Whole grains beyond fibre: what can metabolomics tell us about mechanisms?

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Whole grains beyond fibre: what can metabolomics tell us about mechanisms?

Alastair B. Ross

Food and Nutrition Science, Department of Life Science Engineering, Chalmers University of Technology, Kemivägen 10, 412 96 Gothenburg, Sweden

Dietary fibre alone does not fully explain the frequent association between greater intake of whole grains and reduced risk of disease in observational studies, and other phytochemicals or food structure may also play an important role. For all the observational evidence for the benefits of a whole-grain-rich diet, we have only limited knowledge of the mechanisms behind this reduction in disease risk, aside from the action of specific cereal fibres on reduction of blood cholesterol and the post-prandial glucose peak. Nutritional metabolomics, the global measurement and interpretation of metabolic profiles, assesses the interaction of food with the endogenous gene–protein cascade and the gut microbiome. This approach allows the generation of new hypotheses which account for systemic effects, rather than just focusing on one or two mechanisms or metabolic pathways. To date, animal and human trials using metabolomics to investigate mechanistic changes to metabolism on eating whole grains and cereal fractions have led to new hypotheses around mechanistic effects of whole grains. These include the role of cereals as a major source of dietary glycine betaine, a possible effect on phospholipid synthesis or metabolism, the role of branched-chain amino acids and improvements in insulin sensitivity, and the possibility that whole grains may have an effect on protein metabolism. These hypotheses help explain some of the observed effects of whole grains, although mechanistic studies using stable isotopes and fully quantitative measures are required to confirm these potential mechanisms.

Whole grains: Metabolomics: Insulin sensitivity: Protein turnover: Betaine: Health benefits

Whole grains are associated with health, but are the effects only due to fibre?

The association between greater intake of whole grains and reduced risk of disease is one of the most consistent findings in nutritional epidemiology[1]. The diseases where whole grains are associated with reduced risk are diverse, including CVD, diabetes and some types of cancer[2]. These associations point to potentially diverse metabolic and physiological effects of whole grains which might not be fully explain by their macro- and micronutrient composition alone. Much focus on explaining the mechanisms behind the lower risk of disease in people who eat the most whole grain has focused on dietary fibre[3,4]. The greater fibre content of whole grains is likely an important factor, but may not explain all observed reduction in disease risk[1]. For example, there are well-established mechanisms for cholesterol lowering by cereal-derived β-glucan[5], yet the whole grains that are most commonly consumed in population studies that find associations between whole grains and health are wheat, maize and rice, grains that have a very low content of β-glucan and other soluble fibres. Other grain components are also likely to play a role in explaining the mechanism behind whole grains and health[6]. This review explores the role of metabolomics in finding new hypotheses that fill the gaps on why eating more whole grains reduces the risk of some diseases.

Abbreviation: BCAA, branched-chain amino acid.

Corresponding author: A. B. Ross, email Alastair.Ross@chalmers.se

Proposed mechanisms behind whole-grain health effects

Prior to the application of metabolomics to research on whole grains and health, explanations for why observational studies consistently found associations between eating more whole grains and reduced risk of many diseases centred on the higher fibre, vitamin and mineral content, as well as phenolic phytochemicals for their antioxidant capacity[7]. Fibre in particular was thought to be important not only for reducing blood lipids, but for promoting the growth of beneficial bacteria and increasing the production of SCFA in the large intestine[7]. Although there is little that is controversial about these proposed mechanisms, there was little explanation or evidence about how the compositional differences would impact on health. One major change in thinking between the early 2000s and the present day is the role of phenolic compounds in cereals as antioxidants, which would in turn reduce long-term disease incidence by prevention of oxidative damage. While an attractive explanation at the time, the theory that phenolic compounds reduce disease by acting as free-radical scavenging antioxidants has not been borne out in clinical intervention trials that have consistently found that high intake of antioxidant compounds has little effect on *in vivo* antioxidant capacity, and no effect on health outcomes[8].

Prior to the advent of systems biology tools, most studies looking to understand the effect of whole grains on health have focused on a few clinically relevant endpoints including blood lipids, glucose and markers of inflammation. This has given some insight into possible mechanisms, but may not have been informative about the global impact of whole grains. For the past decade researchers have been encouraging the greater use of newer methodologies, including metabolomics, in nutrition in the search for a better understanding of the link between whole grains and health.

Metabolomics: an ideal tool to study biological mechanisms

Metabolomics is the field of analytical chemistry focused on measuring a broad spectrum of metabolites, normally to understand the global, or systemic, effects of a biological change, such as disease, environment or diet. Metabolites are the end product of the cascade from genes to proteins and then to metabolites, and as such reflect gene and protein modifications such as epigenetic modifications, and protein phosphorylation and glycosylation. In addition to endogenous metabolites, mammalian plasma and urine also contain metabolites that originate from gut microbial metabolism, and can be a window into the status of the gut microbiota.

A number of different methods are used to collect metabolomics data, the most common being proton NMR spectroscopy and GC or liquid chromatography coupled to MS (GC–MS or LC–MS). Several papers have addressed the key advantages of the various techniques[9]. For the purposes of this review, it should be noted that proton NMR requires less sample preparation and generally has less instrumental drift (instrument response will be very similar for the first or the 100th sample), whereas MS-based techniques are more sensitive and can detect more metabolites, but have higher run to run variation that needs to be corrected for statistically. High resolution MS can detect thousands of metabolites and in recent years have shown promise to greatly enhance our understanding of the breadth of metabolites present in human and food samples. After chemical analysis, the critical steps of post-run data analysis, where the acquired data are aligned and normalised, and statistical analysis are used to determine which of the dozens to thousands of measured metabolic features are predictive of the study question.

Although metabolomics holds much promise for advancing biological understanding, it needs to be emphasised that the goal of metabolomics is to generate hypotheses around metabolic mechanisms, rather than to definitively prove a mechanism or an effect. Because of the statistical and analytical problems in analysing large numbers of metabolites, hypotheses generated based on a metabolomics study need to be followed up with studies specifically powered to test that hypothesis, with analysis carried out using validated quantitative methods.

Metabolomic insights into whole-grain health benefits: animal studies

The first studies on the global impact of a whole-grain diet have been based on samples from animal studies. The first such paper highlighted the potential importance of the methyl donor glycine betaine (betaine) from a whole-grain rye and rye bran enriched bread fed to pigs as a potential mediator of health effects of whole grains and bran-rich cereals[10]. Urinary urea and creatinine were reduced when pigs were fed rye bread, while urinary hippurate was elevated. Later, a decrease in urinary urea and creatinine due to a whole-grain diet were linked to protein turnover in human subjects[11], while urinary hippurate is often described as a product of microbial metabolism of phenolic compounds, although it may also come from metabolism of aromatic amino acids[12]. This work underlined the potential role of whole grains in interacting with gut microbiota, and for the first time linked the high concentration of betaine in rye to improved betaine status in a mammalian model.

Analysis of liver tissue samples from hypercholesterolaemic pigs fed either a high-fibre rye bread diet, or a high-fibre refined wheat diet using magic angle spinning proton NMR indicated differences to liver lipid metabolism, with high-fibre rye leading to an increase in liver free-choline, while the refined wheat diet led to elevated glycerophosphocholine and phosphocholine, suggesting a change in the expression or activity of phospholipases[13]. Notably there was no difference in the signal from trimethylamine oxide/betaine (the NMR signal for both overlaps making differentiation difficult), although plasma betaine was found to be increased in the same pigs[14]. Lipids, possibly TAG, were decreased

in plasma [14], suggesting a possible link between changes to liver phospholipids and lipid transport and circulation in blood. A secondary study on the same hypercholesterolaemic pig model using untargeted LC–quadrupole time-of-flight MS metabolomics also found evidence for remodelling of lipid metabolism, with reduced plasma concentrations of linoleic acid-derived oxylipins and cholesterol on the rye bread-based diet[15]. In the present study, the mammalian lignan enterolactone was detected and highly elevated on the rye diet, a finding backed up by previous studies on similar pig models[16]. In a study designed to compare metabolic responses between pigs and human subjects, both species were fed either whole-grain rye or refined wheat, and post-prandial plasma metabolic profile responses were compared[17]. Pigs and human subjects had similar relative metabolic changes for twenty-one out of twenty-six metabolites that were predictive of the different diet interventions in both species, indicating that pig models give a good reflection of human metabolism of cereals. Among the compounds that were different between the refined and whole-grain rye diets for both pigs and human subjects in this study were phosphatidylcholines 38 : 4 and 36 : 4, and lysophosphocholine 18 : 1, which were all elevated on the rye kernel diet compared with the refined wheat bread diet[17].

These initial studies had focused on a high-fibre rye bread diet, based on whole-grain rye and rye bran, compared with a fibre (wheat cellulose) matched refined wheat diet. A rat study comparing a whole-grain wheat diet *v.* a non-fibre matched refined wheat diet identified several changes in urine, plasma and liver-extract related to the whole-grain wheat diet[18]. Contrary to the results for pigs, urinary creatine was elevated on the whole-grain wheat diet, along with urinary amino acids (tyrosine, tryptophan and phenylalanine) and hippurate. Excretion of central carbon metabolism metabolites citrate and fumarate were increased, while pyruvate excretion was decreased, hinting at a possible change to energy metabolism. A signal attributed to either trimethylamine oxide or taurine was also decreased on the whole-grain wheat diet. Plasma lipids and lysine were slightly elevated ($\sim 10\,\%$ increase) on the whole-grain wheat diet. Feeding whole-grain wheat also led to a decrease in liver lipids, and an increase in glucose, glutathione and the trimethylamine oxide/betaine signal. The present study also found many NMR signals that were significantly different between diets, but could not be identified[18].

The role of gut microbiota in the release and availability of phytochemcials from cereal fibre was well demonstrated in a metabolomics study on mouse urine after feeding with differently processed wheat aleurone layer fractions. A large number of metabolites, including hippurate, dihydrophenolic acids and benzoxazinoids metabolites were released from the fibre matrix when the aleurone layer was milled or ultramilled. Fermentation of the aleurone layer feed led to a different urinary metabolite response altogether, including many unknown metabolites and small phenolic acids[19]. Although this work did not link the different responses to metabolic endpoints in the mice, it is a clear demonstration of the ability of metabolomics to detect differences in gut microbiota-related metabolites, as well as to discriminate between fermented and non-fermented diets.

Together, these first animal studies highlighted the link between the high concentration of betaine in wheat and rye to increases in circulating and tissue betaine, as well as the likely increased metabolism of phenolics by the gut microbiota as indicated by hippurate, enterolactone and a diverse range of other phenolic metabolites released from the fibre matrix. Possible effects on lipid metabolism were also detected in porcine liver and plasma, and as some of these involved phospholipids with choline moieties, these changes may be related to improved betaine status via choline sparing. Unfortunately none of these studies were able to link metabolic changes to health-related outcomes, although do give a picture of the potential scope for the metabolic effects of whole grains.

Metabolomic insights into whole-grain health benefits: human studies

Detecting metabolic changes due to diet in human subjects is more complicated than in animal models due to the wide genetic variation in the population (animals are often siblings) and variation in food intake aside from the intervention. Whole grain brings the additional problem that it inherently cannot be condensed into a pill or extract, so proportionally it cannot make up as large a part of the diet as rats or pigs being fed a fortified bread diet. Nevertheless, several studies have found that whole-grain diets do induce some metabolic differences compared with refined grain-based diets.

