Metabolomics Reveals Differences in Postprandial Responses to Breads and Fasting Metabolic Characteristics Associated with Postprandial Insulin Demand in Postmenopausal Women

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Abstract

Changes in serum metabolic profile after the intake of different food products (e.g., bread) can provide insight into their interaction with the human metabolism. Postprandial metabolic responses were compared after the intake of refined wheat (RWB), wholemeal rye (WRB), and refined rye (RRB) breads. In addition, associations between the metabolic profile in fasting serum and the postprandial concentration of insulin in response to different breads were investigated. Nineteen postmenopausal women with normal fasting glucose and normal glucose tolerance participated in a randomized, controlled, crossover meal study. The test breads, RWB (control), RRB, and WRB, providing 50 g of available carbohydrate, were each served as a single meal. The postprandial metabolic profile was measured using nuclear magnetic resonance and targeted liquid chromatography–mass spectrometry and was compared between different breads using ANOVA and multivariate models. Eight amino acids had a significant treatment effect (P < 0.01) and a significant treatment × time effect (P < 0.05). RWB produced higher postprandial concentrations of leucine (geometric mean: 224; 95% CI: 196, 257) and isoleucine (mean ± SD: 111 ± 31.9) compared with RRB (geometric mean: 147; 95% CI: 186; mean ± SD: 84.2 ± 22.9) and WRB (geometric mean: 190; 95% CI: 207; mean ± SD: 95.8 ± 17.3) at 60 min respectively (P < 0.001). In addition, 2 metabolic subgroups were identified using multivariate models based on the association between fasting metabolic profile and the postprandial concentration of insulin. Women with higher fasting concentrations of leucine and isoleucine and lower fasting concentrations of sphingomyelins and phosphatidylcholines had higher insulin responses despite similar glucose concentration after all kinds of bread (cross-validated ANOVA, P = 0.048). High blood concentration of branched-chain amino acids, i.e., leucine and isoleucine, has been associated with the increased risk of diabetes, which suggests that additional consideration should be given to bread proteins in understanding the beneficial health effects of different kinds of breads. The present study suggests that the fasting metabolic profile can be used to characterize the postprandial insulin demand in individuals with normal glucose metabolism that can be used for establishing strategies for the stratification of individuals in personalized nutrition.

Introduction

Certain functional properties of cereal products, e.g., their postprandial glucose and insulin responses, have been characterized as steps toward obtaining a greater understanding of the interaction between cereal products and the human metabolism. These functional properties can potentially explain some of the findings regarding the association of cereals with lifestyle-related diseases. For example, a low-glycemic-index diet results in decreased postprandial insulin and glucose responses, which is thought to be beneficial for insulin and glucose metabolism (1). For many carbohydrate-rich foods, there is a linear correlation between the glycemic index and the insulimemic index. However, in healthy individuals, it has been shown that rye breads (RBs) produce a lower postprandial insulin response compared with refined wheat breads (RWBs) despite similar glucose responses (2–5). Juntunen et al. (4) suggested that the difference in the structural characteristics between RB and RWBs (less porous...
and mechanically firmer structure in RBs) is a possible explanatory mechanism. However, the underlying mechanism of this discrepancy between insulin and glucose responses to RB, the so-called “rye bread factor,” is still essentially unknown (2–8). Postprandial glucose and insulin responses in peripheral blood represent complex interactions of the food matrix and food components in the human metabolism, and understanding these post-absorptive processes requires methodologies that can comprehend these complexities, i.e., metabolomics, which can provide a comprehensive view of metabolic events (9). A more comprehensive knowledge of postprandial metabolic effects can help to understand the complex interaction between these products and the human metabolism and may help to explain their differences in the intensity of insulin responses.

In the present study, the differences in the postprandial metabolic responses between refined rye bread (RRB) and whole-meal rye bread (WRB) were compared with RWB as the control (4) using NMR and targeted liquid chromatography (LC)–MS metabolomics. In addition, the association between the profile of metabolites in fasting serum and the intensity of postprandial insulin responses was investigated.

**Participants and Methods**

**Participants**
Twenty healthy postmenopausal women without diabetes were recruited for the study. One woman discontinued the study after the first visit because of heart problems. All women had normal glucose tolerance at the time of entry into the study, as determined by a 2-h oral glucose tolerance test (OGTT) and the 3 bread trials. At the time of entry into the study, no impaired fasting glucose on 4 different occasions, i.e., the baseline values at the time of entry to the study, as determined by a 2-h oral glucose tolerance test (OGTT) according to WHO criteria (10). The women had no impaired fasting glucose on 4 different occasions, i.e., the baseline values of the OGTT and the 3 bread trials. At the time of entry into the study, the participants were (mean ± SD) 61 ± 4.8 y old, and their BMI was 26.0 ± 2.5 kg/m². The clinical characteristics regarding blood pressure, serum cholesterol, and TG concentrations and the results of the OGTT have been published previously by Juntunen et al. (4). The protocol for the study was approved by the Ethics Committee of Kuopio University and University Hospital.

**Test breads**
The breads used in this study were RRB and WRB. In addition, a commercial RWB (ElloPakari; Vaasan & Vaasan Oy) was used as the reference (control) bread. Each bread provided 50 g of available carbohydrates. Sourdough containing both yeast and lactobacilli was used in all RBs. Commercial rye endosperm flour and commercial whole-meal rye flour were the only kinds of flour used in the formula of RRB and WRB, respectively (4). The details of the experimental bread formula and the procedures for measuring dietary fiber content, protein content, fat content, and moisture content have been described previously by Juntunen et al. (4). The nutrient composition of the test bread portions is shown in Table 1 (4).

**Study design, sample collection, and biochemical analysis**
The participants fasted for 12–15 h before the postprandial tests. The participants received a test meal containing the test bread, 40 g of cucumber, and 3 mL of a noncaloric orange drink. Six blood samples were taken (antecubital vein of the arm) before (0 min) and after (30, 45, 60, 90, and 180 min) the test meal intake were used for metabolomics and biochemical analysis. The test bread portions were served in random order at intervals of 1–2 wk. The protocols regarding body weight maintenance/monitoring, nutrient intake, and lifestyle throughout the study have been published previously by Juntunen et al. (4).

The details of serum and plasma collection and insulin and glucose measurements have been described previously by Juntunen et al. (4). Plasma glucagon was measured using the Glucagon Ria Kit (catalog no. GL-32K; Millipore).

**NMR metabolomics analysis and identification of metabolites**
NMR-based metabolomics analysis of serum samples was performed as described previously after slight modification (11–14). All NMR analyses of serum samples were performed on a Bruker spectrometer operating at 600 MHz. 1H NMR spectra of serum samples were obtained using the zgexp pulse sequence (Bruker Spectrospin) at 25°C with 128 scans and 65,536 data points over a spectral width of 17,942.58 Hz. Acquisition time was 1.82 s, and relaxation delay was 4.0 s. Forty-eight metabolites were identified, and their concentrations were calculated using NMR spectral data as described previously (14). The identity of NMR signals corresponding to 2-oxoisocaproate was confirmed after spiking with authentic standard.

**Targeted LC–MS metabolomics analysis**
The targeted LC–MS metabolomics was based on measurements using the AbsoluteID Q p180 kit (Biotrend Life Sciences) as described previously (15,16). In total, 189 metabolites were measured: 21 amino acids, 17 biogenic amines, 47 acyl-carnitines, 38 phosphatidylycerolines (PCs), 39 acyl-alkyl PCs, 14 lysophosphatidylcholines, 17,942.58 Hz. Acquisition time was 1.82 s, and relaxation delay was 4.0 s. Forty-eight metabolites were identified, and their concentrations were calculated using NMR spectral data as described previously (14). The identity of NMR signals corresponding to 2-oxoisocaproate was confirmed after spiking with authentic standard.

