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# New insights from a β-glucan human intervention study using NMR metabolomics

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ABSTRACT

This study investigates the plasma metabolic effectiveness from 3.3 g mixed linkage barley or oat β-glucan fibre per day by $^1$H NMR spectroscopy on plasma samples and multivariate data analysis. Three large-scale extracted, equally sized but structurally different β-glucans were tested in a blinded randomised cross-over design using young healthy adults, where the β-glucans were compared to a non-fibre control during a 21 day dietary intervention period. Subject variance was found to dominate the metabolomics data although the variations in subject age and BMI were very small. The second most influential variation was found to be due to gender as characteristic lipoprotein profiles were found for male and female samples. The 3.3 g/day did not perturb the blood homeostasis in healthy adults as no systematic differences between 3-week β-glucan treatments and control were found. The present study constitutes the first metabolomic β-glucan human intervention study which proves complementary and confirmatory with respect to previous nutritional investigations evaluating traditional cardiovascular disease risk markers.



## 1. Introduction

It is well established that viscous soluble dietary fibres like barley and oat mixed linkage β-glucans affect the cholesterol metabolism (Behall, Scholfield, & Hallfrisch, 1997; Brown, Rosner, Willett, & Sacks, 1999; Gunness & Gidley, 2010; Theuwissen & Mensink, 2008; Wood, 2004); however, the underlying mechanisms of the hypocholesterolemic actions of β-glucans are still not fully understood. One proposed mechanism involves interference with lipid and/or bile acid metabolism as the molecular properties of β-glucans may hinder or slow down the bile acid reabsorption in the small intestine leading to increased de novo synthesis of bile acid, with plasma cholesterol as substrate (Gunness & Gidley, 2010; Theuwissen & Mensink, 2008).

The risk of cardiovascular disease is related to the distribution of cholesterol in different lipoprotein fractions (Castelli, 1996). Lipoprotein particles function as transport vehicles for the water insoluble lipids in the human body and they are usually divided into five main classes: chylomicrons and very low, low, intermediate and high density lipoproteins (VLDL, LDL, IDL and HDL, respectively) (Ala-Korpela, 1995). This classification is related to the physiological and physical characteristics of the lipoproteins and their isolation by ultracentrifugation based on their density. Standard analytical measurements are traditionally used for monitoring single biomarkers like VLDL and LDL cholesterol in clinical medicine, but techniques aimed at assessing large numbers of metabolites that are substrates, intermediate or end products in various metabolic pathways are becoming increasingly relevant in the risk assessment of metabolic conditions like cardiovascular disease (Ala-Korpela, Salomaa, & Kvalheim, 2011).

Metabolomics is an omics approach aimed at identifying and monitoring metabolic characteristics, changes and phenotypes with respect to various synergetic factors such as environment, life style, diet and potential pathophysiological processes (Fiehn, 2002; Giovane, Balestrieri, & Napoli, 2008). Recently, the concept of nutritional metabolomics has evoked with the purpose of relating the intake of a specific dietary component to specific metabolic fingerprints (Gibney et al., 2005; Ryan et al., 2013). Mass spectrometry (MS) (Scalbert et al., 2009) and proton nuclear magnetic resonance ($^1$H NMR) spectroscopy (Savorani, Rasmussen, Mikkelsen, & Engelsen, 2013) are the two key analytical platforms in the field. Especially, NMR profiling of blood serum and plasma has shown to be promising in the characterisation and quantification of lipoprotein subclasses and in turn identification of early biomarkers associated with the risk of cardiovascular disease (Ala-Korpela, 2008; Dyrby et al., 2005; Otvos, Jeyarajah, Bennett, & Krauss, 1992; Petersen et al., 2005; Savorani, Kristensen, Larsen, Astrup, & Engelsen, 2010). Common to these lipoprotein studies was the availability of reference lipoprotein measurements from ultracentrifugation, which is the golden standard for determining the lipoprotein profile.

Recently, we investigated the traditional exposure and effect markers from flaxseed dietary fibre intake (Kristensen et al., 2011, 2012) and found a relation between fibre viscosity and cholesterol lowering effect. Using the same experimental design, a full-scale dietary human

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intervention study on differently structured barley and oat β-glucan fibres was conducted and effects on blood lipids and faecal endpoints were reported (Ibrügger et al., 2013). As a further step towards the understanding of the metabolic consequences of such dietary interventions, the present study investigates the full blood metabolic effects of daily supplementation of mixed linkage β-glucans from oat and barley by means of $^{1}$H NMR spectroscopy in combination with multivariate data analysis. The authors hypothesise that an increased dietary fibre intake will perturb the blood metabolome and that by using nutritional metabolomics techniques it may be possible to detect complementary responses to those found in our previous study using the traditional risk markers.

## 2. Materials and methods

### 2.1. The intervention study

In a randomised, blinded, cross-over 3-week intervention study the hypocholesterolemic effects of 3.3 g β-glucan/day from three equally sized but structurally different oat and barley β-glucans (Mikkelsen, Jespersen, Larsen, Blennow, & Engelsen, 2013) were investigated in free-living normocholesterolemic individuals that maintained their habitual diet with a few restrictions (Ibrügger et al., 2013). The number of participants was based on a power calculation from 2 of our previous intervention studies on the effect from flax seed dietary fibres on blood cholesterol levels (Kristensen et al., 2011, 2012). Fourteen (out of 16) young adults (8 F/6 M, age 22.9 ± 2.1 years) completed the 4 intervention treatment periods separated by at least 2 weeks wash-out. Treatments consisted of: oat (O), mutant barley (Bm), mother barley (B) β-glucan and control (C) without fibre. Barley β-glucans showed high ratios of cellotriocyl to cellotetraosyl oligomer units in the polysaccharide chain and low solubility compared to oat β-glucan with less cellotriosyl units and high solubility. For further physico-chemical characterisation of β-glucans and intervention test products please refer to Mikkelsen et al. (2013) and Ibrügger et al. (2013), respectively.