In a cohort of hypercholesterolaemic post-menopausal women partaking in a crossover study feeding either high-fibre refined wheat bread or high-fibre rye bread, several new metabolites were identified as related to the high-fibre rye diet: concentrations of the ribose metabolites ribitol and ribonic acid were elevated, as was the tryptophan metabolite indole acetic acid[20]. The authors correlated ribonic acid with tryptophan, and speculated that this may be because of an increased synthesis of sertotonin, and reduced hunger. Hunger was not measured in the present study, and serotonin was not directly measured, making this link somewhat speculative, although several studies have found that rye is more satiating than other cereals[21,22]. Serum cholesterol increased on the high-fibre rye bread diet, although the fatty acids palmitic acid, oleic acid and myristoleic acid were decreased in plasma during the rye bread period[20] supporting the hypothesis of an effect on fatty acid metabolism beyond cholesterol.

Elevated plasma betaine has also been found in metabolomics studies in human subjects. In an intervention with a high intake of whole-grain rye bread with added rye bran, carried out in males diagnosed with the early stage prostate cancer, elevated plasma betaine and dimethylglycine, the post-methyl donation metabolite of betaine, were measured, backed up by a trend for reduced homocysteine during the intake of rye bread[23]. Elevated plasma 3-hydroxybutyric acid and acetone was suggested

to be related to a shift in energy metabolism from anabolic to catabolic state while fasting. This same study found that the rye bread diet improved glucose metabolism (reduced urinary C-peptide and plasma insulin) and reduced plasma prostate-specific antigen, a marker of prostate cancer risk[24]. Other studies specifically analysing betaine found that whole-grain-rich or cereal betaine-enriched diets led to an increased amount of circulating betaine[25,26], and that with very high doses of cereal betaine from the wheat aleurone layer, downstream effects on reducing homocysteine and increasing dimethylglycine and methionine could be measured[25].

In work carried out on post-menopausal women, a group of the population at risk of developing type 2 diabetes, both fasting and post-prandial plasma samples had lower leucine and isoleucine concentrations when fed a whole-grain rye bread compared with refined grain bread[27,28]. These results are the first indication that whole grains may have an impact on branched-chain amino acid (BCAA) metabolism, which may in part explain the mechanism how whole grains reduce insulin resistance (see further discussion later).

Although not a whole-grain study *per se*, Bondia-Pons *et al.*[29] studied the possible metabolic mechanism behind why sourdough endosperm rye bread gives a lower post-prandial insulin compared with refined wheat bread, using two-dimensional GC–MS based metabolomics. Similar to studies where whole-grain rye/rye bran has been the major intervention, amino acids increased (methionine and phenylalanine), as well as ribitol, a breakdown product of tryptophan, increased after the rye endosperm bread. Organic acids (butenedioic acid, ascorbic acid, picolinic acid and succinic acid) were increased after the refined wheat bread meal. The authors noted that both ribitol and picolinic acid are breakdown products of tryptophan, but with different responses to either rye endosperm bread or refined wheat bread. Their possible biological effects are also quite different, with picolinic acid suggested to induce inflammatory proteins in macrophages[29]. The similar finding of a possible effect on tryptophan metabolism as earlier described[20] gives greater weight to the hypothesis that eating cereal-based foods has an effect on aromatic amino acid metabolism.

A study comparing a refined-cereal based diet with either a whole-grain-based diet or a 'healthy diet' with whole grains, fish and Nordic berries found that the healthy diet intervention led to both changes to glucose response (2 h glucose and glucose area under the curve), and several changes to the lipidomic response, including *n*-3 fatty acids that would be expected to increase with increased fish intake, and many changes to TAG, phosphatidylcholine species and phosphatidylethanolamine species, indicating a wide ranging remodelling of circulating lipids due to the healthy diet[30]. Conversely, the whole-grain-based diet, which included both wheat- and rye-based products, did not lead to any changes to the lipidome relative to the control diet[30]. Although it is difficult to decipher differences in mixed diet interventions, it would appear that in this free-living study design, whole grains were not the main driver of changes in lipids in the healthy diet intervention. No health-related endpoints changed with the whole-grain diet (although a trend for decreased 2 h glucose was observed), which may fit with the observation that there was no major metabolic response to the intervention diet. These data support the idea that analysing the metabolome in samples from interventions where no primary outcomes change may be pointless, as any measured change in primary outcome should be reflected in a change in metabolic homeostasis. Null results are not published as frequently as 'difference' results, and it can be interpreted from the results found here that unless the primary goal is to find diet-specific biomarkers, metabolomics analysis of samples where no primary or secondary outcomes have changed will be unlikely to find any further changes to the metabolome. An additional observation is that multivariate models generated from metabolomics analyses whole-grain-based clinical trials is that they are often weak. This indicates a wide inter-individual variation in overall metabolic response, and that other factors such as other types of food intake are stronger drivers of metabolic differences than the whole-grain intervention itself. Care needs to be taken to ensure validation of multivariate models and to confirm results using correction for multiple testing.