**Statistical analysis**

**Multivariate data analysis.** Multivariate data analysis, i.e., principal component analysis (PCA), hierarchal cluster analysis (HCA), orthogonal partial least-squares analysis (OPLS) and orthogonal partial least-squares–discriminant analysis (OPLS–DA), were performed using SIMCA-P+ 13.0 software (Umetrics) as described previously (17). The significance of the OPLS and OPLS–DA models were tested using cross-validated ANOVA (18). At each postprandial time point, the metabolic profile was compared between 2 breads using separate OPLS–DA models (total of 3 comparisons per time point). First, the NMR data including 48 metabolites were analyzed, followed by the targeted LC–MS data including 189 metabolites. Variable influences on projection values were used to determine the most important discriminative metabolites in each comparison. Metabolites with variable influences on projection > 1 and for which the corresponding jackknife-based 95% CIs were not close to or including 0 were considered discriminative.

It was further investigated whether fasting metabolic status is predictive of the intensity of postprandial insulin responses. The aim was to look for metabolic subgroups identified on the basis of their fasting metabolic profile that differ in the intensity of their postprandial insulin responses. As a first step, the association between the fasting concentrations of 189 metabolites (0 min before intake of RWB, Y variable) measured by targeted LC–MS and the postprandial insulin concentration at 30 min after intake of RWB (Y variable) was investigated using an OPLS model. In parallel, HCA was performed using the fasting concentrations of 189 metabolites (0 min before RWB intake), OPLS–DA was performed to identify discriminative metabolites between the 2 subgroups in fasting serum (0 min before RWB intake) identified in the previous steps using OPLS and HCA. The metabolic characteristics of the subgroups over different days was investigated by fitting a PCA model using the metabolic profiles of serum collected from each woman on 3 different occasions (0 min, RRB, WRB, and RWB).

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**TABLE 1 Nutrient composition of the test bread portions**

<table>
<thead>
<tr>
<th></th>
<th>Refined rye bread</th>
<th>Whole-meal rye bread</th>
<th>Refined wheat bread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portion size, g</td>
<td>111.9</td>
<td>142.7</td>
<td>105.5</td>
</tr>
<tr>
<td>Available carbohydrate, g</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Total dietary fiber, g</td>
<td>6.1</td>
<td>15.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Insoluble dietary fiber, g</td>
<td>3.1</td>
<td>10.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Soluble dietary fiber, g</td>
<td>3.0</td>
<td>4.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Protein, g</td>
<td>4.9</td>
<td>11.1</td>
<td>9.0</td>
</tr>
<tr>
<td>Fat, g</td>
<td>3.4</td>
<td>7.8</td>
<td>5.2</td>
</tr>
<tr>
<td>Moisture, g</td>
<td>46.7</td>
<td>58.2</td>
<td>38.2</td>
</tr>
<tr>
<td>Energy content, kJ</td>
<td>1056</td>
<td>1295</td>
<td>1177</td>
</tr>
</tbody>
</table>

1 All participants received the same amount of meal.
ANOVA comparing breads at each time point. The concentrations of 48 metabolites calculated from NMR spectra were compared between the 3 breads at each postprandial time point using a general linear model (ANOVA), with participants as a random factor, followed by a Bonferroni’s multiple-comparison test (Minitab statistical software, version 16; Minitab). For the ANOVA, a Bonferroni’s correction was performed to take into account multiple testing, using the number of metabolites calculated from the NMR spectrum as the number of variables \((n = 48)\), giving \(\alpha = 0.001\). Only metabolites that were significantly different between the breads at least at 1 postprandial time point \((\alpha = 0.001)\) or had a trend for significance \((\alpha = 0.05)\) are presented in the tables. Serum amino acid concentrations measured by the targeted LC–MS analysis were compared at each postprandial time point between the 3 breads using ANOVA and Bonferroni’s multiple-comparison tests.

The values for absolute concentrations of the metabolites were log-transformed before the ANOVA when the distribution was skewed (Anderson-Darling test, \(P < 0.05\)).

To normalize for the protein content of breads, the leucine-to–total amino acid and isoleucine-to–total amino acid ratios were compared between different breads at 45 and 60 min using ANOVA and Bonferroni’s multiple-comparison tests.