### 2.2. Collection and preparation of plasma samples

Blood was collected as fasting (0 h) and postprandial samples (2 and 4 h) after a β-glucan or control test meal at the start (day 1) and end (day 22) of each treatment period. A schematic overview of the 4-armed cross-over study design and blood sampling performed in the treatment periods is presented in Fig. 1. Since only 8 samples were lost, a total of 328 fasting and postprandial blood samples were collected from the 14 subjects.

The blood was collected into 4 ml vials containing heparin as anticoagulant and centrifuged at 3000 *g*, 4 °C for 10 min. The plasma fraction was stored at −80 °C until NMR analysis. Fasting concentrations of serum total, and LDL cholesterol along with both fasting and postprandial plasma triglycerides (TG) were measured using colorimetric test kits (Roche TG, Roche Diagnostics GmbH). Results on blood lipids, dietary intake and faecal parameters are reported by Ibrügger et al. (2013).

### 2.3. NMR data acquisition and preprocessing

Plasma samples were thawed on ice and 300 μl plasma was transferred to 5 mm NMR tubes together with 300 μl phosphate buffer (pH 7.4) containing trimethylsilyl propionate (TSP) as reference signal and 10% $D_2O$ for the lock signal. NMR profiles were acquired on a Bruker Avance III 600 spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) operating at a Larmor frequency of 600.13 MHz for protons, equipped with a double tuned cryo-probe (TCI) set for 5 mm sample tubes and a cooled autosampler (SampleJet). Spectra were acquired from all plasma samples using the Carr–Purcell Meiboom–Gill (cpmg)

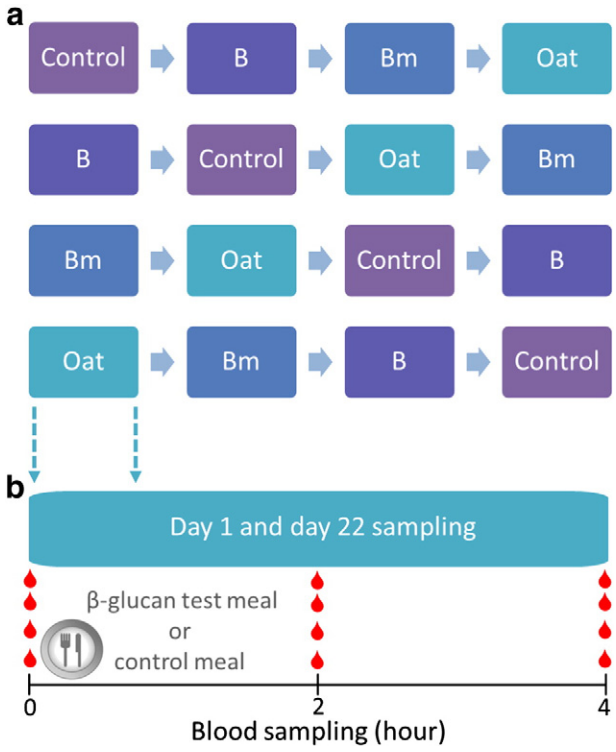


**Fig. 1.** (a) Schematic overview of the 4-armed cross-over study design with oat (O), mutant barley (Bm), mother barley (B) β-glucan or control (C) 3-week treatment periods separated by 2–3 weeks wash-out. All treatments were represented in each of the four 3-week periods. (b) Blood sampling (0 h, 2 h and 4 h) preformed at the beginning (day 1) and end (day 22) of each treatment period.

experiment according to Beckonert et al. (2007). All experiments were performed at 310 K with a fixed receiver gain (RG), which was determined through initial tests. Each free induction decay (FID) was collected using a total of 128 scans. Prior to Fourier transformation the FID's were zerofilled to 64 K points and apodised by 0.3 Hz Lorentzian line broadening and thereafter baseline- and phase-corrected automatically. The spectral area chosen for multivariate data analysis ranged between 0.0 and 8.0 ppm with exclusion of the 4.4–4.9 ppm region dominated by the residual water signal (Fig. 2). To correct for spectral misalignment the entire dataset was globally aligned with respect to the α-D-glucose signal around 5.25 ppm using the icoshift algorithm (Savorani, Tomasi, & Engelsen, 2010). 1-Norm and TSP area normalisation of data were evaluated, however, the aligned non-normalised data was chosen as the most unbiased data block.

### 2.4. Data analysis

The general variance structure of the blood plasma spectral data was investigated by principal component analysis (PCA) (Wold, Esbensen, & Geladi, 1987) and Partial Least Squares (PLS) regression (Wold, Martens, & Wold, 1983) was used to relate spectral data to reference biomarker measurements, α-glucose and triglycerides. Prior to the multivariate data analysis the spectral data were mean centred. The PLS regression models were developed using 250 samples for calibrating the model and 78 samples for testing the model performance and to determine the optimal number of components to be used. Data were orthogonalised (Smilde et al., 2005) according to subject in order to minimize the inter-individual variations not related to the intervention.

As a further data mining approach, multilevel PLS-DA (van Velzen et al., 2008) was used to analyse individual contrast between β-glucan interventions and control diet. Let for example: