berries with special reference to compositional, geographical and nutritional aspects.
21. **ANNE PIHLANTO-LEPPÄLÄ (1999)** Isolation and characteristics of milk-derived bioactive peptides.
22. **MIKA TUOMOLA (2000)** New methods for the measurement of androstenone and skatole – compounds associated with boar taint problem. (Biotechnology).
23. **LEEA PELTO (2000)** Milk hypersensitivity in adults: studies on diagnosis, prevalence and nutritional management.
24. **ANNE NYKÄNEN (2001)** Use of nisin and lactic acid/lactate to improve the microbial and sensory quality of rainbow trout products.
25. **BAORU YANG (2001)** Lipophilic components of sea buckthorn (*Hippophaë rhamnoides*) seeds and berries and physiological effects of sea buckthorn oils.
26. **MINNA KAHALA (2001)** Lactobacillar S-layers: Use of *Lactobacillus brevis* S-layer signals for heterologous protein production.
27. **OLLI SJÖVALL (2002)** Chromatographic and mass spectrometric analysis of non-volatile oxidation products of triacylglycerols with emphasis on core aldehydes.
28. **JUHA-PEKKA KURVINEN (2002)** Automatic data processing as an aid to mass spectrometry of dietary triacylglycerols and tissue glycerophospholipids.

29. **MARI HAKALA (2002)** Factors affecting the internal quality of strawberry (*Fragaria x ananassa* Duch.) fruit.
30. **PIRKKA KIRJAVAINEN (2003)** The intestinal microbiota – a target for treatment in infant atopic eczema?
31. **TARJA ARO (2003)** Chemical composition of Baltic herring: effects of processing and storage on fatty acids, mineral elements and volatile compounds.
32. **SAMI NIKOSKELAINEN (2003)** Innate immunity of rainbow trout: effects of opsonins, temperature and probiotics on phagocytic and complement activity as well as on disease resistance.
33. **KAISA YLI-JOKIPII (2004)** Effect of triacylglycerol fatty acid positional distribution on postprandial lipid metabolism.
34. **MARIKA JESTOI (2005)** Emerging *Fusarium*-mycotoxins in Finland.
35. **KATJA TIITINEN (2006)** Factors contributing to sea buckthorn (*Hippophaë rhamnoides* L.) flavour.
36. **SATU VESTERLUND (2006)** Methods to determine the safety and influence of probiotics on the adherence and viability of pathogens.
37. **FANDI FAWAZ ALI IBRAHIM (2006)** Lactic acid bacteria: an approach for heavy metal detoxification.
38. **JUKKA-PEKKA SUOMELA (2006)** Effects of dietary fat oxidation products and flavonols on lipoprotein oxidation.
39. **SAMPO LAHTINEN (2007)** New insights into the viability of probiotic bacteria.
40. **SASKA TUOMASJUKKA (2007)** Strategies for reducing postprandial triacylglycerolemia.
41. **HARRI MÄKIVUOKKO (2007)** Simulating the human colon microbiota: studies on polydextrose, lactose and cocoa mass.
42. **RENATA ADAMI (2007)** Micronization of pharmaceuticals and food ingredients using supercritical fluid techniques.
43. **TEEMU HALTTUNEN (2008)** Removal of cadmium, lead and arsenic from water by lactic acid bacteria.
44. **SUSANNA ROKKA (2008)** Bovine colostral antibodies and selected lactobacilli as means to control gastrointestinal infections.
45. **ANU LÄHTEENMÄKI-UUTELA (2009)** Foodstuffs and medicines as legal categories in the EU and China. Functional foods as a borderline case. (Law).
46. **TARJA SUOMALAINEN (2009)** Characterizing *Propionibacterium freudenreichii* ssp. *shermanii* JS and *Lactobacillus rhamnosus* LC705 as a new probiotic combination: basic properties of JS and pilot *in vivo* assessment of the combination.
47. **HEIDI LESKINEN (2010)** Positional distribution of fatty acids in plant triacylglycerols: contributing factors and chromatographic/mass spectrometric analysis.
48. **TERHI POHJANHEIMO (2010)** Sensory and non-sensory factors behind the liking and choice of healthy food products.
49. **RIIKKA JÄRVINEN (2010)** Cuticular and suberin polymers of edible plants – analysis by gas chromatographic-mass spectrometric and solid state spectroscopic methods.
50. **HENNA-MARIA LEHTONEN (2010)** Berry polyphenol absorption and the effect of northern berries on metabolism, ectopic fat accumulation, and associated diseases.
51. **PASI KANKAANPÄÄ (2010)** Interactions between polyunsaturated fatty acids and probiotics.
52. **PETRA LARMO (2011)** The health effects of sea buckthorn berries and oil.
53. **HENNA RÖYTIÖ (2011)** Identifying and characterizing new ingredients *in vitro* for prebiotic and synbiotic use.
54. **RITVA REPO-CARRASCO-VALENCIA (2011)** Andean indigenous food crops: nutritional value and bioactive compounds.
55. **OSKAR LAAKSONEN (2011)** Astringent food compounds and their interactions with taste properties.
56. **ŁUKASZ MARCIN GRZEŚKOWIAK (2012)** Gut microbiota in early infancy: effect of environment, diet and probiotics.
57. **PENGZHAN LIU (2012)** Composition of hawthorn (*Crataegus* spp.) fruits and leaves and emblic leafflower (*Phyllanthus emblica*) fruits.
58. **HEIKKI ARO (2012)** Fractionation of hen egg and oat lipids with supercritical fluids. Chemical and functional properties of fractions.
59. **SOILI ALANNE (2012)** An infant with food allergy and eczema in the family – the mental and economic burden of caring.
60. **MARKO TARVAINEN (2013)** Analysis of lipid oxidation during digestion by liquid chromatography-mass spectrometric and nuclear magnetic resonance spectroscopic techniques.