Unifying or common mechanisms for whole grains and health benefits

Betaine and phospholipids

Although there are many disparate findings between the different studies reported here, there are some common themes for changes to metabolic pathways. The increase in plasma betaine was the first major metabolite change due to a whole-grain cereal diet that was highlighted using metabolomics, and this may explain some of the other metabolic observations related to changes to phospholipid metabolism and even insulin resistance. Betaine, as a methyl donor, remethylates homocysteine to methionine, a change that has been demonstrated in human subjects using betaine-rich wheat aleurone porridge[25]. In the presence of adequate supplies of betaine, less choline is used for the remethylation of homocysteine, allowing choline to be used for the production of phospholipids that make up a large proportion of cell membranes[31]. Postprandial plasma phospholipid concentrations of two phosphatidylcholines increased in both pigs in human subjects [17], and although betaine was not measured, a post-prandial rise in betaine following eating whole grains has been measured in human subjects[26]. Whole-grain intake was associated with lower fasting plasma phosphatidylcholine concentrations in the European Prospective Investigation into Cancer and Nutrition cohort[32], although post-prandial and fasting samples reflect very different metabolic states. Changes to the enzyme activities related to phospholipid biosynthesis may also explain these differences, although interaction between cereal components and lipid biosynthesis enzymes are yet to be tested. The composition of phosphatidylcholine in

biomembranes may be important for health, as this can impact on membrane fluidity, which consequently can alter cell integrity and the activity of membrane-based enzymes, with possible long-term consequences for cellular function and repair[33].

Recent work using a mouse model of diet-induced obesity found that mice supplemented with betaine not only increased plasma, muscle and liver betaine, but also led to an increase in carnitine and short-chain acetylcarnitines in liver and muscle[34]. These increases were not associated with any phenotypic changes, but do provide evidence for an interaction between betaine and lipid metabolism, and potentially a further link to glucose metabolism (see later).

Betaine may be of additional interest as a universal mediator of whole-grain health benefits, as a methyl donor for epigenetic modifications. Although whole grains have yet to be tested directly for epigenetic effects, betaine supplementation in rodents is able to change DNA methylation patterns with possible effects on metabolic enzymes[35,36], and given that whole-grain wheat, rye and quinoa are among the best dietary sources of betaine[37], they will be important epigenetic mediators if betaine's role in DNA methylation is confirmed in human subjects.

Branched-chain amino acids, aromatic amino acids, acyl-carnitines and glycaemic control

The findings by Moazzami *et al.* that rye-based diets decrease circulating BCAA[23,27,28] may explain the reduced risk of diabetes seen in observational studies. However, many of the observational studies have been carried out in populations with low intake of rye, suggesting that an effect on glycaemic control may not be unique for rye-based foods. Two recent studies have found that a diet based mainly on whole-grain wheat led to improved measures of glucose control[38,39], although no analyses of BCAA has been reported in these studies. Newgard reported that elevated BCAA were clustered with C_3 and C_5, acylcarnitines, as well as aromatic amino acids phenylalanine, tryptophan and tyrosine[40]. This formed the basis for a hypothesis of an interaction between elevated BCAA and muscle mitochondrial utilisation of energy[40]. There may be some evidence for whole grains interacting with carnitine metabolism, with carnitine and acetylcarnintine excretion being reduced on a mixed whole-grain diet[41] although exact assignment of chain length of a carnitine by NMR can be difficult. Supporting the hypothesis that a whole-grain diet leads to lower concentrations of BCAA, a metabolomic analysis of the European Prospective Investigation into Cancer and Nutrition-Potsdam cohort, whole-grain intake was associated with lower plasma valine and isoleucine concentrations, along with the aromatic amino acid tyrosine[32]. This same metabolite pattern was also associated with reduced risk of type 2 diabetes, adding weight to the idea that whole grains may play a role in amino acid metabolism.

