Diets rich in whole grains increase betainized compounds associated with glucose metabolism

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ABSTRACT

Background: Epidemiologic evidence suggests that diets rich in whole grains are associated with a reduced risk of developing chronic diseases and all-cause mortality. However, the molecular mechanisms behind these beneficial metabolic effects are poorly understood.

Objective: Our aim was to investigate novel trimethylated (betainized) compounds from mice and humans, and their association with whole grain–rich diets and insulin resistance and insulin secretion.

Design: Fasting plasma samples were obtained in a mouse (C57BL/6J male) feeding trial and a controlled dietary intervention. The mouse trial involved feeding the mice a rye and wheat bran–enriched feed which was compared with a high-fat diet. In the human trial, participants recruited from Kuopio, Finland ($n = 69$) and Naples, Italy ($n = 54$) with characteristics of the metabolic syndrome were randomly assigned to either a whole grain–enriched diet or a control diet for 12 wk. Plasma concentrations of betainized compounds were analyzed with the use of liquid chromatography-tandem mass spectrometry. Insulin resistance and insulin secretion were assessed in an oral-glucose-tolerance test and a meal-glucose-tolerance test.

Results: The betaines that were increased in mouse plasma after bran-enriched feeding were identified de novo via chemical synthesis and liquid chromatography-tandem mass spectrometry, and confirmed to be associated with an increased intake of whole-grain products in humans. In particular, the concentrations of pipecolic acid betaine were increased at the end of the whole-grain intervention in both the Kuopio cohort ($P < 0.001$) and the Naples cohort ($P < 0.05$), and these concentrations inversely correlated with the postprandial glucose concentration. Furthermore, the concentration of valine betaine was substantially increased during the intervention in Naples ($P < 0.001$) with an inverse correlation with the postprandial insulin concentration. In addition, the concentrations of other betaines, e.g., glycine betaine and proline betaine, correlated with glucose and insulin concentrations at the end of the intervention.

Conclusions: Novel betainized compounds in humans are associated with diets rich in whole grains, and they improve insulin resistance and insulin secretion. These results suggest that these novel compounds may contribute to the beneficial effects of whole grain–rich diets. The studies were registered at clinicaltrials.gov as NCT00945854 (Naples) and NCT00573781 (Kuopio). *Am J Clin Nutr* 2018;108:971–979.

INTRODUCTION

There is mounting epidemiologic evidence that a diet rich in whole grains can reduce the risk of chronic diseases including cardiovascular diseases, type 2 diabetes, and cancer (1–5). Prospective cohort studies have associated a whole-grain intake with a 20–30% reduced risk of developing type 2 diabetes (6). Furthermore, a very recent meta-analysis focusing on 12 major food groups indicated that, of these food groups, whole grains have one of the strongest associations with reduced risk of all-cause mortality and that this is evident in a dose-dependent manner (7). In addition to prospective cohort studies,

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Supplemental Figures 1–12, Supplemental Tables 1 and 2, and Supplemental Methods are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: gm%, grams per 100 g; HF, high fat; LC, liquid chromatography; MS, mass spectrometry; WGED, whole grain–enriched diet.

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numerous controlled clinical trials with diets rich in whole grains have demonstrated improvements in risk factors, including postprandial glucose and insulin concentrations, blood pressure, body fat mass, and inflammatory markers, as well as in the blood lipid profile, e.g., fasting concentrations of plasma cholesterol, LDL cholesterol, and triglycerides (8–14). However, there are also reports that have not detected any reduction in risk factors; these discrepancies are most likely related to differences in study setups, analytic approaches, and populations, as well as reflecting differences in the definition of whole-grain foods (15, 16).

The bran and germ compartments are removed when cereal grains undergo the refining process, although these are the most nutrient-dense parts of cereals. These compartments contain many beneficial compounds, e.g., micronutrients, B vitamins, and various phytochemicals, and therefore they are most likely involved in the health-promoting mechanisms related to the consumption of whole grains (16–21). As yet, no comprehensive biological mechanisms or clear causality have been revealed for any of the molecular species known to be present in whole grains.

Cereal grains, especially whole-grain rye and wheat, are some of the most important dietary sources of glycine betaine (22), which has numerous physiologic and biochemical functions including the regulation of cellular osmolarity and the donation of methyl groups in 1-carbon metabolism in many enzymatic reactions (23–26). Humans are not capable of synthesizing methyl groups; for that reason, the maintenance of endogenous homeostasis in methylation reactions is essential, and the main sources, choline and glycine betaine, need to be obtained from the diet (22, 24). Indeed, elevated concentrations of glycine betaine in plasma have been reported in several studies after increased intakes of whole grain or bran (27–29).

In our current study, we describe the de novo identification of a group of trimethylated, or betainized, compounds; the concentrations of these compounds were increased in plasma samples of both mice and humans after consumption of whole grain- or bran-rich diets. Furthermore, we demonstrate that the concentrations of several of the novel betainized compounds correlate with those of biomarkers related to glucose metabolism. We propose that the increase in the concentrations of these betainized compounds after consumption of whole-grain diets could be associated with their beneficial effects as demonstrated in epidemiologic studies.

METHODS

Animal experiments

C57BL/6J male mice were obtained from the National Laboratory Animal Center (Kuopio, Finland) at the age of 9 wk. The mice were acclimatized for 1 wk and housed as 3–4 mice/cage during weeks 1 to 12 and in single cages from week 13 until the end of the study. The environment in the animal facility was regulated: temperature 22°C ± 1°C, relative air humidity 55% ± 15%, and 12/12-h light/dark cycle with lights on at 0700. After 1 wk of acclimatization, the mice were fed ad libitum with a commercial high-fat (HF) diet (D12451, Research Diets Inc.) for 9 wk in order to induce obesity. After 9 wk of prefeeding, the mice were randomly assigned into study groups (n = 9–14) and were fed with either D12451, D12450B, or the rye bran– or wheat aleurone–containing HF diets for an additional 9 wk.

The rye diets contained either rye bran or bioprocessed, enzymatically treated, and yeast fermented rye bran whereas the aleurone diets contained wheat aleurone or ground and enzymatically treated (xylanase and ferulate esterase) aleurone. All diets had similar compositions of fat [23–24 gm% (grams per 100 g)], protein (23–24 gm%), carbohydrate (40–41 gm%), and fiber (5–6 gm%). A more detailed description of the diets can be found in earlier publications (30, 31).

Nine weeks after rye bran or wheat aleurone feeding, the mice were deprived of food for 8 ± 0.5 h and killed by decapitation after being rendered unconscious by CO_2 gas. The mice were 28 wk old at the end of the study. Blood was collected into EDTA-coated tubes (K2-EDTA Microtainer Tubes with BD Microgard Closure, Beckton Dickinson Oy) and centrifuged for 10 min at 16,000 × g at 22°C. Tissues were rinsed with physiologic saline, wrapped in aluminium foil, and snap-frozen in liquid nitrogen. All samples were kept at −80°C until further processing.

Frozen tissue samples were cryo-ground either in 10-mL grinding steel jars with a stainless steel ball for 60 s at 15 Hz (liver, jejunum, ileum, caecum, colon, subcutaneous adipose tissue), or in 2-mL microcentrifuge tubes with 4- or 7-mm stainless steel beads in precooled 2 × 24 adapters that were shaken for 45 s at 30 Hz (muscle, heart, pancreas, visceral adipose tissue, brown adipose tissue) with the use of TissueLyser II (Qiagen Finland, Helsinki, Finland). Samples containing 100 mg (±2 mg) of tissue powder were cryo-weighted into 1.5-mL microcentrifuge tubes and 90% methanol was added (vol:vol H_2O, LC-MS Ultra CHROMASOLV, Fluka) in a ratio of 300 μL solvent to 100 mg tissue. The samples were shaken for 20 min. Colon contents were weighed, mixed with 90% methanol (500 μL methanol per 100 mg content), and shaken with 4-mm stainless steel beads for 45 s at 20 Hz. All samples were centrifuged for 10 min at 4°C (16250 x g) and supernatants were filtered with the use of 0.2-μm Acrodisc Syringe Filters with a polytetrafluoroethylene membrane (PALL Corporation) and stored at −20°C until mass spectrometric analyses. The mouse model was used in order to investigate if betainized compounds are also found in organs.

Clinical trials

HealthGrain Kuopio intervention

Altogether 131 participants with impaired glucose metabolism and features of the metabolic syndrome were recruited from the Kuopio area for a 12-wk dietary intervention with a randomized parallel design (**Supplemental Figure 1**A). Details on the inclusion criteria and study design have been published elsewhere (32, 33). The participants were randomly assigned to one of the following groups: HealthyDiet, whole grain–enriched diet (WGED), or Control. This study is restricted to only the WGED and Control groups. Compositions of the study diets are shown in **Supplemental Table 1** and clinical characteristics are shown in **Supplemental Table 2**.

In the WGED group (n = 34), the participants replaced their habitually used cereal products with breads having a low postprandial glucose and insulin response, contributing ≤20–25% of total energy intake (40% share of endosperm rye bread, 10% share of sourdough wholemeal wheat bread, and 50% share of a selection of commercial rye breads). The recommended intake of wholemeal pasta was ≥3.5 dL (uncooked)/wk. In

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addition to the aforementioned cereal products, the participants were permitted to consume 1 portion of their habitually used cereal product each day, e.g., porridge, cereals, or pastries. In addition, they were given whole-grain oat biscuits to be consumed at 1 portion/d on a voluntary basis.

In the Control group ($n = 35$), the participants replaced their habitually used breads with refined-wheat breads (dietary fiber 3–4.3 g/100 g) and their other cereal products, e.g., porridge or pasta, with low-fiber products (<6 g/100 g dietary fiber). The participants were allowed to eat a maximum of 1–2 portions of rye products/d. The intake of bilberries and fish was restricted. Otherwise, the habitual diet and exercise habits were kept unchanged in all groups.

All of the groups kept a 4-d food record (consecutive days, 1 weekend day) once during the run-in period (baseline), when the participants were consuming their habitual diet, and 3 times during the intervention.

HealthGrain Naples intervention

Sixty-one overweight/obese men and women with the metabolic syndrome aged 40–65 y were recruited for a 12-wk dietary intervention study which had a randomized, controlled, parallel design (Supplemental Figure 1B). Subjects were randomly assigned to an isoenergetic diet based on either whole-grain or refined cereal products. Fifty-four subjects completed the study, 28 in the whole grain group and 26 in the control group. Compositions of the study diets are shown in Supplemental Table 1 and clinical characteristics are shown in Supplemental Table 2.

Details on the study design have been published elsewhere (34). In short, the participants were advised not to modify their habitual consumption of meat, dairy products, eggs, fish, fruit, and vegetables, and fat intake during the study. The only difference between the whole-grain and the control diets was that the former contained a fixed amount of whole grain (all-bran breakfast cereals, whole-wheat bread, wholemeal pasta, barley, oat biscuits, and a small portion of endosperm rye bread) whereas the latter had refined cereal products (rice crispies, white-wheat bread, pasta, rice, pizza, and cornmeal porridge) as the main carbohydrate source. Cereal products provided ~60–80% of the daily total carbohydrate intake. The diets were designed to have the same nutrient composition (18% protein, 30% fat, 52% carbohydrates), although their cereal food sources and cereal fiber intakes were different.

At baseline and at the end of the intervention, fasting and postprandial blood samples (over 3 h after a specific lunch test) were collected for the evaluation of the glucose and insulin concentrations. Dietary compliance was assessed by a 7-d food record at baseline and every 4 wk during the intervention.

Glucose concentrations and insulin metabolism were measured as previously described (33, 34).

Synthesis of betaines

All amino acid betaines were synthesized by the reaction of an amino acid and an excess of methyliodide in the presence of an inorganic base. The reaction mixture was briefly heated in a microwave instrument and the product was separated after solvent evaporation by washing with acetone to remove sodium iodide and impurities. The product was dried in vacuum and used as such in the mass spectrometry (MS) analyses. Details of the synthesis and information about the nuclear magnetic resonance characterization of the betaines are available in **Supplemental Methods**.

Liquid chromatography-mass spectrometric analysis

The samples were analyzed by liquid chromatography-quadrupole time-of-flight MS (LC-QTOF-MS; Agilent Technologies; a 1290 LC system, a Jetstream electrospray ionization source, and a 6540 QTOF-MSultra high definition accurate-mass quadrupole time-of-flight spectrometer). We used hydrophilic interaction chromatography. The sample tray was kept at 4°C during the analyses. The data acquisition software used was MassHunter Acquisition B.04.00 (Agilent Technologies).

An aliquot (3 μL) of the sample solution was injected into the column (Acquity UPLC BEH Amide column, 2.1 × 100 mm, 1.7 μm; Waters Corporation) and maintained at 45°C. The mobile phases, delivered at 0.6 mL/min, consisted of 50% acetonitrile (vol:vol; eluent A) and 90% acetonitrile (vol:vol; eluent B), respectively, both containing 20 mmol/L ammonium formate, pH 3 (Sigma-Aldrich). The following gradient profile was used: 0–2.5 min, 100% B; 2.5–10 min, 100% B→0% B; 10–10.1 min, 0% B→100% B; 10.1–14 min, 100% B.

The MS conditions were: Jetstream electrospray ionization source, operated in the positive ionization mode, drying gas temperature of 325°C with a flow of 10 L/min, a sheath gas temperature of 350°C and a flow of 11 L/min, a nebulizer pressure of 45 pounds per square inch (310 kpa), capillary voltage of 3500 V, nozzle voltage of 1000 V, fragmentor voltage of 100 V, and a skimmer of 45 V. In the data acquisition, a 2-GHz extended dynamic range mode was used, and the instrument was set to acquire over the range *m/z* 50–1600. Data were collected in the centroid mode at an acquisition rate of 2.5 spectra/s (i.e., 400 ms/spectrum) with an abundance threshold of 150.

Quality control samples were used for the automatic data-dependent MS/MS analyses. From every precursor scan cycle, the 4 most abundant ions were selected for fragmentation. These ions were excluded after 2 product ion spectra and released again for fragmentation after a 0.25-min hold. The precursor scan time was based on ion intensity, ending at 20,000 counts or after 300 ms. The product ion scan time was 300 ms. The collision energies were 10, 20, and 40 V in subsequent assays. The continuous mass axis calibration was performed by monitoring 2 reference ions from an infusion solution throughout the assays. The reference ions were *m/z* 121.050873 and *m/z* 922.009798.

Data processing was done with the use of Profinder (Agilent B.08.00), which enabled manually selecting MS peaks associated with betaines. Betaines were identified by comparison of the retention times and the use of targeted MS/MS spectra, which were compared to the retention times and spectra of commercial glycine betaine (CAS 107-43-7, B-2629 Sigma-Aldrich), proline betaine (CAS 4136-37-2, 20,506 Cayman Chemical), trigonelline chloride (CAS 6138-41-6, 11,904 Cayman Chemical), and synthetized chemical standards (4-amino valeric acid betaine, alanine betaine, pipecolic acid betaine, phenylalanine betaine, tryptophan betaine, and valine betaine).

Ethics

The mouse experiments were approved by the Institutional Animal Care and Use Committee of the Provincial Government

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of Finland (license number 04,1003). Clinical trials were approved by the Research Ethics Committees of the Hospital District of Northern Savo (HealthGrain Kuopio) and Federico II Naples University 1 (HealthGrain Naples) and they followed Helsinki Declaration guidelines. All participants provided written informed consent. The studies were registered at clinicaltrials.gov (NCT00573781, HealthGrain Kuopio; NCT00945854, HealthGrain Naples).

Statistics

Statistical testing in the mouse feeding trial was undertaken with Welch's 1-factor ANOVA. In the statistical testing in the clinical trials, we used repeated-measures ANOVA (with Greenhouse-Geisser correction) or *t* test to evaluate differences between the study groups. Cohen's *d* effect sizes were calculated between treatment and control groups in the clinical studies to compare differences in the mean change in betaine concentrations during the intervention. Associations between whole-grain bread product intake and concentrations of betainized compounds were evaluated with Spearman rank correlations in the Kuopio and Naples HealthGrain studies. Spearman rank correlation analysis was used to test for correlations between the concentrations of betaines and clinical markers at the end of the Naples and Kuopio HealthGrain interventions. The α level was adjusted via Bonferroni's method to 0.0055 to account for multiple testing. We used IBM SPSS statistics (version 23, IBM Inc., Armonk, NY) in the statistical analyses.

RESULTS

Identification of novel betainized compounds and effect of bran-enriched feed on their concentrations in the mouse feeding trial

Our recent investigation in C57BL/6J mice fed with rye bran–enriched feed revealed several novel urinary metabolites that were tentatively assigned as trimethylated metabolites (30). Here, we have successfully defined the chemical structure of these compounds via chemical synthesis and LC-MS/MS analysis, and concluded that they include 5-aminovaleric acid betaine, alanine betaine, valine betaine, phenylalanine betaine, tryptophan betaine, and pipecolic acid betaine (**Supplemental Figure 2**). We established a semitargeted LC-MS method for analyzing these trimethylated, or "betainized" compounds and examined plasma samples from a mouse feeding trial comprising native or bioprocessed rye bran (30), or native or enzymatically modified wheat aleurone fractions added to the basal HF diet (31). Plasma concentrations of all of these compounds were significantly higher in the groups receiving the bran-enriched feed than in the HF control group, selected as a control group to mimic the typical HF Western diet (**Figure 1**). Pipecolic acid betaine, valine betaine, tryptophan betaine, and phenylalanine betaine were detected only in the groups consuming the bran-enriched feed, the latter 2 only being found in the plasma samples from the mice in the rye bran–fed groups (Figure 1). Furthermore, the concentrations of other methylated compounds including trigonelline and proline betaine were increased in the plasma samples of mice fed with rye or wheat bran–enriched feed (Figure 1). In addition to the fasting plasma, we also analyzed several tissue samples from the mice, and observed that all of the betainized compounds were also found with varying distributions in intestinal, internal organ, and fat samples (**Supplemental Figures 3–11**).

Betainized compounds in fasting plasma after interventions with whole grain–rich diets in humans

After identifying the betainized compounds in the mouse feeding trial, we focused on the human dietary intervention studies. We analyzed the concentrations of betainized compounds in fasting plasma collected from 2 populations with characteristics of the metabolic syndrome before and after their increased consumption of whole-grain products, i.e., their participation in the HealthGrain dietary intervention study conducted in Kuopio and Naples (32–34). The concentrations of several of the fasting plasma betainized compounds were increased in the groups on whole grain–rich diets when compared with the control groups whose diet was low in whole-grain products (**Figure 2**). In particular, the concentration of pipecolic acid betaine was increased at the end of the study in the Kuopio cohort ($P < 0.001$) and the Naples cohort ($P = 0.038$). Additionally, the concentration of valine betaine was increased after the intervention in the Naples cohort ($P < 0.001$). Furthermore, 5-aminovaleric acid betaine concentrations were elevated in the Kuopio cohort ($P = 0.007$). Notably, although whole-grain bread is known to be a rich dietary source of glycine betaine, the concentration of glycine betaine did not differ from those of controls in either of the study populations.

The fasting plasma concentrations of pipecolic acid betaine correlated with whole-grain bread and pasta intakes in both the Kuopio and Naples cohorts; this was the most consistent marker of whole-grain intake (Figure 2). In particular, phenylalanine betaine concentrations correlated with whole-grain bread intake in the Kuopio cohort, whereas the valine betaine concentrations correlated with the intakes of whole-grain bread and pasta, especially in the Naples cohort.

In order to investigate the potential source of the betainized compounds, we analyzed their amounts in 2 bread products tailor-made at the VTT Technical Research Centre of Finland Ltd. (**Supplemental Figure 12**). Glycine betaine was the dominant compound in both whole-grain wheat and rye breads, whereas valine betaine, proline betaine, and trigonelline were found in considerably lower amounts, with the rest of the betaines being observed as trace signals.

Concentrations of betainized compounds correlate with glucose and insulin values in the dietary intervention study on humans

The HealthGrain intervention trial in both Kuopio and Naples involved an assessment of glucose and insulin metabolism, as reported earlier (32–34). In the Naples cohort, the study participants displayed reduced postprandial insulin responses after the whole grain–rich dietary intervention (34), whereas in the Kuopio cohort, there was only a trend towards a reduced 2-h glucose concentration in the oral-glucose-tolerance test after the whole grain–rich intervention (32). Here, we analyzed

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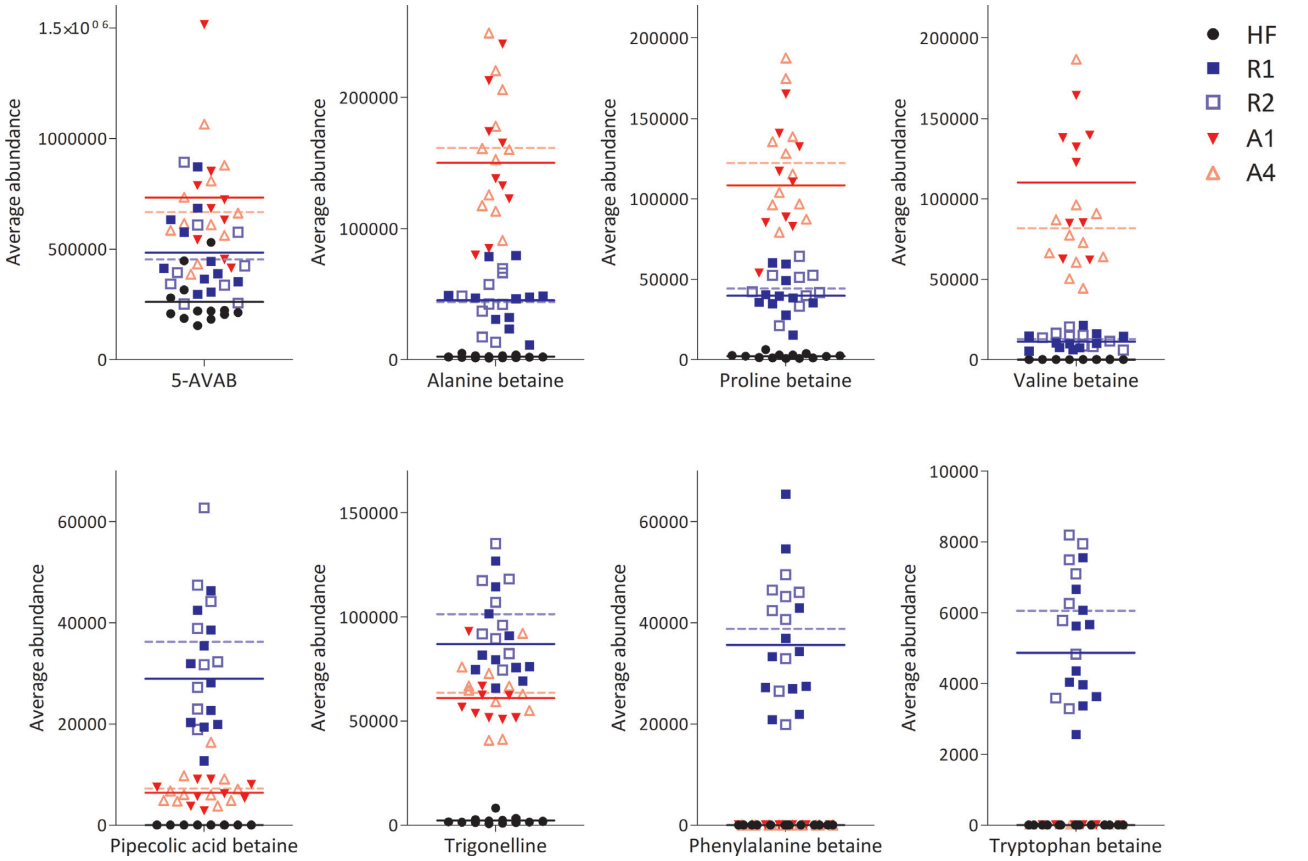