61. **JIE ZHENG (2013)** Sugars, acids and phenolic compounds in currants and sea buckthorn in relation to the effects of environmental factors.
62. **SARI MÄKINEN (2014)** Production, isolation and characterization of bioactive peptides with antihypertensive properties from potato and rapeseed proteins.
63. **MIKA KAIMAINEN (2014)** Stability of natural colorants of plant origin.
64. **LOTTA NYLUND (2015)** Early life intestinal microbiota in health and in atopic eczema.
65. **JAAKKO HIIDENHOVI (2015)** Isolation and characterization of ovomucin – a bioactive agent of egg white.
66. **HANNA-LEENA HIETARANTA-LUOMA (2016)** Promoting healthy lifestyles with personalized, *APOE* genotype based health information: The effects on psychological-, health behavioral and clinical factors.
67. **VELI HIETANIEMI (2016)** The *Fusarium* mycotoxins in Finnish cereal grains: How to control and manage the risk.
68. **MAARIA KORTESNIEMI (2016)** NMR metabolomics of foods – Investigating the influence of origin on sea buckthorn berries, *Brassica* oilseeds and honey.
69. **JUHANI AAKKO (2016)** New insights into human gut microbiota development in early infancy: influence of diet, environment and mother's microbiota.
70. **WEI YANG (2017)** Effects of genetic and environmental factors on proanthocyanidins in sea buckthorn (*Hippophaë rhamnoides*) and flavonol glycosides in leaves of currants (*Ribes* spp.).
71. **LEENAMAIJA MÄKILÄ (2017)** Effect of processing technologies on phenolic compounds in berry products.
72. **JUHA-MATTI PIHLAVA (2017)** Selected bioactive compounds in cereals and cereal products – their role and analysis by chromatographic methods.
73. **TOMMI KUMPULAINEN (2018)** The complexity of freshness and locality in a food consumption context
74. **XUEYING MA (2018)** Non-volatile bioactive and sensory compounds in berries and leaves of sea buckthorn (*Hippophaë rhamnoides*)
75. **ANU NUORA (2018)** Postprandial lipid metabolism resulting from heated beef, homogenized milk and interesterified palm oil.
76. **HEIKKI AISALA (2019)** Sensory properties and underlying chemistry of Finnish edible wild mushrooms.
77. **YE TIAN (2019)** Phenolic compounds from Finnish berry species to enhance food safety.
78. **MAIJA PAAKKI (2020)** The importance of natural colors in food for the visual attractiveness of everyday lunch.
79. **SHUXUN LIU (2020**) Fermentation with non-*Saccharomyces* yeasts as a novel biotechnology for berry wine production.
80. **MARIKA KALPIO (2020)** Strategies for analyzing the regio- and stereospecific structures of individual triacylglycerols in natural fats and oils.
81. **JOHANNA JOKIOJA (2020)** Postprandial effects and metabolism of acylated anthocyanins originating from purple potatoes.
82. **NIINA KELANNE (2021)** Novel bioprocessing for increasing consumption of Nordic berries.
83. **NIKO MARKKINEN (2021)** Bioprocessing of berry materials with malolactic fermentation.
84. **GABRIELE BELTRAME (2021)** Polysaccharides from Finnish fungal resources.
85. **SALLA LAITO (2022)** Bioactive compounds in oats and gut health.

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