The changes to BCAA and insulin resistance may also help explain the finding that protein catabolism appears to be reduced on a whole-grain diet compared with a refined grain diet[41], and may also lend some support to the possibility that whole grains can improve body composition[42–44], given that increased insulin sensitivity improves muscle mass, probably via insulin-mediated phosphorylation of the enzymes used for muscle synthesis[45]. Although there is good observational evidence for whole grains reducing the risk of type 2 diabetes, there is only limited information on the effect of whole grains on glucose metabolism kinetics and insulin response. In a single meal study, whole-grain barley improved peripheral insulin sensitivity as indicated by an increased rate of glucose disappearance[46]. Recent work supports the present hypothesis that whole grains reduce peripheral insulin resistance compared with refined grains[39], even though no effects on body composition were found in the 8-week study. Further work is required to determine if these well-characterised changes in glucose metabolism also match the metabolite changes found in metabolomics studies. At present it appears as though whole grains may improve peripheral insulin uptake, but a direct impact on or role for skeletal muscle would still need to be established.

All these studies taken together would form a basis for supporting that a whole-grain-rich diet improves insulin sensitivity by suppressing the suggested BCAA-driven pathway for increased insulin resistance. Findings that tyrosine and tryptophan metabolism may also be altered by whole-grain interventions[20] adds further interest as these along with the BCAA are often associated with insulin resistance[47–49].

The relatively low number of studies carried out on highly disparate study designs is a weakness in trying to establish common mechanisms for disease reduction. Studies where detailed clinical analyses and comprehensive metabolomics analyses are performed and data analyses focus on linking the two will allow better understanding of the link between metabolism and clinical outcomes.

A role for gut microbiota

Although the commonly found microbial metabolite hippurate was found in several studies, there were few other clear indications of the effect of gut microbiota on the metabolome. One study, which also measured changes in gut microbiota composition, also found a decrease in the microbial metabolites of choline, dimethylamine and trimethylamine present in urine when eating whole grains[41]. This link is of current interest given the potential role of gut microbial metabolism of choline in the evolution of CVD risk[50]. The same study also measured increased faecal output of acetate and butyrate using metabolomics, the concentrations of which were associated with decreased faecal water pH. The increased production of SCFA by gut microbiota is often stated as being one of the mechanisms for how whole grains may reduce insulin resistance, cholesterol synthesis and increase satiety; however changes to plasma SCFA are rarely found after whole-grain interventions. Effects on microbial metabolism of choline have not been reported

in other studies, and it will be of interest if this were confirmed in future work.

Other indications of interactions with gut microbiota are the diversity of phenolic compounds found in plasma and urine after the intake of phenolic rich wheat aleurone-based diets in mice[19]. Although linking the relatively low concentrations of these compounds to reduced risk of disease will prove challenging, it does provide evidence that gut microbiota are metabolising the diverse array of phenolic compounds present in whole grains, and it can be speculated that this may drive greater microbial diversity to adapt to the more diverse substrate, a factor implicated in the prevention of obesity[51].