FIGURE 1 Betainized compounds in mouse plasma. Panel figures show peak area abundance of different betaines in mouse plasma. Results from 5 groups are shown: HF controls ($n = 14$), R1- ($n = 11$), or R2- ($n = 9$) fed mice, and A1- or A4-fed mice (A1, $n = 9$; A4, $n = 11$). Horizontal lines represent mean values of the investigated groups (black = HF, solid blue = R1, dotted blue = R2, solid red = A1, and dotted red = A4). *P* values from ANOVA comparisons were <0.001 in all comparisons of betainized compound concentrations between the study groups (α level was adjusted via Bonferroni's method to 0.0055 to account for multiple testing). A1, diet including native wheat aleurone; A4, diet including bioprocessed wheat aleurone; HF, high-fat; R1, diet including native rye bran; R2, diet including bioprocessed rye bran; 5-AVAB, 5-aminovaleric acid betaine.

the correlations between the concentrations of the betainized compounds and these findings in both centers and found that especially in the Naples cohort, the concentration of glycine betaine was inversely correlated with the postprandial glucose ($r = -0.321$, $P = 0.020$) and insulin concentrations ($r = -0.323$, $P = 0.021$), and the AUC for insulin in a meal-glucose-tolerance test ($r = -0.346$, $P = 0.012$) (**Figure 3**). Furthermore, the concentration of glycine betaine correlated positively with the Matsuda insulin sensitivity index ($r = 0.334$, $P = 0.022$) and the Quantitative insulin sensitivity check index ($r = 0.302$, $P = 0.035$). Furthermore, the concentrations of trigonelline and pipecolic acid betaine correlated inversely with the postprandial glucose concentration ($r = -0.302$, $P = 0.030$ and $r = -0.285$, $P = 0.041$, respectively) and that of valine betaine correlated inversely with the 2-h insulin concentration ($r = -0.300$, $P = 0.032$) and AUC for insulin ($r = -0.368$, $P = 0.007$) in the meal-glucose-tolerance test. In the Kuopio cohort, the concentration of proline betaine correlated inversely with the 2-h concentration after an oral-glucose-tolerance test ($r = -0.270$, $P = 0.028$) and with the AUC for insulin ($r = -0.368$, $P = 0.002$) as well as with HOMA-IR ($r = -0.256$, $P = 0.049$) (Figure 3).

DISCUSSION

Recent meta-analyses compiling several prospective observational studies have revealed an inverse association between whole-grain consumption and the risk of chronic diseases and all-cause-mortality (1, 2, 7). However, the molecular mechanisms underpinning the beneficial effects of diets rich in whole grains are largely unknown. Here we have described a group of de novo–identified betainized compounds the concentrations of which are increased after consumption of whole grain–rich diets in both mice and humans. Furthermore, these compounds seem to associate favorably with insulin sensitivity markers and postprandial glucose metabolism. Therefore, we suggest that the betainized compounds introduced here could be potential contributors to some of the health benefits linked with whole-grain diets.

Betainized compounds are associated with whole-grain intake

It is well known that whole grains are a rich dietary source of glycine betaine (22), but as far as we are aware,

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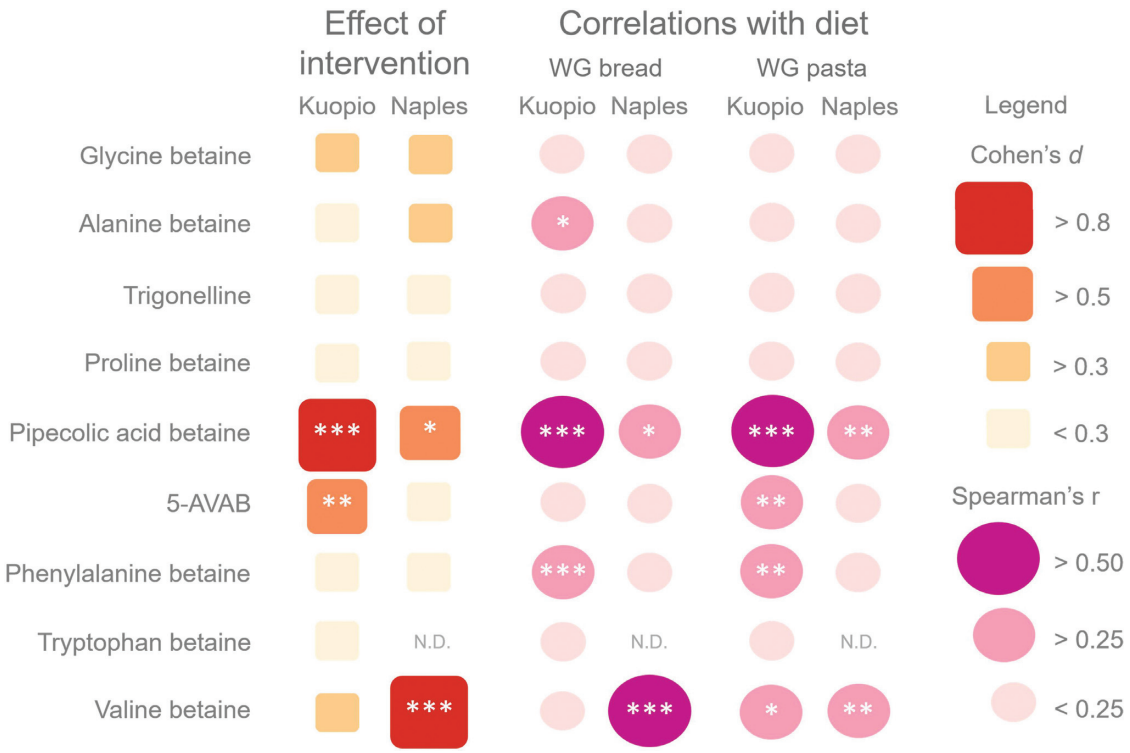

