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Characterisation of Dietary Fibre Properties to Optimise the Effects on Human Metabolism and the Transcriptome

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Abstract

It is well established that dietary fibre, especially soluble dietary fibre, has beneficial effects and can prevent diseases associated with the modern lifestyle. This has been explained by the viscous effects of soluble fibre, which can reduce or delay the absorption of carbohydrates and fat in the small intestine, resulting in lower blood concentrations of glucose, insulin and cholesterol. Oats, rye, sugar beet fibre and barley, all recognised for their high content of soluble fibre, were investigated in this work.

The ability of fibres to form viscous solutions is determined by the concentration, solubility, the polymer molar mass and aggregate formation. The fibre releasability (solubility) and solution behaviour were analysed under physiological conditions to examine the effects of processing, the surrounding matrix and gastrointestinal conditions. Less than half of the β-glucans was released from oat bran when analysed with an *in vitro* method imitating gastrointestinal digestion, and the same was found regarding the release of pectin from coarse particles of sugar beet fibre. Reducing the particle size by milling improved the releasability, while mixing the milled fractions with a solid food matrix (protein, fat or starch) decreased or delayed it. The effect of food processing on barley β-glucan aggregation was studied using asymmetrical flow field-flow fractionation. Boiling was shown to disrupt the largest aggregates, while higher temperatures also seemed to degrade the polymer chain. Freeze-thaw cycles resulted in cryogelation, which could lead to reduced viscosity. The low pH in the *in vitro* gastric digestion phase also disrupted the aggregates, but this was followed by re-aggregation/gelation at the neutral pH mimicking the conditions in the small intestine. This may entrap nutrients in the aggregate or gel matrix, thereby reducing absorption.

A human study was performed with oats, rye and sugar beet fibre, and mixtures of these three. Fibre-rich meals were optimised to promote high releasability of fibre and retention of aggregates. Changes in traditional biomarkers, as well as in the transcriptome, in response to the fibre-rich meals, were examined in healthy men and women. The postprandial glucose levels were lowered by rye bran, while oat bran lowered the insulin concentrations. The lowering effects may be related to fibre source, the amount of soluble and insoluble fibre and pre-processing of the fibre. In contrast, a spray-dried oat drink increased the postprandial levels of insulin, possibly due to its low fibre content in combination with certain amino acids and a high amount of carbohydrates in the liquid matrix. Women showed, on average, a more pronounced glucose lowering response than men, indicating that different amounts of dietary fibre should be recommended for men and women. Changes in gene expression in peripheral blood mononuclear cells after the intake of oat bran were investigated with microarray analysis. Gene sets and pathways related to insulin secretion were significantly suppressed in response to the oat bran meal compared with the control meal.

In conclusion, many factors may influence the properties of dietary fibre. It is therefore valuable to use *in vitro* methods to simulate gastrointestinal digestion to characterise and optimise fibre functionality prior to testing their effects on human metabolism and the transcriptome.

Key words   Oats, rye, sugar beet fibre, β-glucan, pectin, solubilisation, aggregation, gastrointestinal method, field-flow fractionation, postprandial responses, microarray gene expression

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LIST OF PAPERS

This thesis is based on the following scientific papers, which will be referred to in the text by their Roman numerals.

I **Ulmius M**, Johansson A, Immerstrand Nordén T, Bergenståhl B, Önning G. Following the gastrointestinal release of soluble dietary fibers using an *in vitro* method. *Submitted for publication*

II **Ulmius M**, Önning G, Nilsson L. Solution behavior of barley β-glucan as studied with asymmetrical flow field-flow fractionation. *Submitted for publication*

III **Ulmius M**, Adapa S, Önning G, Nilsson L. Gastrointestinal conditions influence the solution behaviour of cereal β-glucans *in vitro. Manuscript*

IV **Ulmius M**, Johansson A, Önning G. The influence of dietary fibre source and gender on the postprandial glucose and lipid response in healthy subjects. *European Journal of Nutrition (2009) 48:395-402*

V **Ulmius M**, Johansson A, Krogh M, Olsson P, Önning G. An oat bran meal influences blood insulin levels and related gene sets in peripheral blood mononuclear cells of healthy subjects. *Submitted for publication*

MY CONTRIBUTIONS TO THE PAPERS

Paper I   I planned the experiment, and performed the experimental work to-gether with Anna Johansson. I evaluated the results and wrote the manuscript.

Paper II   I planned and performed the experiment, and wrote the main part of the manuscript.

Paper III   I planned the experiment and took part in the experimental work. I evaluated the results and wrote the main part of the manuscript.

Paper IV   I took part in the planning of the study design and protocol, and was responsible for the day-to-day management of the study. The analysis of blood samples and the results of the study were evaluated together with Anna Johansson. We also wrote the manuscript together.

Paper V   I took part in the planning of the study design and protocol, and per-formed the blood sample analysis. I evaluated the results of the study together with Morten Krogh. I wrote the manuscript.

ABSTRACT

It is well established that dietary fibre, especially soluble dietary fibre, has beneficial effects and can prevent diseases associated with the modern lifestyle. This has been explained by the viscous effects of soluble fibre, which can reduce or delay the absorption of carbohydrates and fat in the small intestine, resulting in lower blood concentrations of glucose, insulin and cholesterol. Oats, rye, sugar beet fibre and barley, all recognised for their high content of soluble fibre, were investigated in this work.

The ability of fibres to form viscous solutions is determined by the concentration, solubility, the polymer molar mass and aggregate formation. The fibre releasability (solubility) and solution behaviour were analysed under physiological conditions to examine the effects of processing, the surrounding matrix and gastrointestinal conditions. Less than half of the β-glucans was released from oat bran when analysed with an *in vitro* method imitating gastrointestinal digestion, and the same was found regarding the release of pectin from coarse particles of sugar beet fibre. Reducing the particle size by milling improved the releasability, while mixing the milled fractions with a solid food matrix (protein, fat or starch) decreased or delayed it. The effect of food processing on barley β-glucan aggregation was studied using asymmetrical flow field-flow fractionation. Boiling was shown to disrupt the largest aggregates, while higher temperatures also seemed to degrade the polymer chain. Freeze-thaw cycles resulted in cryogelation, which could lead to reduced viscosity. The low pH in the *in vitro* gastric digestion phase also disrupted the aggregates, but this was followed by re-aggregation/gelation at the neutral pH mimicking the conditions in the small intestine. This may entrap nutrients in the aggregate or gel matrix, thereby reducing absorption.

A human study was performed with oats, rye and sugar beet fibre, and mixtures of these three. Fibre-rich meals were optimised to promote high releasability of fibre and retention of aggregates. Changes in traditional biomarkers, as well as in the transcriptome, in response to the fibre-rich meals, were examined in healthy men and women. The postprandial glucose levels were lowered by rye bran, while oat bran lowered the insulin concentrations. The lowering effects may be related to fibre source, the amount of soluble and insoluble fibre and pre-processing of the fibre. In contrast, a spray-dried oat drink increased the postprandial levels of insulin, possibly due to its low fibre content in combination with certain amino acids and a high amount of carbohydrates in the liquid matrix. Women showed, on average, a more pronounced glucose lowering response than men, indicating that different amounts of dietary fibre should be recommended for men and women. Changes in gene expression in peripheral blood mononuclear cells after the intake of oat bran were investigated with microarray analysis. Gene sets and pathways related to insulin secretion were significantly suppressed in response to the oat bran meal compared with the control meal.

In conclusion, many factors may influence the properties of dietary fibre. It is therefore valuable to use *in vitro* methods to simulate gastrointestinal digestion to characterise and optimise fibre functionality prior to testing their effects on human metabolism and the transcriptome.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Genom att inta en bärdryck med extra fiber från råg eller havre steg inte blodsockret och insulinnivån lika snabbt jämfört med samma måltid utan fiber i drycken. Dessutom visade ett test två timmar efter havreklimåltiden att avläsningen av insulinrelaterade gener (genuttrycket) hade nedreglerats. Andra gener som påverkades av havremåltiden var kopplade till proteinsyntesen och cancersjukdomar. Denna avhandling beskriver hur lösliga kostfiber från olika grödor påverkas av tillverkningsprocesser och matlagning, och hur de beter sig i en simulerad mag-tarmkanal. Hälsoeffekterna av fibrerna undersöktes därefter i en måltidsstudie på människa.

Det är framför allt lösliga fibrer som fördröjer näringsupptaget i tunntarmen genom att öka viskositeten, dvs. göra tarmvätskan mer tjockflytande. Ett långsammare och lägre upptag av kolhydrater gör att blodsockerhöjningen efter en måltid inte blir lika stor (lägre glykemiskt index, GI), vilket i längden kan motverka välfärdssjukdomar som fetma och diabetes.

Olika tillverkningsprocesser kan påverka de lösliga fibrernas effekt på hälsan. Med en analysmetod som inte bryter ner fiberaggregat (hopslagning av fiberkedjor) kunde vi visa att kokning separerade aggregaten men vid högre temperaturer bröts även de enskilda fiberkedjorna ner. En vecka i frys påverkade inte fibrerna medan upprepad frysning och upptining gjorde att det bildades en oönskad gel.

En modell av mag-tarmkanalen anpassades för att undersöka hur stor andel av den lösliga fibern som frisätts i magen respektive tarmen. En hög frisättning leder till ökad viskositet. Från de grova partiklarna frisattes 20 % β-glukan från havrekli och 50 % pektin från sockerbetsfiber, men genom att mala partiklarna ökades frisättningen till 55 % respektive 70 %. I nästa steg undersöktes malda fiber som blandats med olika livsmedel, som kokt äggvita (protein), smör (fett) eller kokt potatisstärkelse (stärkelse). Både protein och stärkelse orsakade en betydligt långsammare frisättning av fibern.

Malning, kokning och frysning är alltså olika processer som påverkar kostfibers möjliga hälsoeffekter. Dessutom påverkas de av vilka livsmedel man blandar dem med. Sedan 1997 tillåts hälsopåståenden för fiberrika produkter i USA, dvs. man får skriva att produkten är hälsosam på förpackningen. Sverige tillåter sådana för specifika produkter medan EUs krav är under beslut och förväntas innehålla skrivningar om vilka processer kostfibern får utsättas för om hälsopåståendena ska gälla. En modell av mag-tarmkanalen kan användas för att undersöka hur olika fiber och fiberrika produkter beter sig, vilket vi tror är en bra metod innan man går vidare till att undersöka hälsoeffekter på försökspersoner.

I måltidsstudien undersöktes malda fiber av antingen havrekli, spraytorkad havredryck, rågkli, sockerbetsfiber eller en blandning av fibrer. De olika fibrerna blandades i kall svartvinbärsdryck och blev därmed varken kokade eller frysta. Arton friska, unga försökspersoner deltog. En morgon i veckan under sju veckor, efter en natts fastande, kom

de till studiecentrumet för att äta en fibermåltid eller en kontrollmåltid utan fiber. Blodprov togs före och under tre timmar efter för att mäta effekterna på blodsocker, insulin och triglycerider (ett blodfett). Prov före och två timmar efter måltider med havrekli respektive kontroll användes också för att isolera mRNA från vita blodkroppar för att studera förändringar i genuttrycket för 24 000 gener med microarrayteknik.

Drycken med rågkli sänkte blodsockret signifikant medan havrekli åstadkom en signifikant sänkning av insulinnivåerna. Det fanns en tendens att kostfibrerna gav bättre sänkning av blodsockret hos kvinnor än hos män. Sockerbetsfiber och fiberblandningen visade inga tydliga effekter, medan den spraytorkade havredrycken höjde insulinnivåerna, möjligen på grund av låg halt kostfiber och att kolhydraterna enbart var i flytande form. Fibermåltiderna påverkade inte nivån av triglycerider signifikant. Måltiden med havrekli ändrade genuttrycket redan efter två timmar. Gener som är relaterade till insulinproduktion verkar ha inaktiverats, vilket i sin tur sänkte insulinnivån i blodet. Även gener relaterade till proteinsyntesen och cancersjukdomar påverkades, vilket skulle kunna vara relaterat till de sänkta insulinnivåerna. Dessa effekter efter en enda fibermåltid kan vara en tidig indikation på långtidseffekter av kostfiber.

Sammanfattningsvis visar denna avhandling att behandlingen av kostfibrerna innan de intas kan leda till förändringar som påverkar hälsoeffekterna och att det är viktigt att kartlägga detta för att kunna få önskad hälsoeffekt. Genom att använda nya tekniker för analys av fiberaggregat och mag-tarmmodeller följt av studier på människa finns det stora möjligheter att kunna erbjuda konsumenter livsmedel som innehåller optimalt hälsosamma kostfiber.

ABBREVIATIONS

AsFlFFF	Asymmetrical flow field-flow fractionation
AUC	Area under the curve
BMI	Body mass index
cRNA	Complementary RNA
DNA	Deoxyribonucleic acid
FAO	Food and Agricultural Organization of the United Nations
FC	Fold change
FDA	US Food and Drug Administration
FDR	False discovery rate
GI	Glycaemic index
GSEA	Gene set enrichment analysis
HDL	High-density lipoprotein
HPSEC	High-performance size-exclusion chromatography
IAUC	Incremental area under the curve
LDL	Low-density lipoprotein
M	Molar mass
M_w	Weight-average molar mass
MALS	Multi-angle light scattering
mRNA	Messenger RNA
NuGO	The European Nutrigenomics Organisation
PBMC	Peripheral blood mononuclear cell
RI	Refractive index
RNA	Ribonucleic acid
SCFA	Short-chain fatty acid
SEM	Standard error of the mean
USP	United States Pharmacopeia
WHO	World Health Organization

CONTENTS

1 INTRODUCTION

An energy-dense diet low in dietary fibre, common in many parts of the world today, is believed to play a major role in the development of conditions such as obesity, cardiovascular disease and type 2 diabetes. Eben Hipsley was probably the first to use the term "dietary fibre" to describe non-digestible plant cell walls in 1953[1], and in the 1970s, Dennis Burkitt and Hugh Trowell adopted Hipsley's term in association with beneficial effects on cardiovascular disease, type 2 diabetes and cancer[2]. Today, dietary fibre is one of the most widely studied food components, and considerable research is being devoted to the development of fibre-rich raw materials for use in new food products, and the enrichment of food with low fibre content.

The beneficial effects of dietary fibre, especially soluble fibre, on diseases associated with the Western lifestyle are primarily attributed to their ability to modify the viscosity of the intestinal content. The reduction of blood cholesterol level by the ingestion of dietary fibre is well documented, and this health claim was approved by the US Food and Drug Administration (FDA) in 1997 for use in association with several types of dietary fibre[3]. Despite the strong evidence of the benefit of dietary fibre, the results of ingesting fibre vary, depending on the source and the way in which it is produced and processed[4,5]. Differences were seen in the blood-cholesterol-lowering effects of oat and barley in an EU project that was completed in 2005 by our research group[6]. Consequently, there is a need for analytical methods that can identify active fibre fractions in order to optimise the health-promoting properties of fibre-rich foods.

Our knowledge and understanding of how dietary fibre regulates molecular events at the gene level are limited[7]. Systems biology makes it possible to perform genome-wide assays to study gene and protein functions in a single analysis. Within the field of nutrition, the technique is referred to as *nutrigenomics,* and enables screening for the effects of a food component on multiple minor changes in gene regulation, protein synthesis or the pattern of metabolites. The use of nutrigenomic techniques together with traditional measurements of biomarkers such as blood glucose and insulin levels, can provide further knowledge on the correlation between dietary fibre and health.

The first topic presented in this thesis is the characterisation of soluble fibre from different sources, by the analysis of viscosity-related properties under physiological conditions. The second topic is the documentation of the health effects of the characterised fibre fractions in a human study, including changes in the transcriptome using nutrigenomic techniques. The studies aimed at obtaining more knowledge of the fibre properties that are important for desirable health effects, and to help the food industry to develop new, healthy, fibre-rich food products.

2 BACKGROUND

It is acknowledged that the intake of dietary fibre is good for the health, but *dietary fibre* is poorly defined. There are several kinds of dietary fibre, having different properties and, therefore, different health effects. The relation between dietary fibre and the prevention of contemporary lifestyle-related diseases is presented in this chapter. The sources and kinds of fibre studied, especially the soluble fibre fractions, are discussed. Furthermore, a presentation of nutrigenomics and microarray analysis is given.

INTAKE OF DIETARY FIBRE

By analysing DNA plant remnants in human coprolites (fossilised excrement), researchers have estimated that prehistoric populations that were hunter-gatherers in present-day Texas, USA, had a dietary fibre intake of 150-225 g per day[8]. The adoption of agriculture, about 10 000 years ago, signified the start of cereal grains being man's staple food. It is believed that fibre intake decreased due to a reduction in fibre content as a result of processes such as drying and grinding[9], but the diet was still mainly composed of unrefined grains and vegetables, and was high in dietary fibre compared with today.

Industrialisation and Westernisation played a major role in the development of several present-day lifestyle-associated diseases and have simultaneously decreased the dietary fibre content in foods. The dietary fibre intake in Sweden in 1998 has been estimated to be about 16-18 g per day[10], somewhat higher in the whole Europe, with a range of 16-29 g per day[11], but only 15 g per day in the USA[12]. The major source of dietary fibre in Sweden is bread based on wheat flour and whole grains, fruits and berries[10], while in the USA it is wheat and potatoes[12]. The recommendations for dietary fibre intake are 25-35 g per day in the Nordic countries[13], at least 25 g per day in Europe[14] and 28-36 g per day in the USA[15], all of which represents almost a doubling of the present intake. The Nordic recommendation, which dates from 2004, suggests that the sources of fibre should be cereal products, potatoes, vegetables, fruits and berries, while the American recommendation of 2010 states that dietary fibre can be both naturally occurring in foods and isolated fibre fractions that have been shown to have positive health effects. In my view, this difference may be due to the newly adopted definition of dietary fibre.

DEFINITION OF DIETARY FIBRE

The definition of dietary fibre used in the European Union since 2008 is[16] "*fibre means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:*

- ◦ *edible carbohydrate polymers naturally occurring in the food as consumed;*
- ◦ *edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic, or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence; or*
- ◦ *edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence.*"

The definition published by the United Nations' Food and Agriculture Organization and World Health Organization (FAO/WHO), Codex Alimentarius from 2008[17] is similar, but includes only carbohydrate polymers with ten or more monomers, and thus excludes oligosaccharides. This difference, together with the beneficial physiological effects that should be included, has resulted in heated debates at conferences and in scientific papers[18-20]. Some of the beneficial physiological effects, such as reduced postprandial blood glucose and insulin levels and reduced blood cholesterol, are defined in the EU Commission Directive[16], while others, for example short-chain fatty acid production and satiety, are only discussed in general terms.

2.1. Dietary fibre in the prevention and management of disease

METABOLIC SYNDROME, CARDIOVASCULAR DISEASE AND TYPE 2 DIABETES
Metabolic syndrome is a term used to describe several metabolic disorders, and is identified by the presence of three or more of the following: obesity (body mass index (BMI) > 30 kg/m^2), elevated blood pressure, low high-density lipoprotein (HDL) cholesterol, high triglyceride concentration or high fasting plasma glucose[21]. The prevalence of metabolic syndrome in middle-aged Swedes in 2004 was 15 %[22]. Obesity was reported to be the major factor, and the frequency of obesity in Sweden has doubled from 1980 to 2003[23]. About 10 % of Swedish adults were obese in 2007, but this is still low in an international perspective (the average in the EU countries was reported to be 15.5 % in 2010, and the rate of obesity in the USA was 27.5 % in 2008)[24,25]. A combination of several disorders included in the metabolic syndrome and elevated low-density lipoprotein (LDL) cholesterol increase the risk of developing type 2 diabetes and cardiovascular disease. According to WHO, cardiovascular diseases are the major cause of death worldwide[26].

The proposed beneficial effects of dietary fibres are presented in Table 1. Products rich in dietary fibre have a low energy density, thus reducing the energy content of a meal. This is due to the increased bulk arising from the fibre, which contributes to satiety[27], and can prevent or reduce obesity. Dietary fibre can delay gastric emptying and reduce or delay the uptake of carbohydrates from the small intestine. These effects result in lower blood concentrations of glucose and insulin after a meal. Improved insulin responses can lead to enhanced insulin sensitivity[28], which may prevent, or have beneficial effects on, type 2 diabetes. It has also been suggested that dietary fibre can reduce the uptake of cholesterol, or the re-absorption of bile acids, which results in the synthesis of new bile acids from cholesterol[29].

Table 1. Physicochemical properties of dietary fibre and proposed effects on human health

Physicochemical property	Proposed effect	Effect on health
Soluble dietary fibre Viscosity	Delays gastric emptying, prolonging intestinal phase[27]	Contributes to satiety
	Prevents or delays nutrient uptake in the small intestine[28]	Lowers glucose, insulin and lipid levels after a meal
	Prevents the reabsorption of bile acids[29]	Lowers blood cholesterol levels
	Prevents the reabsorption of oestrogen[30]	Protects against breast cancer
	Prevents digestive enzymes from reaching their substrates/inhibits enzyme activity[31]	Lowers glucose, insulin and lipid levels after a meal
Interaction/"binding"	Binding to bile acids (mostly demonstrated *in vitro*)[32]	Lowers blood cholesterol levels
	Interaction with digestive enzymes (only demonstrated *in vitro*)[33]	Lowers glucose, insulin and lipid levels after a meal
Fermentation	Growth of health-promoting bacteria[34]	Protects against inflammation and colorectal cancer
	Production of short-chain fatty acids[29]	Lowers blood cholesterol levels, protects against colorectal cancer
Insoluble dietary fibre Intact particles	Increase stool weight[35]	Reduces the incidence of colorectal cancer and intestinal diseases
	Accelerate transit time[36]	Reduces time for nutrients to be absorbed; lowers glucose, insulin and lipid levels after a meal
Water-holding capacity/"viscosity"	"Particle viscosity" reduces or delays nutrient uptake (animal study)[37]	Lowers glucose, insulin and lipid levels after a meal

Several kinds of dietary fibre can be fermented by bacteria in the large bowel, which results in the production of short-chain fatty acids (SCFAs) which can be absorbed by the body and may lower LDL cholesterol[29].

Obesity, and probably also type 2 diabetes and cardiovascular disease, are associated with a chronic inflammatory state[38,39]. The intake of dietary fibre is correlated with the suppression of inflammation by many interrelated mechanisms, as illustrated in Figure 1. Apart from lowering the glucose, insulin and lipid levels, as mentioned previously, it has also been suggested that the intake of fibre can stimulate the growth of health-promoting bacteria, such as lactobacilli, which help to prevent inflammation[34]. Phenolic compounds (antioxidants) in the fibre fractions may decrease oxidative stress and thereby inflammation[40,41].

PREVALENCE OF CANCER

It has been reported that dietary fibre can decrease the prevalence of colorectal cancer, inhibit the prognosis of prostate cancer and offer protection against breast cancer, although results vary[30,42]. Protection against colorectal cancer could be due to the presence of phenolic compounds in the fibre fractions, shortened intestinal transit time and the production of SCFAs. The mechanism suggested to be responsible for a decrease in the progression of prostate cancer was lowered insulin levels[43], while protection against breast cancer has been suggested to be due to a reduction in oestrogen levels by the inhibition of re-absorption[30].

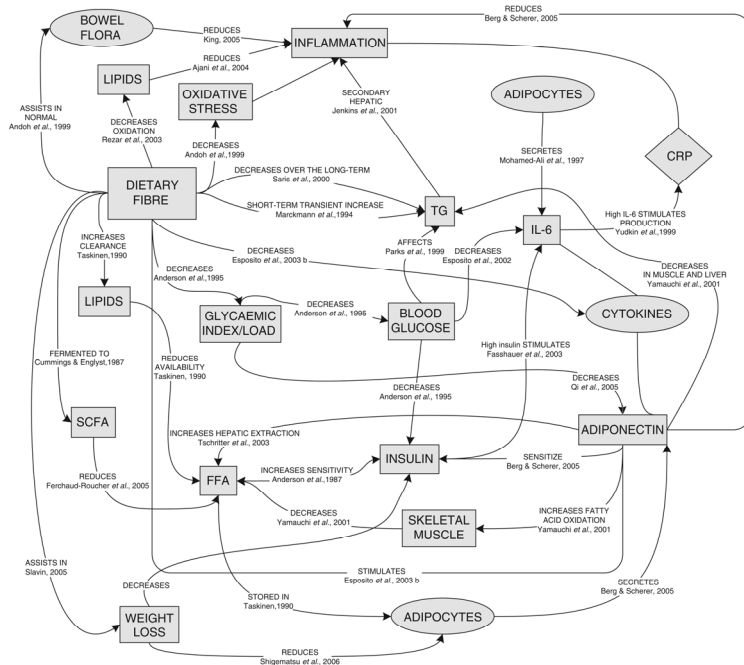**Figure 1.** Schematic illustration of the ways in which dietary fibre may influence inflammation (with permission, North et al.[44])

2.2. Beneficial physicochemical effects

The definition of dietary fibre does not include the physicochemical properties of the fibre, e.g. solubility or fermentability, despite the fact that different properties are responsible for different health effects in the body. The most common way of classifying dietary fibre is to group them according to their solubility, i.e. soluble (fibre from oats, barley, fruits, etc.) and insoluble (fibre from wheat, vegetables, etc.). Many of the physiological effects of soluble dietary fibres are related to their ability to increase viscosity (Table 1), as will be further discussed in this thesis.

The viscous property of soluble dietary fibre refers to the ability of the fibre to thicken solutions when mixed with fluids. Insoluble fibre particles can also increase viscosity, but only to a minor extent. An increase in viscosity along the gastrointestinal tract may result in delayed gastric emptying, the trapping of macronutrients, bile acids, oestrogen or digestive enzymes in the viscous network, and/or slower mixing and diffusion.

FACTORS INFLUENCING VISCOSITY
Several properties determine the viscosity of solutions of dietary fibre.

- ◦ *Solubility.* A high concentration of soluble fibre increases the viscosity. Fibres that have charged groups interact more favourably with polar solvents, such as water, which increases their solubility. Changes in pH affect the charge and polarity, leading to lower solubility and the formation of networks and gels. Side-chains and structural irregularities also lead to less network formation and hence, a higher solubility[45,46]. Milling to a smaller particle size promotes solubility due to the increase in surface area and by opening physical barriers allowing fluid penetration[47].

- ◦ *Molar mass.* A high molar mass contributes to increased viscosity, but at the same time to decreased solubility. The molar mass differs between fibre sources, and can be influenced by processing techniques[48].

- ◦ *Aggregation.* The ability of polymers to associate and form macromolecular aggregates can increase the viscosity[49]. Small molar masses increase the degree of aggregation due to higher diffusion rate[50].

- ◦ *Water-holding capacity.* A high water-holding capacity can also increase viscosity[51].

Mechanical treatment can cause shearing damage, influencing the particle size, the fibre structure and interactions, as well as depolymerisation[52]. High temperatures can also influence the structure causing depolymerisation of fibre chains. These effects may lead to a reduction in the viscosity. On the other hand, smaller fibre particles and/or a smaller molar mass will increase the solubility, which is also necessary for increased viscosity. Insufficient heating can result in unsuccessful deactivation of endogenous enzymes, which in turn can depolymerise the fibres. Frozen storage has been reported to decrease the solubility of β-glucans in oat products[53]. Repeated freeze-thaw cycles have also been demonstrated to cause physical changes and gelation, i.e. cryogels, in several polymers, which have been suggested to decrease the viscosity[54].

The ability of some forms of dietary fibre to form gels has also been suggested to delay or inhibit nutrient uptake in the small intestine, although the relationship between gelling and physiological effects is not yet fully understood[48].

2.3. Types of dietary fibre

Dietary fibre is found in plant foods. In the studies described in this thesis, particular fibre sources were selected with help of the food companies supporting the project as these were considered interesting fibre fractions for product development and commercial activities. The sources chosen for the studies were oats, rye and sugar beet, and the different fractions studied were the bran from oats and rye, a spray-dried oat drink and sugar beet fibre with different particle sizes. These fractions contain both soluble and insoluble fibre, but all are known for their high content of soluble fibre.

OATS
Oats (*Avena sativa* L.) are cool-season crops common in the Nordic countries, used mostly for porridge and breakfast cereals made from oat groats. The groat is the edible part remaining after removal of the insoluble fibre-rich hull, and contains about 8 % dietary fibre, about half of which consists of soluble β-glucans[55]. Oats are also good sources of protein, fat, vitamins and phenolic acids (antioxidants). Apart from mechanical treatment to remove the hull, the oat groats are hydrothermally treated (at about 100 °C for 0.5-2 h). This heat treatment is performed to inactivate enzymes, for example lipases and lipoxygenases to prevent rancidity, and β-glucanases to prevent degradation of the β-glucans[55].

Oat bran is the coarse fraction obtained when grinding oat groats to separate oat flour, and contains principally the outermost layers (the pericarp, seed coat and aleurone layer), but also parts of the inner endosperm and the germ (Figure 2). β-Glucans are found in the cell walls of the groat, primarily in the thicker cell walls in the outer layers which are included in the bran[56]. The β-glucan content in some commercial Nordic oat brans has been determined to be about 7 % (Table 2)[57].

The spray-dried oat drink is derived entirely from oats, and is produced by wet-milling of oat kernels followed by enzymatic treatment for the degradation of starch to maltose, separation of the insoluble fractions (including insoluble fibres) and finally spray-drying. The powder is reported to contain about 4 % β-glucan (Table 2)[58].

The soluble fibre β-glucan
β-Glucan is a linear, unbranched polysaccharide that consists of β-D-glucopyranosyl units linked with (1→3) and (1→4) linkages (Figure 3A). The (1→4) linkages occur in groups of two or three, but longer cellulose segments can sometimes be found, separated by a (1→3) linkage. The irregular structure prevents the formation of an ordered crystalline structure, leading to the polysaccharide being water soluble.

Rye (*Secale cereale* L.) consumption varies considerably throughout the world. The highest intake is in north-eastern Europe and the Nordic countries, usually as rye bread or crisp bread[59]. The rye grain contains about 17 % dietary fibre, but increases to 20 % when fructan is included, using the new EU definition of dietary fibre[60]. The main dietary fibre is arabinoxylan, the content being about 10 % (3 % soluble, Table 2), although higher values have also been reported[60]. After the hull is removed during threshing, the grain is either used whole, ground or milled. Rye bran is the coarse fraction obtained after grinding, and contains the pericarp, seed coat and aleurone layers, but also parts of the inner endosperm and the germ (Figure 2).

The soluble fibre arabinoxylan

Arabinoxylan is a branched polysaccharide. The branches prevent the formation of an ordered crystalline structure, rendering the polysaccharide water soluble. The arabinoxylan backbone consists of β-D-xylopyranosyl residues connected via (1→4) linkages (Figure 3B). About half of the residues carry α-L-arabinofuranosyl units, a few percent are double-branched, carrying two arabinose units and some of the arabinose units also carry a ferulic acid (a phenolic acid)[61]. Arabinoxylans in rye are more branched than those in wheat, and thus more soluble. Arabinoxylans in the endosperm are more branched than those in the outer layers[62].
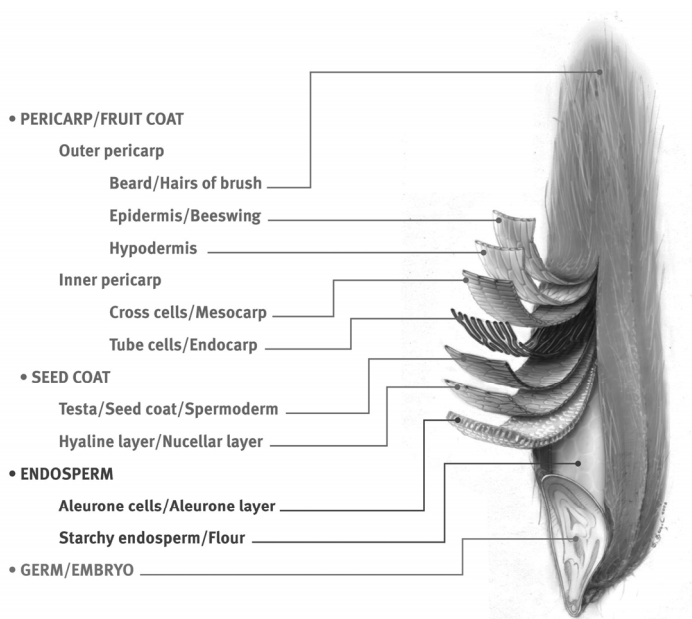

Figure 2. Cross section of an oat kernel. The cross section of a rye kernel is almost identical. (Kampffmeyer Food Innovation GmbH, Hamburg, Germany).

Sugar beet (*Beta vulgaris* L.) is one of the most important crops in southern Sweden[63], and the fibre is obtained from the residue remaining after the extraction of sucrose. The beets are heat treated (typically at 85 °C for 15 min) followed by diffusion in water to extract the sucrose for sugar production, and the remaining pulp is steam dried[45]. The dried sugar beet fibre is sold commercially and contains about 20 % of the soluble fibre pectin (Table 2).

The soluble fibre pectin

Pectin is a charged polysaccharide with side-chains and substituents. Both the charged groups and the branched structure prevent a packed and ordered structure, resulting in a water soluble polysaccharide. The backbone is composed of α-D-galacturonic acids with methylated carboxyl groups and acetylated hydroxyl groups, and connected via (1→4) linkages. These form long smooth regions, interrupted by hairy regions of galacturonic acid and (1→2)-linked rhamnose (Figure 3C). The rhamnose residues carry side-chains with arabinoses and galactans[64]. Beet pectin differs from the pectins obtained from, for example, citrus fruits and apples, in having a lower molar mass, a higher amount of acetyl groups and side-chains, and the presence of ferulic acids at amounts similar to those in cereals. The acetyl groups and neutral sugars reduce the charge effect, resulting in solubility and increased viscosity at low pH, but without gelling[65].

The mechanisms behind the health effects of sugar beet fibre have been questioned, since the molar mass of the soluble fibre pectin in sugar beet is reported to be lower than that of soluble fibres from other sources[45] (Table 2). On the other hand, sugar beet fibre is reported to have a higher water-holding capacity (sugar beet fibre 29 > oat bran 24 > rye bran 23 g/g[66]). A low degree of carboxyl esterification and a high amount of uronic acids have further been suggested to lower the pH in the intestinal tract and reduce the activity of digestive enzymes, leading to slower or reduced nutrient uptake[67].

Table 2. Content of total fibre, and the content and properties of the main soluble fibre in the fibre fractions studied.

	Total fibre[1] (%)	Main soluble fibre			
		Type	Amount[1] (%)	Structure	Molar mass[2] (g/mol)
Oat bran	11-19	β-Glucan	5-8	Unbranched	3×10^6
Spray-dried oat drink	8	β-Glucan	4	Unbranched	n.d.
Rye bran	20	Arabinoxylan	10^3	Branched	2×10^6
Sugar beet fibre	75	Pectin	20	Branched, charged	0.4×10^6

[1]Oat bran[57], spray-dried oat drink[58], rye bran[60], sugar beet fibre[45]
[2]Oat bran[68], rye bran[60], sugar beet fibre[69]
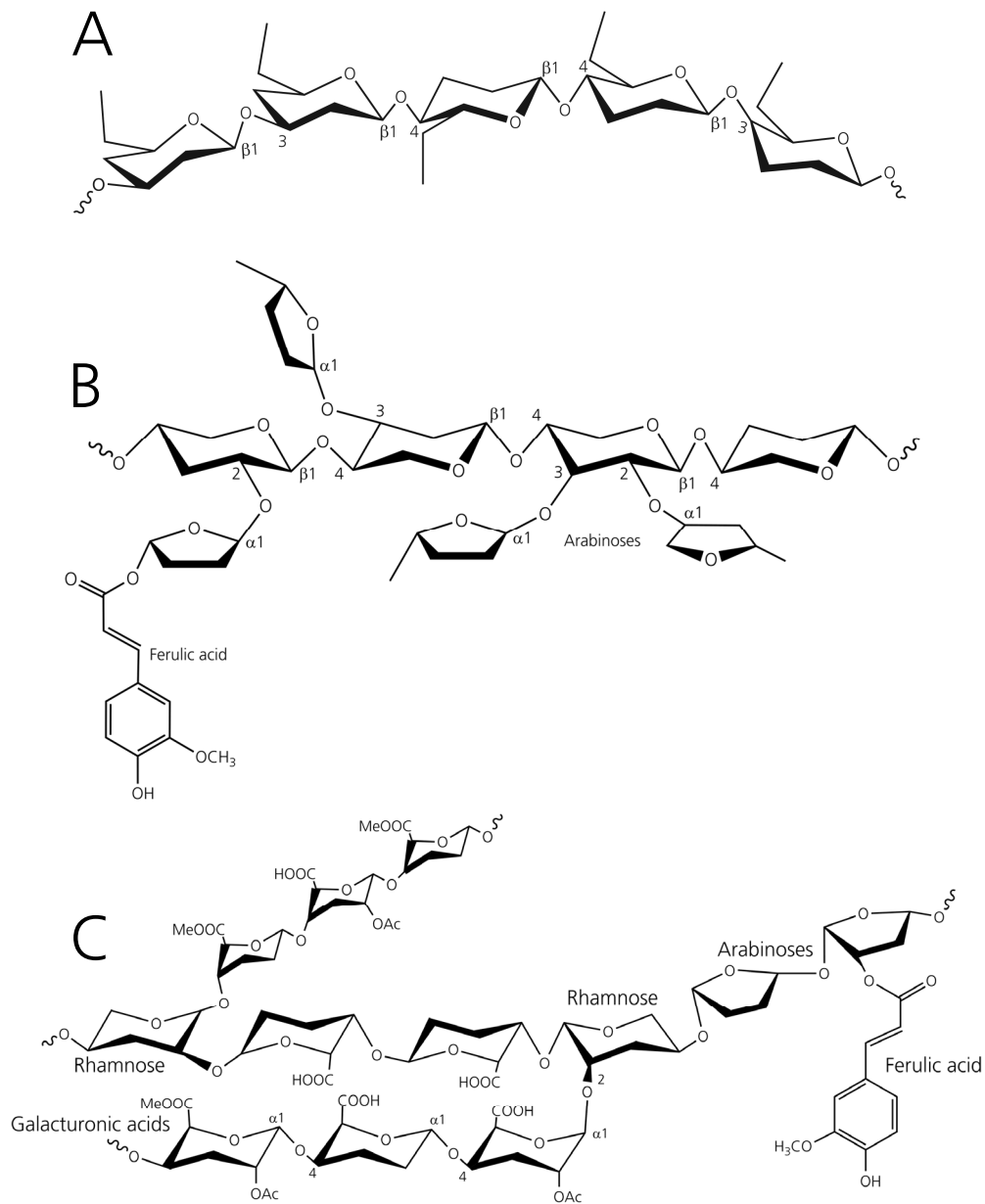[3]Of which 30 % is soluble[60]

Figure 3. Structures of A) β-glucan, modified after Vasanthan and Temelli[70], B) arabinoxylan, modified after Vinkx and Delcour[61], and C) sugar beet pectin, modified after Funami et al.[71] and a figure by Nordic Sugar A/S[65]. The H and OH groups are not shown in the structures.

Several studies have demonstrated that oat bran, rye bran and sugar beet fibre have hypoglycaemic and/or hypoinsulinaemic effects after a fibre-rich meal[72-74], as well as lowering the blood cholesterol after long-term intake[75-77]. No previous studies on the effects of ingesting spray-dried oat drink on glucose and insulin levels after a meal could be found. Oat milk, which is similar to the dispersed spray-dried oat drink, has been demonstrated to have a blood-cholesterol-lowering effect after long-term intake[78].

In 1997, the US FDA approved a health claim stating that soluble fibre from whole oats (oat bran, rolled oats or oatmeal, and whole-oat flour), as part of a diet low in saturated fat, cholesterol and total fat, may reduce the risk of heart disease[3]. The minimum amount of fibre required for a blood-cholesterol-lowering effect was 0.75 g of soluble fibre per serving, based on an intake of 3 g per day. The scientific panel of the EU has reacted favourably to the application for a health claim that 3 g β-glucan from oat and barley or unprocessed or minimally processed β-glucans per day can maintain normal blood cholesterol levels[79]. Recently, a claim that there is a correlation between an intake of 3 g oat β-glucan per day, naturally present or in forms added to foods, and a lowering of blood cholesterol was also positively received[80]. A correlation between β-glucans and long-term maintenance of normal blood glucose concentrations has also been evaluated for a claim, but without establishing a relationship[81].

Until there is general agreement on EU regulations, generic Swedish health claims and the health claims of specific products can be used. One generic claim is approved for oat or barley soluble fibres and reduced risk for cardiovascular disease/atherosclerosis, according to the two-step principle i) *A nutritionally balanced diet high in soluble fibres from oats can contribute to lower cholesterol levels in the blood and thereby reduced risk of cardiovascular disease.* ii) *Product X is high in soluble oat fibres*[82]. For processed foods containing β-glucan, a cholesterol-lowering effect must be demonstrated. Two product-specific health claims have been approved for a muesli product with soluble fibre derived from oats: one related to a lowering of blood glucose, and the other to lowered blood cholesterol levels.

Regarding sugar beet fibre, the EU scientific panel has expressed a positive opinion of claims that 10 g pectin (no given source) per meal can reduce postprandial glycaemic responses, and that 6 g pectin per day maintains normal blood cholesterol levels[83]. Furthermore, applications for health claims that sugar beet fibre balances blood glucose levels, maintains blood cholesterol levels, and improves bowel function have been registered[84].

A request for the approval of a health claim within the EU regarding the effects of rye fibre on carbohydrate metabolism and insulin sensitivity has also been registered[84].

2.4. Nutrigenomics

Nutrigenomics is the study of the interaction of dietary components with the genome and the resulting changes in gene expression, the function of proteins and metabolites[85]. The techniques used to study these changes can be divided into transcriptomics, proteomics and metabolomics, which are all inter-related (Figure 4). The linking of these different *-omics* is referred to as *systems biology*. The ability to measure small changes in pathways can help our understanding of how diets or individual dietary components affect the body.

Transcriptomics is the study of mRNA transcripts produced by the genome at any one time, and provides information on which genes are active and hence, which proteins will be formed. Proteomics is the study of the quantity and categories of proteins present at any one time. It is also possible to identify post-translational modifications resulting from nutritional influences. Metabolomics is the study of all the metabolites present at any one time.
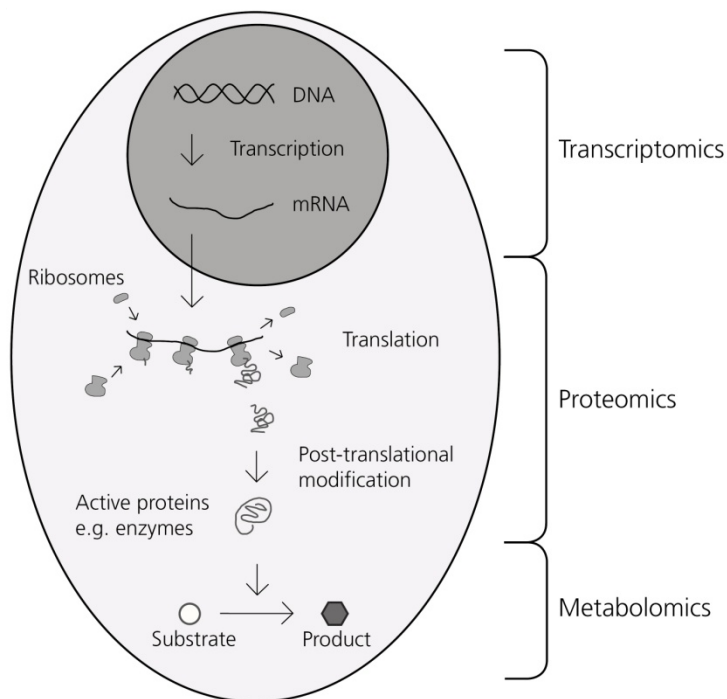
Figure 4. Activated genes are transcribed and the genetic information forms the template for the translation to proteins. Proteins may undergo post-translational modifications, such as glycosylation, leading to activation or inactivation. Furthermore, several active proteins are enzymes that can transform substrates into products (metabolites).

Infobox 1 Microarray analysis

Microarray analysis is a technique used for studying a "snapshot" of gene expression for thousands of genes simultaneously. The array (a chip) has an organised matrix of known spots, containing many short oligonucleotide probes representing one gene. mRNA isolated from the sample is converted to a complementary DNA and back to a complementary, single-stranded RNA (cRNA) which is fluorescently labelled. The cRNA fragments are hybridised with their complementary probe on the chip (base pair) while unhybridised cRNA is washed away (Figure 5).

A fluorescence scanner is used to quantify the intensity of the spots, which is directly related to the amount of target mRNA, and the resulting image is analysed to extract raw data on the gene expression.