Identification of novel metabolites related to whole-grain cereal exposure

Identifying cereal-related compounds linked to whole-grain intake can be a valuable tool in the search for novel biomarkers of food intake. Biomarkers of food intake can help improve estimates of food intake from questionnaires in observational studies, and be used to check compliance in dietary intervention studies. Using metabolomics, Beckmann *et al.* identified novel benzoxazinoid metabolites, 2-hydroxy-*N*-(2-hydroxyphenyl) acetamide and *N*-(2-hydrxyphenyl) acetamide, in urine as being specific biomarkers of sourdough rye intake[52]. The benzoxazinoid metabolite 2,4-dihydroxy-1,4-benzoxazin-3-one sulphate was also linked to intake of rye bread based on metabolomic analysis of urine samples[53], whereas 2-hydroxy-*N*-(2-hydroxyphenyl) acetamide sulphate and *N*-(2-hydrxyphenyl) acetamide sulphate were identified in post-prandial plasma samples, discriminating between rye sourdough and rye bran bread, and refined wheat bread[54]. While previously known as plant allelochemicals, benzoxazinoids were rarely mentioned among the potentially beneficial compounds present in cereal grains. Metabolomics has helped to rekindle interest in benzoxazinoids and how they may play a role in the health benefits of rye in particular[6]. Metabolomics has also confirmed that cereal alkylresorcinols are useful markers of whole-grain intake, with two studies confirming that alkylresorcinol metabolites in urine are related to both whole-grain wheat and rye intake[53,55].

Research on whole grains tends to focus on health benefits, although eating the outer layers of the grain can also lead to greater exposure to toxic contaminants that may be present in the outer layers. The toxic fungal metabolite deoxynivalenol, when present, is found at highest concentrations in the bran fraction of the grain, and has been suggested to be potentially deleterious for human health[56]. A small scale metabolomics study comparing urine from people with high *v.* low deoxynivalenol exposure found that urinary hippurate was the most discriminating metabolite, with highest concentrations being present in people with greatest deoxynivalenol exposure[56]. Hippurate is frequently associated with a greater exposure to plant-based foods, and this case is more likely to be a marker of greater intake of cereal foods, which also increases the chance for deoxynivalenol exposure, rather than a direct metabolite from microbial metabolism of the fungal toxin.

What does the future hold?

Metabolomics is proving to be a useful tool for identifying novel pathways that are impacted by whole-grain diets, although as for the results on disease markers and other clinical chemistry endpoints, the results are mixed. However, there are some fairly consistent changes observed in several of the studies, including changes that can be related to energy metabolism, lipid metabolism and one-carbon metabolism. For these observations to be credible it is necessary to move beyond 'changes' and to start quantifying metabolites from the relevant pathways to confirm their role in the observed outcomes. This would require greater advances in the use of semi- and fully-quantitative metabolomics as standard, which would facilitate easier comparison between different datasets. At present few methods are quantitative due to the difficulty in applying standard curves that cover many hundreds of metabolites, even though NMR is inherently a quantitative method, and MS can be, provided a suitable spectrum of internal standards are used.

Notwithstanding future improvements in metabolomics methodology, at the heart of conclusive research on the mechanisms behind whole-grain health benefits lies greater consistency in how pre-clinical and clinical trials are carried out, including what type of grains and grain products are used. With the enormous heterogeneity of populations studied, intervention duration on top of the different products used, we should expect heterogeneous results. Another key gap is knowledge about metabolic response to grains aside from wheat and rye. At present there is a lack of understanding on how commonly consumed grains such as rice and maize may be beneficial for health, and lack of biomarkers for their intake. If it is possible to determine if there is commonality in metabolic responses between different grains then it will aid in understanding if it is necessary to discuss whole grains and health, or study the effect of individual grains.

Future work also needs to include a greater emphasis on molecular mechanisms. Unfortunately intact whole grains are not so amenable to *in vitro* studies commonly used to test mechanisms, so work may be limited to pure compounds or digests to try and best mimic what is absorbed from whole grains.

The application of metabolomics to studies where the effects of whole-grain or related interventions have been studied has broadened our view of how whole grains may mediate health benefits. Instead of just focusing on established clinical markers often associated with fibre, a researcher in the field can now consider a deeper role for impacts on lipid, glucose and protein metabolism, as well as the emerging field of epigenetics. Greater work is needed to establish if there is any causality between the phytochemicals found in grains and these metabolic effects, but we can now start adding more detail to the previously hypothesised mechanisms behind whole-grain health benefits.

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Authorship

The author was solely responsible for all aspects of preparation of this paper.

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