FIGURE 2 Effect of HealthGrain dietary intervention performed in Kuopio and Naples on betainized compounds and their correlation with whole-grain bread and pasta intakes at the end of the intervention studies. HealthGrain interventions were arranged in Kuopio ($n = 69$) and in Naples ($n = 54$) with diets rich in whole grains resulting in increased concentrations of some betaines as evaluated with repeated-measures ANOVA and Cohen's d effect sizes (d = mean change between baseline and week 12 in WG group – mean change in control group/SD of change in all subjects). Spearman correlations were calculated between concentrations of betaines and whole-grain bread or whole-grain pasta intake at the end of the Kuopio and Naples studies. $***P < 0.001$, $**P < 0.01$, $*P < 0.05$; α level was adjusted to 0.0055 to account for multiple testing. ND, not detected; WG, whole-grain; 5-AVAB, 5-aminovaleric acid betaine.

the other betainized compounds described here have not been identified previously from whole-grain products. Interestingly, a recent study identified methylated compounds including trigonelline, proline betaine, valine betaine, and pipecolic acid betaine in commercial cereal flours (35). These compounds were also detected in our current study when analyzing 2 different breads, and pipecolic acid betaine was detected only in the rye bread sample, as was reported in the case of the flours (35).

Interestingly, the fasting plasma concentrations of the novel betainized compounds correlated much more clearly with the intake of whole-grain bread and pasta than was evident for glycine betaine, which is the main betaine in the breads. The fact that the pipecolic acid betaine concentration correlated with the intake of whole-grain bread especially in the intervention conducted at Kuopio agrees well with the observations that pipecolic acid is present only in rye bread and this was the type of bread consumed mainly by the study participants in the Kuopio cohort. However, the concentration of pipecolic acid betaine also correlated with the intake of whole-grain pasta in the Kuopio cohort, which cannot be directly due to the diet, because the wholemeal pasta consumed in the Kuopio cohort was based on whole-grain wheat. This could be related to the fact that in the Kuopio cohort the study participants consumed both whole-grain pasta and rye bread. Likewise, an examination of the internal organs of the mice revealed that the concentration of pipecolic acid betaine was elevated in particular after the animals consumed the rye bran–enriched feed.

Betainized compounds correlate with insulin and glucose concentrations

The fasting plasma concentrations of valine betaine and glycine betaine correlated with the postprandial insulin response in the Naples cohort but not in the Kuopio cohort. This is an important notification, because a reduction in postprandial insulin is one of the most consistently reported metabolic phenomena related to whole-grain intake (11). In the Naples study population, also the concentrations of glycine betaine, trigonelline, and pipecolic acid betaine were inversely correlated with fasting and postprandial glucose concentrations, further evidence that these betainized compounds may have a role in glucose metabolism. It is notable that the data from the mouse organs highlighted the fact that pipecolic acid betaine was the compound with the most intense accumulation in the pancreas, a fact which clearly warrants further studies in relation to pancreatic insulin secretion, glucose metabolism, and pipecolic acid betaine.

The minor discrepancies between the studies conducted in Kuopio and Naples are most likely due to differences in background diets, and emphasize that differently nutritional whole-grain cereals may exert distinct metabolic effects. In the Naples cohort, the main bread was sourdough whole-grain wheat