Data analysis and algorithms are then used to determine which genes are significantly up-regulated or down-regulated with respect to a control sample.
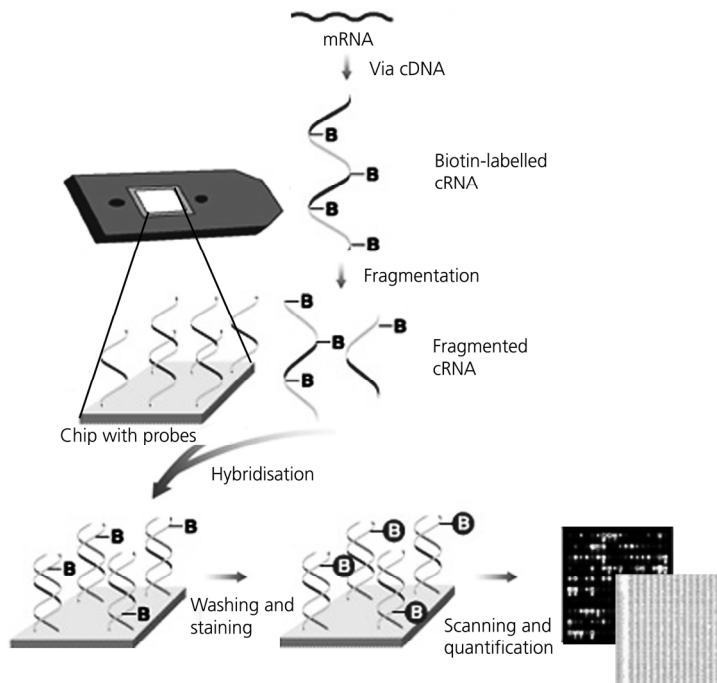
Figure 5. Microarray analysis, modified after Staal et al.[86] and the Affymetrix webpage[87]

In 2008, Rideout et al. pointed out how limited our understanding was of the ways in which dietary fibre regulates molecular events at the gene level, and called for more studies on dietary fibre using -omics approaches[7]. At an international symposium in the Netherlands, *nutritional regulation of gene transcription* was voted the third greatest recent discovery in nutrition, and *controlling obesity and insulin resistance through activity and diet* the most important future challenge[88]. By studying gene transcription with microarray analysis (Infobox 1), changes in the expression levels of thousands of genes, in response to a dietary component, can be measured in one analysis. The technique not only allows the analysis of changes that are already known to be related to an active food component, but also those where no function is known, or for which no involvement has been described previously[89].

Transcriptomics was only recently introduced into nutrition research. Published microarray studies within nutrigenomics research on humans have, for example, demonstrated that a diet rich in rye and pasta could decrease the expression of genes related to insulin signalling in adipose tissue of subjects with metabolic syndrome[90]. In the same study, oat-wheat-potato diet, ingested by another group, was found to activate genes responding to stress. Another study has demonstrated that the long-term intake of polyunsaturated fatty acids (fish-oil) decreased the expression of inflammatory genes in blood cells of healthy subjects[91].

3 OBJECTIVES

The aim of the work described in this thesis was to analyse the characteristics of soluble fibre under conditions similar to those in the gastrointestinal tract or in a food product, with as little impact as possible of the analytical methods. Based on the hypothesis that particle size, food matrix and processing are important factors for the viscous effects, the specific objectives of the fibre characterisation studies were:

1   to investigate how much soluble fibre is released from fibre particles using an *in vitro* method mimicking the gastrointestinal tract, including the effects of particle size and the surrounding food matrix (Paper I), and

2   to study the solution behaviour and aggregates of β-glucans, including the effects of simulated food processing (Paper II), as well as the effect of gastric and intestinal digestion using the *in vitro* method (Paper III).

The results obtained from the fibre characterisation studies were used to select active functional fibre fractions for use in test meals in a human study. The specific objectives here were:

3   to evaluate postprandial glucose, insulin and triglyceride responses in healthy subjects after the intake of meals containing rye bran, sugar beet fibre, spray-dried oat drink or a mixture of these three (Paper IV), and

4   to investigate changes in gene expression in the same subjects after an oat-bran-rich meal, using microarray analysis (Paper V).

4 *IN VITRO* CHARACTERISATION OF FIBRE

The physicochemical properties of soluble fibres are analysed in order to understand the impact of fibre source and treatment, as well as to ensure a high-quality product. However, many analyses include procedural steps that can influence the results *per se*. For example, extraction methods often involve enzymes, acids or bases at high temperatures, and polymer size is conventionally determined by high-performance size-exclusion chromatography (HPSEC) involving high shear forces[92,93]. This chapter describes the analysis of viscosity-related properties performed under physiological conditions, i.e. conditions similar to those in the gastrointestinal tract or in a food product.

4.1. Materials and methods

COMPOSITION OF THE SELECTED FIBRES
The fibre fractions; oat and rye bran (Lantmännen Food R&D AB, Järna, Sweden), spray-dried oat drink, which is hereafter denoted oat powder (Oatly AB, Landskrona, Sweden) and the sugar beet fibre (Nordic Sugar A/S, Copenhagen, Denmark) were analysed with regard to their nutrient composition (Table 3).

The oats contained more protein and fat than the other cereals, while the production of the oat powder removes not only the insoluble fibre but also the protein and fat, thus increasing the proportion of carbohydrates. Rye bran contains a high relative amount of insoluble fibre, while the sugar beet fibre consists almost only of dietary fibre and has a low energy content.

IN VITRO GASTROINTESTINAL METHOD
Measurements of digestion along the gastrointestinal tract are not easily performed in humans or animal models. However, since dietary fibre is neither digested nor absorbed in the gastrointestinal tract, it may be possible to study some processes using a simple *in vitro* method. A "dissolution tester", used to assess the dissolution properties of tablets in the pharmaceutical industry, was used (Figure 6). The experiments were performed in parallel vessels, in which the settings and fluids were standardised according to the United States Pharmacopeia (USP)[94].

Table 3. Nutrient composition (g/100 g) of the different types of fibre studied, together with measured/reported values of the specific soluble fibre (g/100 g).

Component	Oat bran[1]	Oat powder	Rye bran	Sugar beet fibre
Energy (kJ/100 g)	1452	1635	904	503
Carbohydrate	44.9	72.9	28.2	13.4
Fat	9.1	6.0	4.9	3.2
Protein	20.7	10.2	14.3	9.2
Moisture	6.9	4.3	8.9	6.8
Ash	3.0	1.2	4.3	3.9
Total dietary fibre	15.4	5.4	39.4	63.5
Insoluble fibre	9.3	1.0	33.9	36.7
Soluble fibre	6.1	4.4	5.5	26.8
β-Glucan[2]	6.5	4.3		
Soluble arabinoxylan[3]			5.0	
Pectin[4]				27.5

[1] Materials reported in Paper V. Another batch was reported in Papers I and III, with a β-glucan content of 8.4 g/100 g (9.1 g/100 g dry weight, moisture 7.4 g/100 g).
[2] Measured using an enzymatic method described in Paper I.
[3] Reported value from the same supplier but not the same batch[95]: it was estimated that 30 % of arabinoxylans are soluble (Section 2.3).
[4] Measured using a calorimetric method described in Paper I (29.5 g/100 g dry weight).

The USP method was modified in order to avoid transfer between vessels, by adding an intestinal boost to transform the simulated gastric fluid (pH 1.2, containing NaCl, HCl and pepsin) to small intestinal fluid (pH 6.8, containing the gastric fluid, KH_2PO_4, NaOH, bile acid, lecithin and pancreatin). Bile acid and lecithin were added to the fluid to emulsify fat from the fibre particles and foods and to further mimic the *in vivo* conditions.

Fibre fractions were added to the gastric fluid. After 1 h, the gastric fluid was converted into small intestinal conditions by adding the boost, and digestion continued for up to 2 h. Samples were withdrawn from both gastric and intestinal digestion and the in-soluble fractions were separated. When oats were digested, the samples were also boiled to inactivate degrading enzymes.

Releasability in the gastrointestinal tract
Three sizes of oat bran and sugar beet fibre particles were studied; one fraction consisting of coarse particles, and two fractions that were milled and sieved (oat bran: 1 mm^2, < 1 and < 0.5 mm particle size, and sugar beet fibre: 18 mm^2, < 0.125 and < 0.032 mm particle size). The intermediate fractions were also mixed 1:1 with egg white (protein), potato starch (starch) and butter oil (fat), respectively.
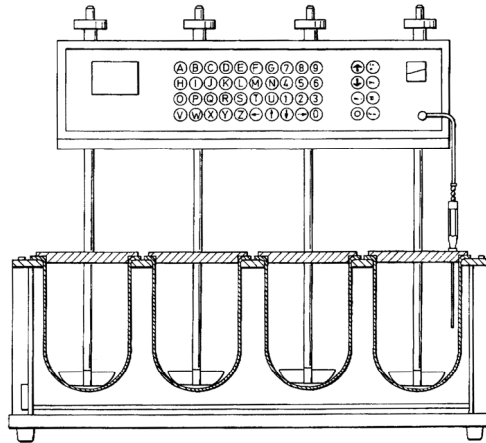
Figure 6. Dissolution tester with paddles for stirring and surrounding water bath to maintain a constant temperature. Modified after Müller[96].

ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION

Asymmetrical flow field-flow fractionation (AsFlFFF) was pioneered by Karl-Gustav Wahlund, Lund University[97]. The method allows the fractionation of molecules varying greatly in size, making the method suitable for high molar mass soluble fibre and their aggregates. The absence of a stationary phase results in low shear forces (Infobox 2), which enables the solution behaviour of the fibre to be studied with minimal impact of the analytical method. The absolute molar mass distribution of β-glucan was studied by combining AsFlFFF with multi-angle light scattering (MALS) detection and refractive index (RI) detection. AsFlFFF combined with fluorescence detection was used to identify which molecules/aggregates were β-glucans in multi-component samples. Calcofluor was used as the fluorescent label via a t-junction after the fractionation.

Influence of food processing on solution behaviour of dietary fibre

A pure barley β-glucan molar mass standard (molar mass in g/mol is the same as molecular weight or relative molecular mass in u or Da[98]) was used in this study. To retain the aggregates, the β-glucans were dissolved under mild conditions by stirring in a 70 °C water solution (10 mM $NaNO_3$) for 30 min. One sample was divided into several aliquots for different processing:

- ◦ no processing, direct testing
- ◦ microwave heating at 100 °C for 4 or 10 min
- ◦ microwave heating at 121 °C (autoclave temperature) for 4 or 10 min
- ◦ freezing for 1 week
- ◦ 5 or 10 freeze-thaw cycles.

Infobox 2 The AsFlFFF separation technique

Separation using field-flow fractionation is based on differences in diffusion coefficient, which in turn results from the size and the shape of the molecules. Asymmetrical flow field-flow fractionation (AsFlFFF) is performed in a thin channel without any packing, where the upper wall is a glass plate (Figure 7). The lower channel wall, called the accumulation wall, consists of a membrane which allows liquid to flow through, but not the solute. The flow into the channel, F_{in}, makes up both the axial flow, F_{out}, and the perpendicular cross-flow, F_c.

Molecules have a tendency to distribute themselves as evenly as possible throughout the available volume by diffusion (D). Smaller molecules diffuse more rapidly than larger ones. When the components in the sample are forced towards the accumulation wall by the cross-flow, diffusion counteracts this force, and at equilibrium the size of the molecule determines its distance from the accumulation wall (ℓ). Smaller molecules will have a greater average distance from the wall, and will be affected by a higher axial flow, and will thus be transported through the channel faster than large particles.
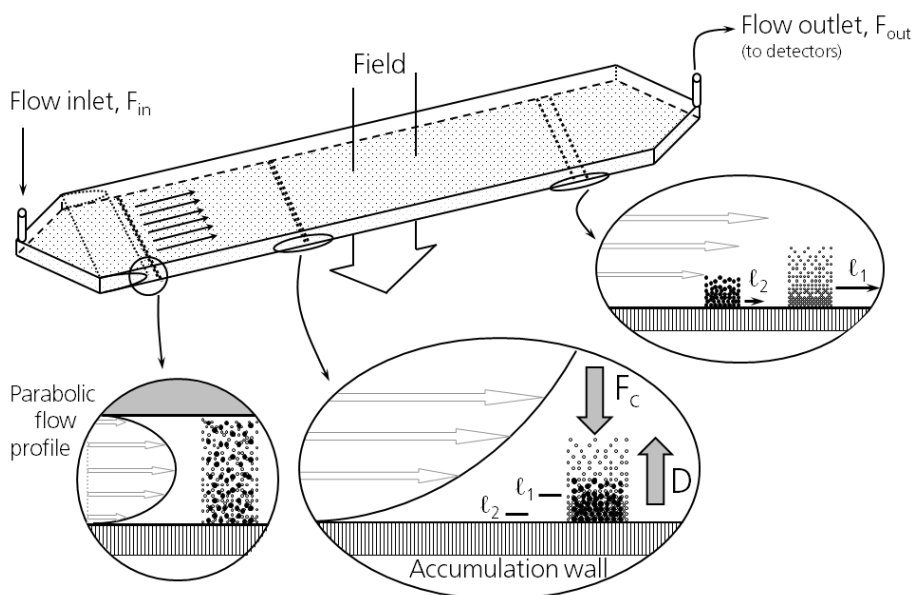

Figure 7. Illustration of a field-flow fractionation channel with two sample components at distances $\ell 1$ and $\ell 2$ from the accumulation wall. In AsFlFFF, the field applied is a cross-flow (F_c) passing through the accumulation wall counteracted by molecular diffusion (D). Modified after Andersson[99].

The effect of storage at room temperature was investigated by analysing samples once a week for up to three weeks (unprocessed samples and samples heated to 121 °C).

Influence of gastrointestinal conditions on solution behaviour of dietary fibre
Oat bran samples (< 0.5 mm) treated in the dissolution tester that mimicked the gastrointestinal tract were withdrawn after gastric and small intestinal digestion for analysis with AsFlFFF. β-Glucan concentrate from oat and barley were digested and analysed in the same way. The fibre fractions were also dispersed in water and in gastrointestinal fluids without digestive enzymes and bile acids to isolate the impact of the gastrointestinal conditions, and to establish whether there was any binding of β-glucans to digestive enzymes or bile acids (see Table 1, page 5).

4.2. Results and discussion

RELEASABILITY IN THE GASTROINTESTINAL TRACT
Studying the release of soluble fibre from the fibre fractions showed that unmilled oat bran released 15 % β-glucan during gastric digestion and a total of 20 % had been released after small intestinal digestion. Digestion of the coarse sugar beet fibre fraction showed that 30 % of the pectin was released during gastric digestion and 50 % after small intestinal digestion (Figure 8). Other studies on the release of β-glucan from oat bran after digestion *in vitro* and *in vivo* (cannulated pigs) have demonstrated similar results (10-15 % of the β-glucan was released after gastric digestion and 10-30 % after small intestinal digestion, depending on the type of oat bran)[53,100]. Despite the fact that low releasability is reported in the literature, optimisation is seldom discussed. No reports on the releasability of sugar beet pectin could be found in the literature.

Influence of particle size and food matrix
The releasability was significantly increased by reducing the particle size by milling. The total release of β-glucan for the two milled oat bran fractions increased to 55 %, compared with 20 % for the unmilled fraction, and the release of pectin from milled sugar beet fibre increased to 65-75 % for the milled fractions, compared with 50 % for the coarse fraction (Figure 8).

Mixing oat bran with protein or starch significantly decreased the releasability of β-glucans: 7 % β-glucan was released from the protein matrix and 4 % from the starch matrix after digestion, compared with 55 % without matrix. For sugar beet fibre, 30 % of the pectin was released from the fraction mixed with protein after digestion, and 40 % was released from the fraction mixed with starch, compared with 70 % without matrix (Figure 8). The release from both types of fibre took place primarily during intestinal digestion, probably due to the action of pancreatic enzymes. These results indicate that the amount of soluble fibre released in the upper small intestine may not have been sufficient to increase viscosity of the intestinal content. Mixing the fractions with fat, which melted at 37 °C, resulted in slower release, but there was little influence on the total amount of fibre released.

The fact that pectin is a small, charged, branched molecule may have increased its re-leasability compared with the linear, unbranched, large β-glucan molecule. Further-more, the treatment of sugar beet fibre during production makes the structure more porous[101], which may facilitate water penetration and thus releasability, compared with the more compact oat bran particle. Indeed, the coarse sugar beet particles (18 mm^2) released more fibre than the small oat bran (1 mm^2). The milled sugar beet fibre parti-cles were also smaller than the milled oat bran particles (it was not possible to obtain smaller oat bran particles with the mill used), which may have increased the amount of fibre released.
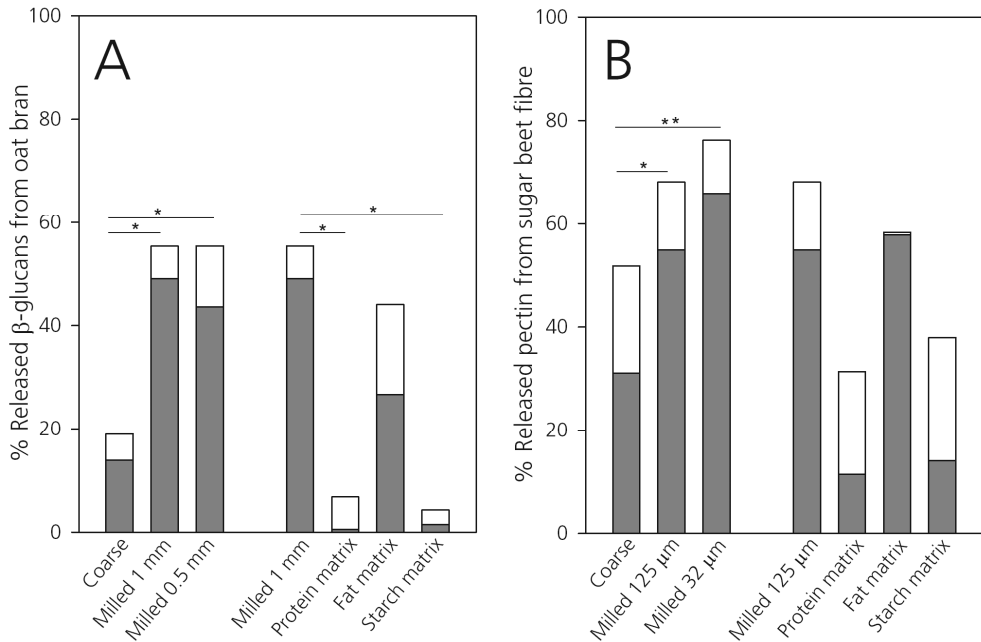
Figure 8. Percentage of soluble fibre released during gastric (grey) and intestinal (white) *in vitro* digestion. A) β-glucans released from oat bran from different particle fractions (left) and with or without food matrices consisting of protein, fat or starch (right). B) Pectin re-leased from sugar beet fibre from different particle fractions (left) and with or without food matrices consisting of protein, fat or starch (right). * p < 0.05. ** p < 0.01. The complete release profiles can be found in Paper I: Figures 2 and 3.

The pure β-glucan samples that were unprocessed and analysed directly using AsFlFFF showed a weight-average molar mass (M_w) close to 3×10^6 g/mol, compared with the value of 0.4×10^6 g/mol reported by the producer. (M_w is the total sum of the mass of each chain of a particular length divided by the total mass of the entire sample.) The difference suggests the presence of aggregates when using AsFlFFF, while HPSEC used by the producer probably resulted in individual molecules.

Influence of food processing

The aggregates seemed to form directly when the β-glucans were dissolved, and were present irrespective of how quickly the analysis was performed after dissolution. Storage at room temperature for up to 3 weeks had no significant effect on the molar mass.

A temperature of 100 °C appeared to disrupt only the largest aggregates, while 121 °C reduced the size markedly, and may even have degraded the individual molecules (Figure 1A in Paper II). Freezing of the sample did not affect the molar mass noticeably, while freezing and thawing the sample several times resulted in a substantial increase in the molar mass. Moreover, this increased molar mass did not result in a slower transportation through the AsFlFFF channel, indicating that the aggregates had a more dense structure which may be cryogelation.

It has also been reported that some rye arabinoxylans can form labile aggregates, and oxidative coupling of ferulic acid residues can cause gel formation[62]. Sugar beet pectin has also been reported to be able to form micelle-like aggregates or to aggregate via a protein component[102].

Influence of gastrointestinal conditions

The β-glucan aggregates from oat bran as well as oat and barley β-glucan concentrates were disrupted by the gastric conditions, compared with when they were dispersed in water. The subsequent small intestinal digestion resulted in the re-formation of aggregates. No difference was seen when bile acids and the digestive enzymes were absent, suggesting that the probable cause of the changes in aggregate size was the pH (1.2 under gastric conditions and 6.8 under the small intestinal conditions). No indications were found of binding of β-glucan to bile acids or the enzymes.

While β-glucans from oat bran returned to the same molar mass as when dispersed in water, the pure β-glucans increased considerably in molar mass under intestinal conditions but without a slower transportation through the AsFlFFF channel. This demonstrated denser aggregate structures, possibly indicating a first stage of gel formation. A possible explanation could be depolymerisation of the pure, exposed β-glucans under gastric conditions and hence, a greater ability to form aggregates and ultimately gels[50]. The same effect was not seen for oat bran, where the β-glucans may be more protected by the surrounding particle. Oat bran samples after intestinal digestion in this *in vitro* study were also analysed using HPSEC as part of a study by Immerstrand et al.[103]. No difference in molar mass was detected between digested and undigested oat bran.

The ability of pure β-glucans to form a gel in the small intestine could lead to the entrapment of macronutrients in the gel matrix, similar to that reported for oil drops in the gel matrix of the fibre chitosan[104]. This mechanism could contribute to the reduction or delay in the uptake of nutrients by the body, and may be a mechanism for the unexplored relationship between gelling and physiological effects (Section 2.2).

5 *IN VIVO* EFFECTS OF FIBRE INTAKE

This chapter focuses on the performance and results of the postprandial study conducted on healthy volunteers to document the health effects of the different fibre sources. Fibre fractions with small particles were used to improve the releasability of soluble fibres (according to the results presented in Paper I). The fibres were mixed in a cold beverage prior to serving, to obviate the need for a solid food matrix or processing, allowing high releasability and retention of aggregates (according to Paper I and II).

The study was divided into two parts. The primary endpoint of one part was to examine the effects of fibre-rich meals on postprandial glucose concentrations (Paper IV), while the aim of the other part was to study the effects of a fibre-rich meal on gene expression with microarray analysis (Paper V). Secondary endpoints were the study of postprandial insulin and triglyceride levels (as well as postprandial glucose levels in Paper V).

5.1. Fibre fractions studied

Four different fibre fractions were studied separately: oat bran, spray-dried oat drink (oat powder), rye bran and sugar beet fibre. An additional meal contained a mixture of oat powder, rye bran and sugar beet fibre.

The fibres were mixed with a blackcurrant juice containing pulp (Figure 9), since this matrix was determined to give the best taste and colour. The aim was to use a dose of 5 g soluble fibre per meal, which is well above the quantity reported to lower postprandial blood glucose and insulin levels[6,74]. However, due to the high carbohydrate content of the oat powder and the high amount of insoluble fibre in rye bran, lower soluble fibre contents were used in these meals (2.7 and 1.67 g, respectively). The mixted meal contained 1.67 g soluble fibre from each fibre fraction, resulting in a total content of 5 g. The control meal was the beverage without added fibre.

White wheat bread and/or dextrose powder was added in order to balance the amount of carbohydrates in each meal. Dextrose was included when the amount of bread would have been too large. Rapeseed oil was added to balance the amount of lipids. The protein content was not balanced. Due to the high carbohydrate content of oat powder, no extra carbohydrates were necessary. Similarly, the lipid content was high in oat bran and no oil was added. The meals were served together with a 200 ml glass of tap water. The nutrient content of the different meals is presented in Table 4.

Table 4. Nutrient content of the standardised test meals (g).

Standardised test meals	Control	Oat bran	Oat powder	Rye bran	Sugar beet fibre	Mix- ture
Amount (g)	334	348	316	347	347	343
Energy (kJ)	1640	1865	1665	1695	1664	1725
Carbohydrates	75.0	75.0	75.0	75.0	75.0	75.0
Lipids	7.9	7.9	7.9	7.9	7.9	7.9
Protein	5.4	18.2	6.3	8.5	6.7	10.0
Total fibre	1.4	12.9	3.3	13.0	13.2	18.3
Insoluble fibre	1.1	7.8	0.6	11.0	7.9	13.1
Soluble fibre	0.3	5.1	2.7	2.0	5.3	5.2
Soluble fibre from added fibre	**0.0**	**5.0**	**2.7**	**1.7**	**5.0**	**5.0**

5.2. Subjects and methods

Inclusion criteria for participating in the study were BMI 18-30 kg/m^2 and an age range of 20-65 years. Eighteen healthy subjects, 10 men and 8 women aged 20-28 years, were recruited, with the aim of ensuring at least 10 participants completed the whole study, who declared themselves to be healthy at the time of sampling for microarray analysis. The number of subjects was based on a study by van Erk et al., who reported a significant change in gene expression after meals rich in protein and carbohydrates with 8 subjects[105]. The advertisement was published among university employees and students, which explains the low age of the subjects.

The participants ate the test meals in a randomised single-blinded manner, at one-week intervals and after an overnight fast. Control meals were served twice to reduce the intra-individual variation, and the mean of these was used in the statistical analysis of traditional biomarkers. Although the content of the meals was not revealed to the participants, some of the test meals were visually fibre-rich (Figure 9).

Glucose, insulin and triglycerides, indicating changes in the metabolism, were measured before each meal, and then every 30 min for 180 min after the meals. Blood samples for gene expression analysis were taken before and 120 min after the meals.

MICROARRAY ANALYSIS
Samples for the investigation of gene expression were taken from peripheral blood mononuclear cells (PBMCs), from which mRNA was isolated. Apart from being easily obtained from the study subjects, PBMCs interact with every organ and tissue in the body[106]. Gene expression in PBMCs is furthermore reported to be stable within subjects, although there is large interindividual variation[107,108]. However, in studies where responses are compared within one subject, interindividual variations are of no consequence.

Figure 9. Two beverages used in the meal study: left, blackcurrant juice and right, blackcurrant juice mixed with oat powder.

The effects of the oat bran and control meal were analysed using microarray analysis, to examine the up- or down-regulation of genes resulting from the oat-bran-rich meal. The gene chips used were custom made for the European Nutrigenomics Organisation (NuGO) with 23 941 genes. For forty gene chips, samples from 5 men and 5 women (before and after the oat bran meal and before and after the control meal) who reported no illness were chosen. The selection was performed after discarding samples with haemolysis or turbidity, by choosing those which had the highest quantity and quality of mRNA. Hybridisation was performed at the SCIBLU Swegene Centre for Integrative Biology at Lund University.

Data analysis
The raw data on gene expression were analysed using linear mixed models (Infobox 3) to determine differentially expressed genes in response to either the meal intake (independent of meal content) or to the specific effect of oat bran. The *Meal Intake Model* was applied to the oat bran meal and control meal separately, and was corrected for gender and individual effects:

$$\text{Gene expression}_{gti} = \mu_g + \text{Gender}_{g,gender(i)} + \text{Meal effect}_{gt} + \text{Individual}_{gi} + \varepsilon_{gti}$$

where *Gene expression* is the base-2 logarithm of the background-corrected, normalised intensity of the g*th* gene (1,..., 23 941) with the t*th* meal effect (before or after the meal) of the *i*th individual (1,..., 10). *Gender* and *Meal effects* were defined as fixed effects and *Individual* as a random effect.

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Infobox 3 Linear mixed models

The intake of a meal influences which genes are expressed. The gene expression is measured and statistics are used to explain the values as a function of variables (e.g. gene, individual, before/after meal).

A linear mixed model is used when one independent variable, in this case gene expression, can be explained by a set of dependent variables. It is assumed that the independent variable can be explained by a sum of terms (hence the term linear). The terms, or effects, are of two types; *fixed effects* and *random effects* (hence the term mixed), where fixed effects are those used to make interpretations or draw conclusions, such as the effect of the meal. An example of a linear mixed model is:

$$Y_{gti} = \mu_g + T_{gt} + I_{gi} + \varepsilon_{gti} \qquad g = 1,...,100, t = 1,2, i = 1,...,5$$

where Y_{gti} is the intensity of the g*th* gene (of a total of 100) in the t*th* treatment group (1 or 2) of the i*th* individual (of a total of 5). μ is the grand mean, and ε is the normally distributed error term, with standard deviation σ_g. T is the effect of the treatment and I is the random effect of individuals, which is normally distributed with the standard deviation $\sigma_g^{individual}$.

</div>

The *Meal Content Model*, determining the specific effect of the oat bran meal, took both meals into consideration and was corrected for gender, the meal effect and the interaction between the individual and the week they were served the oat bran meal:

$$\text{Gene expression}_{gtfi} = \mu_g + \text{Gender}_{g,gender(i)} + \text{Meal effect}_{gt} + \text{Oat bran}_{gf} + \text{Individual}_{gi} + \text{Week}_{g,week(i)} + \varepsilon_{gtfi}$$

where *Gene expression* is the base-2 logarithm of the background-corrected, normalised intensity of the g*th* gene (1,..., 23 941) with the t*th* meal effect (before or after the meal) eating the f*th* meal (oat bran or control) of the i*th* individual (1,..., 10). *Gender*, *Meal effect,,* and *Oat bran* were defined as fixed effects, while *Individual* and *Week* were random effects.

These analyses gave information on the up- or down-regulation of all the genes on the chip (the magnitude of expression, fold change, FC), together with the probability (p-value) resulting from the intake of the meal or the specific oat bran meal. A false discovery rate (FDR) was calculated for the significantly expressed genes, correcting for multiple testing. The Gene Set Enrichment Analysis (GSEA) program was then used to estimate overrepresented predefined gene sets/pathways in the data, belonging to various databases and publications. All genes were ranked according to the FCs arising from meal intake and meal content (oat bran), respectively, using the Meal Content Model. GSEA was used to determine whether the genes in a gene set were randomly distributed or over-represented at the top (up-regulated) or bottom (down-regulated)

of the ranked list. Small changes in many genes belonging to the same pathway will result in a large change in the total set.

5.3. Results and discussion

A total of 15 subjects (8 men and 7 women) completed the study involving the two meals included in the gene expression analysis (oat bran and control), while 13 subjects (6 men and 7 women) completed the study of all the meals.

During the final study visit, the subjects were asked to complete a questionnaire about their impression of the meals. Comments on taste and appearance varied from sweet and pleasant to repulsive. Most women reported feeling satiated after the standardised fibre-rich meals, while more than half of the men indicated that they were still hungry. Two women complained of "feeling bloated" after some meals, but there were no other reports of gastrointestinal discomfort.

EFFECTS ON GLUCOSE, INSULIN AND TRIGLYCERIDE LEVELS
All fibre-rich meals lowered the incremental peak glucose response after 30 min, compared with the control meal, and this difference was significant for the oat bran ($p = 0.027$) and rye bran ($p = 0.021$) meals (Figure 10). None of the meals significantly reduced the incremental area under the curve (IAUC) for glucose in the 0-120 min interval compared with the control. Several significant differences were found with regard to the oat powder meal, as will be discussed below. The significant lowering of the glucose peak after the intake of oat bran (compared with the control) in the 13 subjects completing all the meals was not detected when 15 subjects were included for the calculations in Paper V.

The largest differences in insulin levels were found 60 min after the meals, where the oat bran meal resulted in significantly lowered insulin levels ($p = 0.008$) compared with the control (Figure 10). The IAUC for insulin was significantly reduced following the oat bran meal in the 0-90 and the 0-120 min intervals, and was somewhat lowered following the rye bran and sugar beet fibre meals, compared with the control.

All fibre meals had a tendency to *increase* the postprandial triglyceride levels compared with the control meal (Papers IV and V). Such a short-term increase in triglycerides has previously been noted as a result of the intake of dietary fibre or foods with low glycaemic index (GI)[109,110].

The effects of oat bran, containing 5 g soluble fibre, on glucose and insulin levels were not as surprising as the effect of rye bran on glucose levels as this contained less than 2 g soluble fibre. This result may be due to the type of fibre or the amount of soluble and insoluble fibre. The rye bran meal contained 11 g insoluble fibre, while the oat bran meal contained approximately 8 g, resulting in the total fibre content being the same in these meals (13 g). Another factor that may have contributed to the effect of rye bran was that this fraction was completely unprocessed. These different factors are discussed further in Chapter 6.
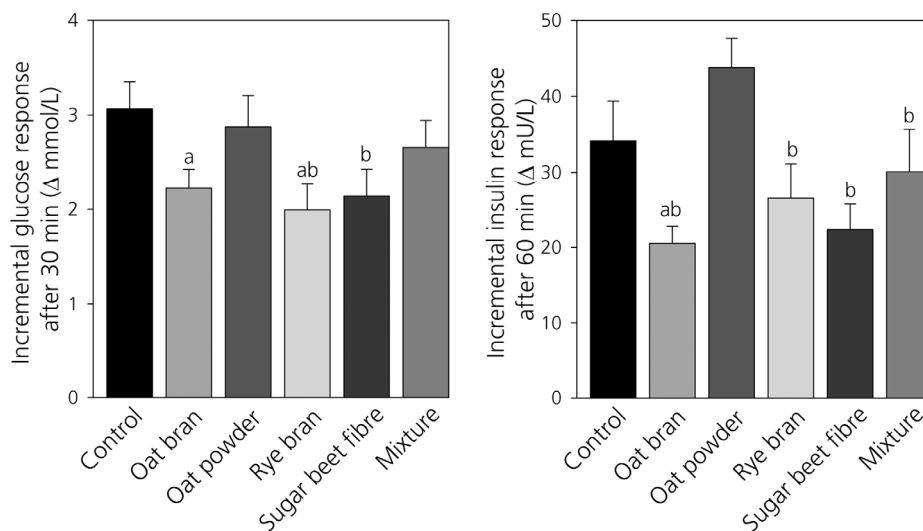
Figure 10. Incremental glucose response after 30 min (left) and incremental insulin response after 60 min (right), mean and SEM (n = 13[1]). *a* indicates a significant difference compared with the control meal (p < 0.05), and *b* indicates significant difference compared with oat powder (p < 0.05).

Effects of oat powder

The oat powder meal resulted in significantly higher peak glucose values than the meals containing rye bran or sugar beet fibre (Figure 10), and the IAUC for glucose in the 0-60 min interval was significantly higher following the oat powder meal than the oat bran, rye bran and sugar beet fibre meals. The oat powder meal resulted in higher insulin levels than the control meal, although the difference was not significant. All other fibre-rich meals thus led to significantly lower insulin levels at 60 min than the oat powder meal (Figure 10). However, the IAUC for insulin was significantly increased after the oat powder meal for all time intervals, however, and consequently oat bran, rye bran and sugar beet fibre led to significantly lower IAUCs than oat powder.

The finding that the *oat powder* meal increased the glucose and insulin levels was unexpected, since the oat powder meal was also quite fibre-rich (3.3 g total fibre, 2.7 g soluble). In spite of its fibre content, the IAUC for insulin was higher than that of the control. Similar results have been reported for milk whey, and were attributed to the amino acid composition[111]. Oats have a similar amino acid composition to whey,

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[1] In order to compare the outcomes of all meals, i.e. including the oat bran meal study described in Paper V, a Friedman test and the Wilcoxon signed rank test were performed on the 13 subjects who had completed the studies of all meals.

which could partly explain the present results. However, *oat bran* clearly decreased the insulin concentration compared with both the control and oat powder meals.

Another interesting effect of oat powder was its influence on the mixed meal. This meal contained as much soluble fibre from rye bran as the rye bran meal alone, and yet, the oat powder attenuated both the glucose- and the insulin-lowering effects.

Different responses in men and women
A post hoc analysis, separating postprandial glucose responses for men and women, was performed. The glucose levels differed after the intake of the fibre meals, but were similar following the control meal (Figure 11). The IAUC for glucose in the 0-120 min interval was significantly lower in women than in men, when eating oat powder, rye bran or the mixed meal. Unfortunately, this study included too few subjects to be able to calculate any significance between fibre-rich meals and the control meal for men and women separately.
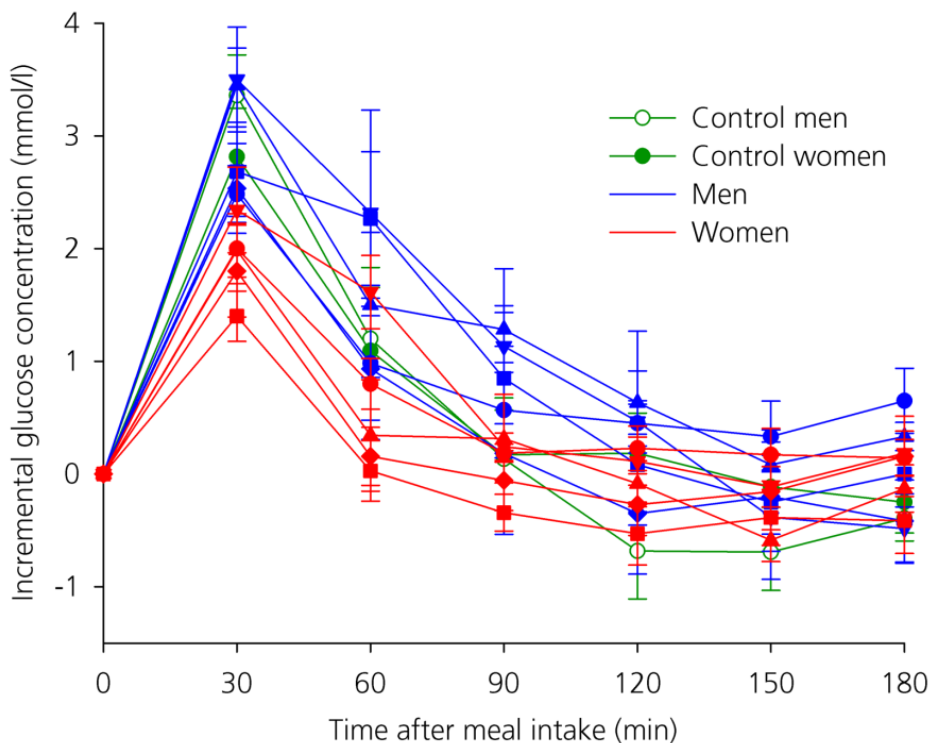
Figure 11. Incremental glucose concentrations after all fibre meals, for men (blue) and women (red), ● denotes oat bran, ▼ oat powder, ■ rye bran, ◆ sugar beet fibre, and ▲ mixture. The results for the control meals are shown in green (mean and SEM, n= 13).

The Meal Intake Model was used to evaluate the effect of meal intake for the oat bran and control meals. The Venn diagram (Figure 12) shows the number of genes that were differentially expressed in response to the two meals separately, and how many genes were expressed as the result of both meals. The higher value for the oat bran meal may indicate that this meal has a greater metabolic effect, but the diagram reveals neither the magnitude of expression nor the identity of the genes.

The genes that were most down-regulated by the oat bran meal were also down-regulated by the control meal, and vice versa for the most up-regulated genes (see Table 2 in Paper V). Only a few genes showed a fold change that reached the common[112] twofold cut-off, FC < 0.5 (down-regulated) or FC > 2 (up-regulated), and these indicated active glucose oxidation and protein synthesis, as well as an increased rate of inflammation. Changes in these genes indicate the transition from a fasting to a satiated state, and have been found after meal intake in several microarray studies[105,113]. The indication of high metabolic stress and inflammation is the normal response to meal intake, regardless of the content of the meal.

The gene sets and pathways that were changed in response to the meal intake, according to GSEA, also indicated activated inflammation pathways, and in addition active gluconeogenesis pathways. Gluconeogenesis is the mechanism maintaining a normal glucose level, and can be explained by low glucose levels in the blood 2 h after the meal. Other pathways influenced were those related to suppressed insulin production, and up- and down-regulation of cancer-related gene sets.

The Meal Content Model corrected for the effects of food intake in order to isolate the specific effects of oat bran. These effects were, however, small compared with the effect of the meal, and single genes were not statistically confirmed as being up- or down-regulated (FDR = 1, Table 3 in Paper V).

Figure 12. Venn diagram of differentially expressed genes after intake of the oat bran and control meal, and both meals.

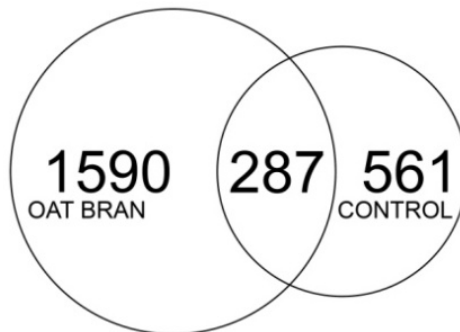Performing gene set enrichment analysis on the response to oat bran specifically revealed that the oat bran meal was responsible for the suppression of insulin synthesis/secretion and β-cell production, compared with the control meal (Table 5). This verifies the finding of lower insulin levels after the oat bran meal compared with the control. The other gene sets influenced by the oat bran meal may, in turn, be related to the insulin level. Inactivation of transcription, translation and ribosomal pathways, i.e. protein synthesis, is probably the result of suppressed insulin levels, as insulin regulates protein synthesis[114]. The cancer-related gene sets influenced by the oat bran meal (Table 5) may also be related to insulin, although it was not possible to determine the direction of regulation as different gene sets were both suppressed and activated. Insulin is a growth factor for oestrogen and other tumour promoters, and decreased insulin levels may thus be beneficial[115].

Changes in gene regulation in PBMCs have been reported to be more sensitive and may appear earlier than other biomarkers[116]. The suppression of pathways related to insulin production was also detected as a decrease in postprandial insulin levels. Lower insulin levels indicate the beneficial effect of a single oat bran meal, and may possibly be correlated to the soluble fibre content. The long-term intake of oat bran may thus contribute to increased insulin sensitivity and, in this way, help prevent type 2 diabetes. However, the relationship between healthy subjects ingesting a single oat bran meal and small effects on cancer-related gene sets is not clear. Long-term and epidemiological studies have indicated that a higher intake of dietary fibre reduces the prevalence and progression of cancer (Section 2.1), but possible short-term protective effects must be further investigated.

Changes in the transcriptome due to the intake of a meal or a dietary intervention are small. Apart from correcting for variables and combining data analysis methods, it is important to standardise as many influencing parameters as possible. Apart from inclusion and exclusion criteria, this study was standardised by, for example, asking the subjects to avoid intense physical activity (this included avoiding strenuous means of travelling to the study centre, and using lifts rather than stairs, etc.), handing out standardised meals to be eaten the evening before tests, and recording illnesses, in particular infections, at every visit. The expression of genes has been reported to vary throughout the day[117], and sampling was conducted at the same time of day at every visit. The nutrigenomics samples were prioritised during handling and analysis (the same person handled these samples). The time at which centrifugation and freezing were performed were noted, as well as analytical problems (e.g. haemolysis and turbidity), in order to be able to exclude potentially defect samples prior to analysis. The precautions and standardisation procedures were essentially the same as those in the NuGO guidelines[118], which was published after this study was completed. The samples selected for microarray analysis were further randomised during extraction and purification, according to subject and day of visit (but with the 0 and 120 min samples in the same batch), and all samples from one subject were hybridised in the same run. No deviations were observed on the arrays, and all passed the quality-control criteria for inclusion in the data analysis.

Table 5. Gene sets down-regulated or up-regulated in response to an oat bran meal, compared with a control meal (p < 0.01, FDR q < 0.01).

Gene set	Short description
Down-regulated	
Reactome GTP hydrolysis and joining of the 60S ribosomal subunit	Ribosomal
Reactome formation of a pool of free 40S subunits	Ribosomal
KEGG ribosome	Ribosomal
Reactome regulation of β-cell development	β-Cell
Reactome peptide chain elongation	Translation
Reactome viral mRNA translation	Translation
Reactome regulation of gene expression in β-cells	β-Cell
Reactome influenza viral RNA transcription and replication	Transcription/replication
Reactome translation	Translation
Reactome influenza life cycle	Replication
Reactome insulin synthesis and secretion	Insulin
Winnepenninckx melanoma metastasis up	Up-regulated in melanoma patients
Huttmann b-cell poor survival dn	Down-regulated in B-cell chronic leukaemia
Pujana BRCA2 PCC network	Genes related to breast cancer
Pujana XPRSS INT network	Genes related to breast cancer
Up-regulated	
Verhaak AML with NPM1 mutated up	Up-regulated in acute myeloid leukaemia
Huttmann b-cell poor survival up	Up-regulated in B-cell chronic leukaemia
Raghavachari platelet specific genes	Genes specific to platelets
Hsiao housekeeping genes	Housekeeping genes in 19 normal tissues
Mullighan MLL signature 2 up	Up-regulated in acute myeloid leukaemia
Rickman metastasis dn	Down-regulated in metastasic carcinoma
Rutella response to CSF2RB and IL4 dn	Down-regulated in PBMCs by e.g. IL4
Reactome RNA polymerase I promoter opening	Transcription
Jaatinen hematopoietic stem cell dn	Down-regulated in CD133+ stem cells
Mullighan MLL signature 1 up	Up-regulated in acute myeloid leukaemia
Schuetz breast cancer ductal invasive up	Up-regulated in invasive ductal carcinoma
Rutella response to HGF vs. CSF2RB and IL4 up	Up-regulated in PBMCs by e.g. IL4
Ross AML with AML1 ETO fusion	Top probe sets for acute myeloid leukaemia
Smirnov circulating endotheliocytes in cancer up	Up-regulated in endothelial cells in cancer patients
Tonks targets of RUNX1 RUNX1T1 fusion hcd dn	Down-regulated in acute myeloid leukaemia
Hoshida liver cancer subclass S1	Genes from hepatocellular carcinoma
Jaatinen haematopoietic stem cell up	Up-regulated in CD133+ stem cells
Rodwell aging kidney up	Increased expression with age in kidney
Heller HDAC targets up	Up-regulated in multiple myeloma cells
Jison sickle cell disease up	Up-regulated in PBMCs from sickle cell disease
Rutella response to HGF dn	Down-regulated in PBMCs by e.g. IL-10
Osman bladder cancer up	Up-regulated in bladder cancer patients
McLachlan dental caries up	Up-regulated in carious teeth
Yanagihara EXS1 targets	Down-regulated in osteosarcoma
Delys thyroid cancer up	Up-regulated in thyroid carcinoma
Ren alveolar rhabdomyosarcoma dn	Down-regulated in rhabdomyosarcoma
Thum systolic heart failure up	Up-regulated in systolic heart failure
Wieland up by HBV infection	Induced in liver during hepatitis B

6 GENERAL DISCUSSION

The FDA-approved health claim concerning the intake of soluble fibre from oats and a reduced risk of heart disease was based on a review of 37 scientific studies, 17 of which demonstrated positive effects. Four studies did not meet the inclusion criteria and the remaining 16 studies showed slight or no effects on blood cholesterol levels, with explanations such as low β-glucan content, processing or low compliance[119]. The report concerning the FDA health claim also contains comments on the critical effects of processing, and the unpredictable changes in viscosity along the gastrointestinal tract. Yet, no restrictions were placed on processing, and no measurements of molar mass or viscosity are required in conjunction with the use of the claim.