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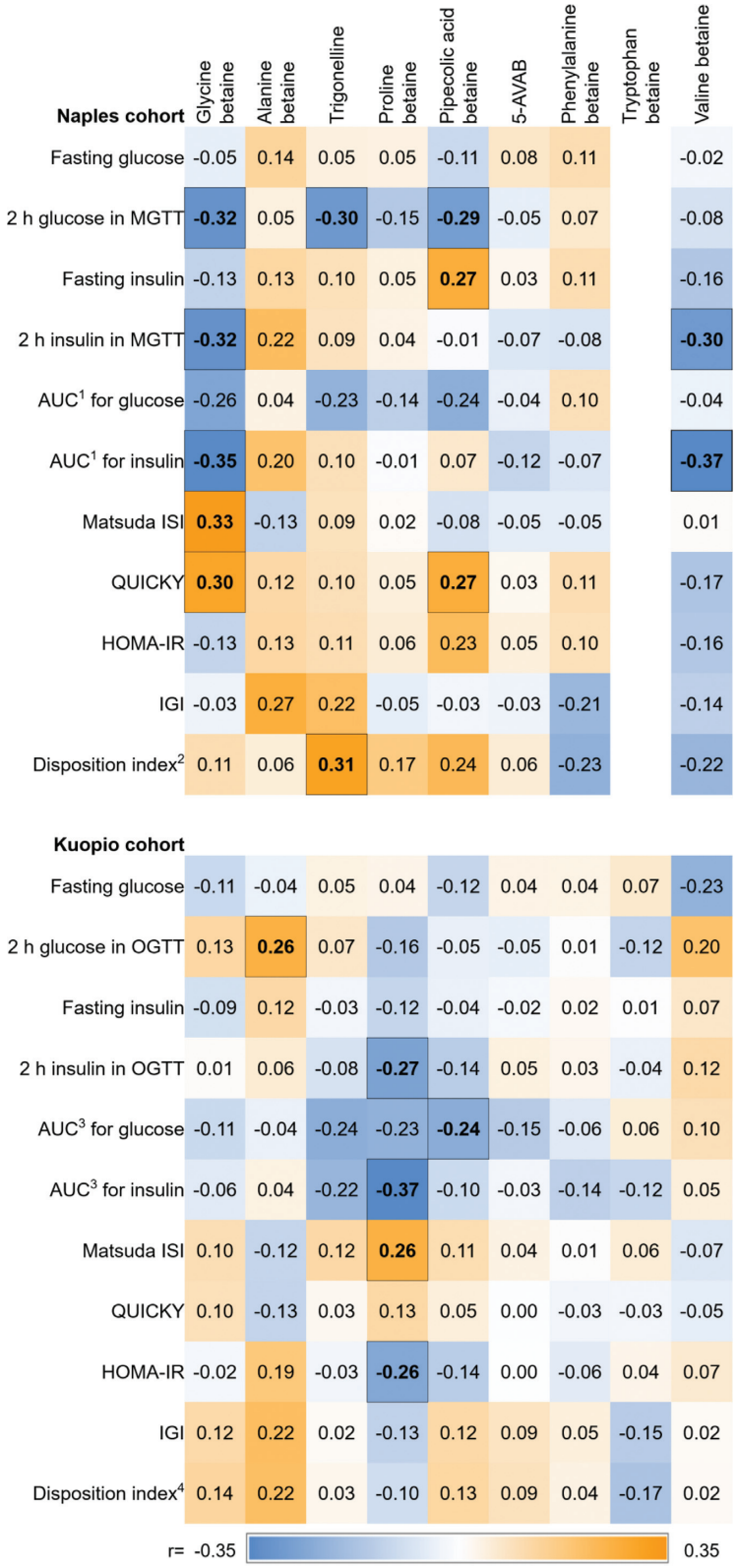

FIGURE 3 Spearman rank correlations between fasting plasma concentrations of betaines and glucose and insulin parameters at the end of the intervention in the whole grain–enriched diet and control groups in the Naples ($n = 54$) and Kuopio ($n = 59$–66) trials. The data on the glucose and insulin parameters have been published earlier (32–34). [1]AUC in 2-h MGTT, [2]Disposition index = ISI × first-phase insulin response (dynamic glucose-stimulated insulin response), [3]AUC in 2-h OGTT, [4]Disposition index = IGI × QUICKY; cell with outside borders, $P < 0.05$; cell with thick outside borders, $P < 0.01$. IGI, insulinogenic index; ISI, insulin sensitivity index; MGTT, meal-glucose-tolerance test; OGTT, oral-glucose-tolerance test; QUICKY, Quantitative insulin sensitivity check index; 5-AVAB, 5-aminovaleric acid betaine.

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bread, whereas in the Kuopio cohort the main bread products were based on rye. Likewise, the quality as well as quantity of pasta products differed between the centres, although in both centres, the pastas were made from whole-grain wheat. In addition, the other food items present in the diet may have contributed to the concentrations of the same or similar compounds in the circulation. For example, proline betaine is a well-known biomarker for the intake of citrus fruits (36). Interestingly, in the Kuopio cohort, the concentration of proline betaine correlated with reduced postprandial insulin values, but did not correlate with whole-grain intake. Notably, in the mouse feeding trial, the concentration of proline betaine was clearly increased after feeding with bran-enriched feed.

Conclusion

In conclusion, the betainized compounds described here are directly and consistently related to the increased consumption of whole-grain cereals. Our results suggest that these betainized compounds could be involved in the biological mechanisms behind the benefits of whole grains, e.g., with regard to glucose metabolism and the risk of type 2 diabetes (37). The fact that these compounds accumulate also in vital organs and are not completely excreted in the urine, as demonstrated in the mouse study, further supports the hypothesis that the betainized compounds may have important roles in tissue metabolism. Moreover, they are novel compounds to be considered in cellular 1-carbon metabolism and methylation homeostasis, which so far has mainly focused exclusively on choline and glycine betaine. Overall, betainized compounds could well be important contributors to the biological mechanism through which diets rich in whole grains achieve beneficial health effects.

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