European health claims are presently being introduced, but there is still no definition of what "minimally processed" β-glucans means, nor any guidelines on how the functionality of dietary fibre can be guaranteed. Numerous factors are known to influence the properties of dietary fibre, which may at least partly explain why some published studies on dietary fibre show no health effects[120-122]. Effects on postprandial glucose and/or insulin have been published for all fibre types included in the present work (Section 2.3), but only the relationship between pectin (10 g per meal) and postprandial glucose has received a positive opinion for a health claim so far[83].

Although it was demonstrated that particle size, food matrix and processing could influence viscosity-related properties (Papers I and II), and these factors were optimised in the *in vivo* study, positive health effects were not seen with all the types of fibre studied. Oat bran lowered postprandial insulin, while rye bran lowered blood glucose levels (Papers IV and V). Furthermore, the gene expression analysis indicated effects related to the suppression of insulin level by oat bran. In contrast, neither the sugar beet fibre nor the mixed fibre meal had any significant effect on any of the postprandial responses, and oat powder even increased the insulin level.

The factors that may contribute to the results are discussed below in order to evaluate which parameters are important for desirable health effects. This will be done by comparing the findings of the physicochemical analyses with the results of the human study, and by comparing the present results with those in publications on the same types of fibre. A discussion on future research is included at the end of this chapter.

PREVIOUSLY PUBLISHED POSTPRANDIAL STUDIES
Recently published postprandial studies including oat bran, processed oat products, rye bran or sugar beet fibre were compiled to allow comparisons with the present results

(Table 6). An extended search was performed in October 2010 in Google Scholar to cover PubMed and articles from the journals *Cereal Chemistry* and *Journal of Cereal Science*. The search terms used were: *oat*, *oat bran*, *rye bran* or *sugar beet fibre* together with *glucose* and *insulin*. Articles were selected based on two criteria: 1) at least one, but preferably several, of the same fibre types as those in the present study included in a human postprandial study, and 2) investigation of the effects on glucose and insulin AUC. The four most recent articles were selected (only three were found for rye). Results from different types of processed oat products were included for comparison with the oat powder studied here, since no publications were found on this particular product. Most studies had been conducted in the Nordic countries or in Canada, and the postprandial studies with sugar beet fibre were rather old. Table 6 includes relevant information concerning study design, meal composition, particle size (when stated) and significant responses for glucose and insulin AUC (lower or higher than the control, denoted by arrows). Significant effects at specific points in time or for the whole observation period are given in parentheses.

6.1. Factors influencing health effects

PARTICLE SIZE

The milling of dietary fibre significantly increased the releasability of soluble fibre from particles, as reported in Paper I, and particle sizes below 0.8 mm were selected for the meal study in order to optimise the potential health effects. The positive effects seen for oat bran and rye bran were therefore expected, while the opposite effect of the fine oat powder was not. This oat powder was rich in carbohydrates, which may have resulted in rapid carbohydrate uptake, increasing blood glucose and insulin levels.

Other *in vitro* studies have also demonstrated that small particle size increased the probability of health effects due to a higher content of soluble fibre (insoluble fibre becoming soluble), higher extraction yield of soluble fibre and improved swelling power[123,124]. However, when different particle sizes are tested *in vivo*, no significant effects of the size have been reported[124,125]. It is possible that milled particles contribute to increased viscosity, but that this effect is difficult to detect *in vivo* due to other factors, including slower gastric emptying in the presence of coarse particles (and whole kernels)[125,126]. Thus, both small and large particles appear to have positive health effects, but through different mechanisms.

Table 6A. Human postprandial studies on oat bran, including the amounts of fibre, the glucose and insulin (incremental) area under the curve (IAUC/AUC) and lipid metabolism.

	First author, year (ref)	No. subjects, gender	Age (y), health status	Amount fibre[1], matrix	Particle size (mm)	Soluble fibre[1] (g)	Insoluble fibre[1] (g)	Control	Available carbohy-drates[1] (g)	Nutrients balanced	Outcome measure	Results
Oat bran	Ulmius (Section 5.3)	6 M, 7 W	20-28, healthy	82 g, liquid	0.8	5.1	7.8	No added fibre	75	Lipids, not protein	Glucose IAUC Insulin IAUC Triglycerides	n.s. (↓) ↓/↓[2] n.s.
	Juvonen 2010[127]	5 M, 15 W	19-33, healthy	30 g, semiliquid (pudding)	n.d.	5.1	5.5	No added fibre	53-57	Lipids, not protein	Glucose AUC Insulin AUC	n.s. (↓) n.s. (↓)
	Juvonen 2009[72]	4 M, 16 W	Mean 23, healthy	30 g, liquid	n.d.	5.1	5.1	Degraded β-glucan (oat bran)	58	Identical	Glucose IAUC[3] Insulin IAUC[3]	n.s. (↓) ↓
	Hallfrisch 2003[128,129]	9 M, 11 W	35-57, healthy	≈ 20-28 g[4] semiliquid (pudding)	n.d.	3-3.7	0.5-1	Glucose	68-84[5]	No	Glucose AUC Insulin AUC	↓ ↓
	Braaten 1994[130]	7 M, 3 W	38-64, healthy	60 g, semiliquid (porridge)	n.d.	9.2	10.8	Wheat porridge	57-62	Lipids, not protein	Glucose AUC[3] Insulin AUC[3]	↓ n.s. (↓)
	Braaten 1994[130]	7 M, 3 W	50-68, type 2-diabetes	60 g, semiliquid (porridge)	n.d.	9.2	10.8	Wheat porridge	57-62	Lipids, not protein	Glucose AUC[3] Insulin AUC[3]	↓ n.s. (↓)

M men; W women; n.d. not determined; n.s. not significant. Results within parentheses are effects measured at certain times (not AUC).
[1]Amount per serving, [2]Significant compared to spray-dried oat drink, [3]AUC 0-180 min, [4]0.33 g/kg body weight, [5]1 g/kg body weight.

Table 6B. Human postprandial studies on processed oat products, including the amounts of fibre, the glucose and insulin (incremental) area under the curve (IAUC/AUC) and lipid metabolism.

First author, year (ref)	No. subjects, gender	Age (y), health status	Processed oat product	Amount fibre[1], matrix	Particle size (mm)	Soluble fibre[1] (g)	Insoluble fibre[1] (g)	Control	Available carbohydrates[1] (g)	Nutrients balanced	Outcome measure	Results
Ulmius (Paper IV)	6 M, 7 W	20-28, healthy	Spray-dried oat drink	62 g, liquid	0.05	2.7	0.6	No added fibre	75	Lipids, not protein	Glucose IAUC Insulin IAUC Triglycerides	n.s. ↑ ↑[2]
Beck 2009[131]	7 M, 7 W	29-45, over-weight[3]	Extruded oat concentrate[4]	n.d., cereal in liquid	n.d.	5.7	2.2	Corn cereal	43	Yes	Glucose AUC Insulin AUC	n.s. ↓[5]
Hallfrisch 2003[128,129]	9 M, 11 W	35-57, healthy	Oat extract[6]	≈ 20-28 g[7] semiliquid (pudding)	n.d.	3.1-3.8	0	Glucose	68-84[8]	No	Glucose AUC Insulin AUC	↓ ↓
Wood 1994[132]	4 M, 5 W	Mean 32, healthy	Oat gum[9]	3.6 g, semiliquid (pudding)	n.d.	2.9	0	Glucose	50	No	Glucose AUC Insulin AUC	n.s. n.s.
Braaten 1994[130]	7 M, 3 W	38-64, healthy	Oat gum[9]	11 g, semiliquid (porridge)	n.d.	9.5	0.6	Wheat porridge	61-62	Lipids, not protein	Glucose AUC[10] Insulin AUC[10]	↓ ↓
Braaten 1994[130]	7 M, 3 W	50-68, type 2-diabetes	Oat gum[9]	11 g, semiliquid (porridge)	n.d.	9.5	0.6	Wheat porridge	61-62	Lipids, not protein	Glucose AUC[10] Insulin AUC[10]	↓ n.s. (↓)

(Left margin label spanning all rows: **Processed oats**)

M men; W women; n.d. not determined; n.s. not significant. Results within parentheses are effects measured at certain times (not IAUC).
[1]Amount per serving, [2]Significant 60 min after meal, [3]Two subjects with hyperinsulinaemia, [4]Extracted with ethanol to 52 % β-glucan, [5]Also significant when hyperinsulinaemic subjects excluded, [6]Nu-trimX oat extract containing 11 % β-glucan and no insoluble fibre, [7]0.33 g/kg body weight, [8]1 g/kg body weight, [9]Containing about 80% β-glucan, [10]AUC 0-180 min.

Table 6C. Human postprandial studies on rye bran, including the amounts of fibre, the glucose and insulin (incremental) area under the curve (IAUC/AUC) and lipid metabolism.

	First author, year (ref)	No. subjects, gender	Age (y), health status	Amount fibre[1], matrix	Particle size (mm)	Soluble fibre[1] (g)	Insoluble fibre[1] (g)	Control	Available carbohy-drates[1] (g)	Nutrients balanced	Outcome measure	Results
Rye bran	Ulmius (Paper IV)	6 M, 7 W	20-28, healthy	31 g, liquid	0.8	2.0	11.0	No added fibre	75	Lipids, not protein	Glucose IAUC Insulin IAUC Triglycerides	n.s. (↓) n.s./↓[2] n.s.
	Rosén 2009[133]	9 M, 3 W	Mean 23, healthy	n.d. solid (bread)	0.8	2.0	10.3	Wheat bread	40[3]	No	Glucose IAUC Insulin IAUC	n.s. n.s./↑[4]
	Juntunen 2003[134]	19 W	51-69, healthy	n.d. solid (bread)	n.d.	4.8	24.1	Wheat bread	50	No	Glucose AUC Insulin AUC	n.s. n.s. (↓)
	Hagander 1987[73]	3M, 4 W	56-73, type 2 diabetes	n.d.[5] solid (bread)	n.d.	2.4	7.4	Wheat bread	48	Yes	Glucose IAUC[6] Insulin IAUC[6] Triglycerides	↓ n.s. ↓[7]
	Hagander 1987[73]	3M, 4 W	56-73, type 2 diabetes	55 g[8] liquid	flakes	1.8	6.7	Wheat bread	48	Yes	Glucose IAUC[6] Insulin IAUC[6] Triglycerides	n.s. n.s. n.s.

M men; W women; n.d. not determined; n.s. not significant. Results within parentheses are effects measured at certain times (not IAUC).
[1]Amount per serving, [2]Significant compared with spray-dried oat drink, [3]Available starch, [4]Significant increased compared with wheat porridge, [5]Milled wholemeal rye flour, [6]IAUC 0-180 min, [7]Significant 20 min after meal, [8]Rye flakes (steamed and flaked grains).

Table 6D. Human postprandial studies on sugar beet fibre, including the amounts of fibre, the glucose and insulin (incremental) area under the curve (IAUC/AUC) and lipid metabolism.

First author, year (ref)	No. subjects, gender	Age (y), health status	Amount fibre[1], matrix	Particle size (mm)	Soluble fibre[1] (g)	Insoluble fibre[1] (g)	Control	Available carbohydrates[1] (g)	Nutrients balanced	Outcome measure	Results
Ulmius (Paper IV)	6 M, 7 W	20-28, healthy	19 g, liquid	0.125	5.3	7.9	No added fibre	75	Lipids, not protein	Glucose IAUC Insulin IAUC Triglycerides	n.s. n.s./↓[2] n.s.
Thorsdottir 1998[74]	15 M	21-42, healthy	7 g, liquid	n.d.	3.7	1.4	No added fibre	51	Yes	Glucose IAUC Insulin IAUC	↓ ↓
Cherbut 1994[135]	6 M	21-32, healthy	15 g, liquid (glucose)	0.6	3.5	9.6	Glucose	25	No	Glucose IAUC[3] Insulin IAUC[3]	↓ ↓
Morgan 1990[136]	12 M	Mean 20, healthy	10 g, honey	n.d.	≈ 3[4]	≈ 4[4]	No added fibre	100	No	Glucose AUC[5] Insulin AUC[5]	↓ n.s.
Hamberg 1989[137]	5 M, 3 W	23-30, healthy	22 g, solid (beef)	n.d.	≈ 6[4]	≈ 8[4]	No added fibre	50	No	Glucose IAUC Insulin IAUC	n.s. n.s.

Sugar beet fibre

M men; W women; n.d. not determined; n.s. not significant. Results within parentheses are effects measured at certain times (not IAUC).

[1]Amount per serving, [2]Significant compared with spray-dried oat drink, [3]IAUC 0-180 min, [4]No data given, estimated from sugar beet fibre in Table 4, [5]AUC 0-90 min.

The rye bran fraction used in the present study, which had not been heat treated during manufacturing, caused a lowering of postprandial glucose levels at a comparably low dose of soluble fibre (2 g). Other studies on unprocessed rye bran could not be found in the literature. Rye bran included in bread has been found to lower postprandial glucose in type 2 diabetic patients (2.4 g soluble fibre), while steamed and flaked rye grains appeared to have no effect (1.8 g soluble fibre)[73]. Healthy subjects showed slightly lower insulin levels, but without effect on glucose, by rye bran included in bread (4.8 g soluble fibre)[134]. It cannot be excluded that components that are normally inactivated during processing and cooking[138] could have contributed to the hypoglycaemic effect of unprocessed rye bran seen in the present study.

As processing was found to affect pure β-glucans (Paper II), cooking and frozen storage of the fibre fractions was avoided in the postprandial study. High temperatures appeared to degrade the individual polymers, although boiling at 100 °C only reduced the largest aggregates, which could lead to higher releasability (Paper II). Similarly, cooking as a porridge has been reported to increase the releasability of oat β-glucans without affecting the molar mass[53,139]. Bread baking increases the releasability, but also results in depolymerisation, due to degrading enzymes in the flour[140]. The results of a long-term intervention study by Kerkhoffs et al. suggested baking as the reason for the lack of effect of oat-bran-rich breads on LDL cholesterol, while the same amount of oat bran in a beverage demonstrated a significant effect[141].

Storage of the pure β-glucan samples in the freezer for one week did not affect the aggregates in the present study (Paper II). Other studies have reported that longer periods of frozen storage resulted in decreased releasability of β-glucans, with no change in molar mass, and similar postprandial effects to those of fresh products[53,142]. Products exposed to freeze-thaw cycles, on the other hand, have been reported to exhibit lower releasability, and reduced ability to lower postprandial glucose[143]. This effect was suggested to be due to cryogelation, as discussed in Paper II.

Processing may increase the releasability of dietary fibre, and thus the viscosity, but it can also lead to depolymerisation. At low concentrations, as in the present *in vitro* studies (Papers I-III) the solution is assumed to be Newtonian. However, if the concentration is increased, as in many food products, the solution develops a shear-thinning behaviour. In the case of β-glucans, doubling the concentration, above a critical level, has been suggested to lead to a 15- to 35-fold increase in viscosity at constant molar mass[144,145]. This effect on the viscous properties has been reported to offset the effect of differences in molar mass[53,131], i.e. dietary fibre with a low molar mass may be as effective as that with a high molar mass if the concentration is sufficiently high.

Several methods of food processing or preparation appear to destroy the properties of soluble fibre responsible for positive health effects. However, high releasability may be more important for viscous effects than retained molar mass, and hence processing may

be advantageous. Boiling dietary fibre may also increase the releasability while maintaining the molar mass.

FOOD MATRIX
The releasability of soluble fibre was decreased when mixed with various solid food components such as protein, fat or starch (Paper I), therefore the fibres were given in a liquid juice in the *in vivo* study. It has been reported in some studies that the intake of dietary fibre in a liquid matrix leads to more pronounced health effects[100,127,141,146], while others report the opposite[147,148]. In two of the studies where the liquid matrix was found to be favourable, bread was used for comparison as a solid meal, and thus processing and degrading enzymes may have attenuated the health effects. It is difficult to conclude whether the health effects are due to the fibre or the food matrix as such. A solid matrix might *per se* slow down gastric emptying and reduce the carbohydrate uptake.

Small differences between the meal matrices may have added to the various health effects observed in the present study. In the oat powder meal, all the carbohydrates were present in the beverage, and no bread was given, which could have resulted in a faster carbohydrate uptake, and thus increased glucose and insulin levels. The sugar beet fibre meal contained less added fibre (19 g) and appeared thinner than the other fibre-rich meals (31-82 g). The oat bran meal tended to form a pudding-like structure after a few minutes, which required the meal to be mixed immediately before serving to keep it liquid and drinkable. In the studies in which the highest effects were found on postprandial IAUC (Table 6) fibre was supplied in the form of a pudding. The results were compared with a liquid glucose load, which makes it difficult to compare the meals.

The amount of protein in the various meals in the present study was not standardised due to the lack of a suitable neutral plant protein. Only 4 of the 14 studies in Table 6 reported that protein and lipid contents were balanced, and most used whey protein or cheese to balance the protein content[73,131]. Variations in protein and fat have been shown to cause negligible effects on postprandial glucose and insulin responses in normal meals, but adding protein to a glucose load can decrease the glucose and *increase* the insulin response[149].

Liquid matrices, such as drinks and soups, may be preferable for fibre-rich meals as they allow hydration and release of the fibre before ingestion. However, when elucidating the effects of dietary fibres, it is more important to use comparable food matrices in both the test and control meals.

FIBRE DOSE
The results of the present meal study showed that a lower amount of soluble fibre from rye bran (2 g) was more efficient than a larger quantity of soluble fibre from sugar beet fibre (5 g, Paper IV). This finding did not confirm the premise that a higher dose of fibre, regardless of source, gives better health effects. Furthermore, the meals containing oat bran and sugar beet fibre had the same contents of soluble (5 g) and insoluble

(8 g) fibres, but oat bran demonstrated more pronounced effects on glucose and insulin responses. Mixing oat powder, rye bran and sugar beet fibre into one meal with a high fibre content did not result in additional health effects, instead the effect of rye bran was lost, probably due to the carbohydrates and amino acids in the oat powder (as discussed above).

Similar results can be seen in Table 6, where studies with the highest amount of soluble and total fibre did not have the most pronounced effects. Juntunen et al. concluded that the effects on postprandial levels of glucose and insulin from different rye breads were not correlated to the amount of soluble fibres (3-5 g)[134]. A tendency towards a dose-response relationship was, however, noted in other studies in Table 6, in which the effects of 2 to 6 g β-glucans in oat bran or processed oats were investigated[127,131,132]. A dose of 4 g β-glucan has been suggested in several studies as the lower threshold for reducing glucose and insulin responses[47,127,150]. The meal containing oat bran in the present study, which lowered glucose and insulin levels, had a β-glucan content above this threshold, while the content in oat powder, which showed no lowering effects, was below 4 g. *Increased* insulin responses at low oat fibre contents has been reported by Juvonen et al.[127] following a meal containing 2.5 g β-glucan, and by Wood et al.[132], who reported a tendency towards increased insulin with 1.5 g β-glucan.

The amount of fibre released in the small intestine, i.e. the "active dose", can be estimated from the final percentage of releasable fibres, reported in Paper I, together with the determined or reported fibre contents (Table 3). Using this method, the amount of β-glucans released from the oat bran meal would be 2.9 g. This dose is the same as the amount required for health claims for improved or maintained blood cholesterol by the US FDA and the EU scientific panel. The EU opinion for pectin health claims requires 10 g pectin per meal to reduce postprandial glucose. The meal containing sugar beet fibre in the present study contained about 5 g pectin, of which 3.6 g was estimated to be released in the small intestine.

Fibre type seems to be as important as the quantity regarding health effects. Adding more fibre or several types of fibre to one meal does not necessarily improve the effects. The findings that different fractions and food matrices release varying amounts of soluble fibre highlight the question of how much fibre must be ingested from different products to obtain desirable effects.

IN VIVO ANALYSES

Some *in vivo* studies, including the present one, demonstrated slightly increased triglyceride levels after fibre-rich meals and diets[109,151], while others have shown decreased postprandial levels[73,152]. The increased concentration has been explained by the ability of the fibre to decrease insulin, which could delay the clearance of triglycerides from the blood[109]. Although this explanation seems logical, the oat powder meal in the present study resulted in both high postprandial insulin and triglyceride levels. Another explanation that has been proposed is that the triglyceride levels may be more influenced by the fat than the carbohydrate content of the meal[110]. The use of triglycerides

as a biomarker for the effects of dietary fibre should perhaps be re-evaluated. The meta-analysis performed by Brown et al., who investigated the long-term intake of several types of soluble fibre, indicated no effect on triglyceride levels[75].

Using nutrigenomic techniques, it may be possible to detect additional responses to those already found with the presently used biomarkers. In this work, it was found that gene sets related to insulin production were suppressed by the intake of the oat bran-rich meal, together with lowered insulin concentration measured in the blood. Cancer-related gene sets were also affected by the oat bran meal but the direction varied, making conclusions uncertain. Bakker et al. used a combination of transcriptomics, proteomics and metabolomics in PBMCs, adipose tissue and urine, and demonstrated that an anti-inflammatory diet (fish-oil, green tea extract, etc.) resulted in numerous subtle changes in inflammation in overweight men, although the traditional inflammation marker C-reactive protein remained unchanged[153].

The optimal time after a meal for gene expression analysis has not been established, and may vary with the food tested as well as the primary endpoints. In a recent study, the gene expression response was elucidated over time for 7 genes, after the intake of meals containing fatty acids[113]. Postprandial samples were taken after 2, 4, 6 and 8 h, and the results indicated that some genes were differentially expressed after only 2 h, while other expressions were found later. In the present work, postprandial samples were collected 2 h after the fibre meals to make the study comparable with the meal study by van Erk et al.[105]. Furthermore, soluble fibre is associated with reduced glucose and insulin concentrations shortly after meal intake, and measurements are usually performed during 2-3 h after ingestion.

PBMCs are involved in inflammatory-related diseases such as obesity and cardiovascular disease[106], which may be prevented or alleviated by a higher intake of dietary fibre. Samples for gene expression analysis (in healthy subjects) have also been taken from adipose tissue, as these cells may provide information on the link between metabolism and inflammation[154]. However, transcriptomics analysis requires high amounts of mRNA, and sampling of blood cells is thus preferable to sampling of tissues[108].

STUDY SUBJECTS
In the present study, the women tended to show a lower glucose peak (Figure 11) and IAUC than the men after standardised fibre-rich meals, and hence, the "dose" required to obtain the same effect may be lower in women. No correlations were found with body weight or BMI. Two of the studies in Table 6 compared gender effects. Hallfrisch et al.[128] detected different glucose and insulin patterns and somewhat lower peak values for women, despite the fact that fibre and carbohydrate intakes were based on body weight. Beck et al.[131] reported that women exhibited significantly higher levels of the satiety hormone cholecystokinin (resulting in prolonged satiety) after meals rich in soluble fibre. In the questionnaire in the present study, most women reported satiety after the fibre-rich meals while men did not.

As in the case of most postprandial studies, the present study was carried out with healthy subjects. Braaten et al. compared healthy subjects with type 2 diabetes patients, and demonstrated similar decreases in glucose and insulin AUCs in both groups[130]. Differences have been detected between healthy subjects and patients in an earlier study, where only patients with type 2 diabetes showed a lower glucose AUC in response to the fibre-rich meal (whole-grain bread and apples, compared with wheat bread and apple juice)[155]. The EU proposal for the health claim that β-glucans can maintain normal blood cholesterol levels is valid for both healthy and hypercholesterolaemic subjects. Patients suffering from type 2 diabetes, hyperinsulinaemia and hypercholesterolaemia are more often recruited in long-term intervention studies[75]. Two meta-analyses, including sixty-seven and ten long-term trials, respectively, have been performed to determine the effects of soluble fibre from oats, pectin, guar gum and psyllium[75,156]. A tendency was found for subjects with a higher initial LDL cholesterol concentration to show a greater decrease in blood cholesterol level. It is possible that the effects of dietary fibre are more easily recorded when the baseline (fasting) level is higher than normal. However, the intake of a meal stresses the body *per se* which may facilitate the detection of changes in postprandial biomarkers, compared to changes in fasting levels.

The recommended β-glucan dose in the FDA-approved health claim and the EU proposal, are the same for both genders, although there may be a difference in health effects between men and women. The recommended intake in the health claims should perhaps be based on energy intake, and hence differ for men and women.

6.2. Future research

To meet today's health recommendations, we should double our intake of dietary fibre. In most parts of the world, it will probably be easier to achieve such a high intake if the fibre is included in new products containing milled fibre. However, noticeably fibre-rich foods are generally accepted in the Nordic countries. Since unprocessed rye bran was found to have beneficial effects on postprandial glucose in the meal study, it would be interesting to compare it with a heat-treated product. Furthermore, in spite of the lack of health effects with the mixed fibre meal, it would be interesting to test other combinations of fibre from sources with documented effects. Provided that they promote accumulated health effects, such combinations may also improve the taste and texture compared to products containing only a single kind of fibre.

The addition of extracted soluble fibre such as β-glucan, arabinoxylan and pectin to food may be useful for certain indications and risk groups, but for the majority of those who want to increase their fibre intake, it should be sufficient to eat a diet containing (milled) brans in various food products together with other naturally fibre-rich products.

It is obvious from Table 6 that most postprandial studies on the effects of soluble dietary fibre include only few subjects. This has probably contributed to the variation in

the results reported. A general problem is distinguishing between the effects of the fibre as such, and other factors that may influence desirable health effects. Factors that appear to be of importance are the surrounding matrix, which should be standardised, the dose of soluble fibre, which should be above certain threshold levels, and testing the effects of fibres on both genders. As recently shown by Juvonen et al.[72], an analogous control meal can be obtained by degrading the soluble fibre for comparison with the natural fraction. Furthermore, it is important to control the effects of processing during manufacturing as well as food preparation, since both may change the properties and thus the health effects of dietary fibre. The large number of factors influencing the effects of dietary fibre calls for larger postprandial studies with sufficient statistical power.

Instead of using microarray chips, where thousands of genes are analysed, it is possible to study the up- and down-regulation of specific genes using the polymerase chain reaction, both to verify the results obtained with microarray analysis, and to investigate the effects of the other fibre meals. Furthermore, the results obtained from the present meal study using transcriptomics could be combined with those obtained using similar techniques in ongoing and future intervention studies to compare the effects on gene expression of a single fibre meal to those resulting from long-term fibre intake. By correlating the changes in gene expression with the results from proteomics and metabolomics, it may be possible to obtain an overall picture and thus gain better insight into the mechanisms behind the effects of dietary fibre.

With the introduction of more general regulations for health claims, it is necessary to verify that the fibre tested in the lab is also functional *in vivo*. This would involve the measurement of several fibre properties, such as the releasability, molar mass and viscosity. For practical reasons, it is relevant to use *in vitro* methods to evaluate how dietary fibre behaves when combined with a food product, and how the fibre-rich product behaves in the gastrointestinal tract. By measuring the releasability of soluble fibres, it would be possible to estimate the "active dose" required in the small intestine, and by optimising the releasability, less fibre should be required for retained physiological effects.

It is important for the food industry that the health-promoting properties of soluble fibre, as well as the factors that may influence these properties, can be evaluated *in vitro* before studies are carried out in humans, or even to eliminate the need for *in vivo* studies for every single product.

7 CONCLUSIONS

It is well accepted that dietary fibre, especially viscous soluble fibre, have beneficial effects on health and/or prevent modern lifestyle-related diseases. The work presented in this thesis first focused on the analysis of the viscosity-related fibre properties, measured under physiological conditions. The results were then used to determine the factors influencing the releasability of dietary fibre and the solution behaviour in order to prepare active fibre fractions for addition to food products. The second step was to document the health effects of several fibre sources in humans using traditional biomarkers. Furthermore, changes in the transcriptome as a result of ingesting a fibre-rich meal were investigated to obtain new insight into the relation between dietary fibre and health. The following conclusions were drawn from the various studies.

A rather small proportion of soluble fibre was released from coarse oat bran (20 % of the total β-glucan) and sugar beet fibre (50 % of the total pectin) in an *in vitro* study designed to model digestion in the gastrointestinal tract. Milling to obtain smaller particles significantly improved the releasability to 55 % of the total β-glucan, and 70 % of the total pectin. Mixing the intermediate fibre fractions in solid food matrices decreased and/or delayed the releasability (Paper I).

The solution behaviour of pure barley β-glucan was affected by processing parameters. Boiling disrupted the largest aggregates, which may lead to increased releasability with retained molar mass, while higher temperatures may have resulted in depolymerisation. Freeze-thaw cycles resulted in a conformational change in the aggregate structure, indicating cryogelation (Paper II).

The low pH in the *in vitro* gastric digestion disrupted aggregates of β-glucan from oat bran, and from oat and barley β-glucan concentrates, while neutralisation of the pH during small intestinal digestion induced re-aggregation. The pure β-glucan aggregates demonstrated a more condensed structure after re-aggregation, indicating difficulties in comparing the solution behaviour of soluble dietary fibre in a food product with that in the small intestine (Paper III).

Postprandial glucose, insulin and triglyceride levels in healthy subjects were affected differently by the intake of fibre with small particle sizes derived from oats, rye, sugar beet fibre or a mixture of these, added to liquid meals. Rye bran had a beneficial effect at a low soluble fibre concentration, while oat powder appeared to increase glucose and insulin levels, both for the separate meal and the mixed meal. There was a tendency

towards more pronounced beneficial glucose responses in women than in men (Paper IV).

Milled oat bran in a liquid meal influenced the gene expression profile in healthy subjects after a single meal. The gene sets influenced indicated suppressed insulin production compared with the control meal, and this was also seen as lowered insulin levels after this meal (Paper V).

The results of these studies can also be discussed in terms of technical, economical and health benefits. When commercial dietary fibre is analysed with more physiological methods, the impact of processing and harsh analytical steps becomes obvious. Physiological conditions can be used to mimic the *in vivo* situation and may, therefore, improve the quality and the economic value of products intended to have beneficial health effects. According to the US FDA regulations for health claims for soluble fibre, e.g. oat and barley, a product must provide a total of 3 g β-glucan per day. There are presently no FDA regulations regarding processing or the physiologically active dose, while the proposed EU health claim accepts natural sources or β-glucan that are non-processed or, even more diffuse, "minimally processed". The health effects of fibre-rich meals in this study appeared to depend on gender, but additional studies will be needed to ascertain whether different doses should be recommended for men and women. Nutrigenomics is still fairly expensive and methodologically complicated, but may reveal complex and early responses.

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