Correlation analyses. After the inclusion of the corresponding values from all 3 breads at each time point, the Pearson correlation coefficient between glucose, leucine, and insulin was investigated (Minitab statistical software, version 16).

ANOVA comparing breads using repeated measurements. The differences in amino acid (LC–MS measurements) responses between the breads at different postprandial time points were analyzed further using ANOVA (general linear model) with repeated measurements (Minitab statistical software, version 16).

ANOVA comparing different subgroups of participants using repeated measurements. The differences in glucose, insulin, sphingomyelin, and phosphocholine (LC–MS measurements) responses between the 2 metabolic subgroups, as identified in previous steps, in response to different breads and at different postprandial time points were analyzed further using ANOVA (general linear model and repeated measurements (Minitab statistical software, version 16).

Results

Multivariate data analysis comparing postprandial metabolic responses between breads. The metabolic profile of serum measured by NMR and targeted LC–MS was compared between pairs of breads at each postprandial time point using separate OPLS-DA models. None of the OPLS-DA models fitted was significant considering their \(R^2_Y\), \(Q^2_Y\), and cross-validated ANOVA \((P > 0.05)\).

Univariate data analysis comparing postprandial metabolic responses between breads. There were no significant differences in concentrations of the 48 metabolites (measured by NMR) at baseline. Amino acids, including leucine, isoleucine (Fig. 1), phenylalanine, tyrosine, proline, valine, methionine (Supplemental Table 1), and leucine catabolic intermediate (2-oxoisocaproate) (Fig. 2), were found to be significantly different between the breads at least at 1 postprandial time point after correction for multiple testing \((\alpha = 0.001)\).

The concentrations of 21 amino acids measured by targeted LC–MS were compared after the intake of different breads at each time point. Leucine, isoleucine, proline, tyrosine, valine, ornithine, tryptophan, histidine, phenylalanine, methionine, citrulline, threonine, and asparagine were found to differ significantly between the breads at least at 1 postprandial time point (Supplemental Table 2).

There were no differences in total amino acid concentrations (the sum of the concentrations of 21 amino acids as measured by LC–MS) between the breads at baseline and 30, 90, and 180

FIGURE 1 Postprandial responses of leucine and isoleucine after intake of refined rye bread, whole-meal rye bread, and refined wheat bread in postmenopausal women. Values are means ± SEMs \((n = 19)\). Mean values at each time point labeled without a common letter are significantly different \((P < 0.05)\). The amino acids were measured in the serum using targeted liquid chromatography–MS.
min. Total amino acid concentration was lower at 45 min \((P = 0.040)\) and 60 min \((P = 0.007)\) after eating RRB compared with RWB, but there were no differences between WRB and either RWB or RRB at 45 and 60 min (data not shown).

In addition, the leucine-to–total amino acid ratio at 45 and 60 min and the isoleucine-to–total amino acid ratio at 60 min were higher after eating RWB compared with both RBs (leucine-to–total amino acid ratio at 45 min: RRB, 0.048 \(\pm 0.006\); WRB, 0.050 \(\pm 0.006\); RWB, 0.047 \(\pm 0.006\); at 60 min: RRB, 0.047 \(\pm 0.006\); WRB, 0.048 \(\pm 0.006\); RWB, 0.056 \(\pm 0.009\); isoleucine-to–total amino acid ratio at 60 min: RRB, 0.023 \(\pm 0.002\); WRB, 0.026 \(\pm 0.003\); RWB, 0.026 \(\pm 0.003\); \(P < 0.001\)).

Eight amino acids, namely leucine, isoleucine, citrulline, ornithine, proline, asparagine, methionine, and lysine, had a significant treatment effect \((P < 0.01)\) and a significant treatment \(\times\) time effect \((P < 0.05)\). For total branched-chain amino acids (BCAAs; leucine, isoleucine, and valine), there was a significant treatment effect \((P < 0.001)\) and a significant treatment \(\times\) time effect \((P = 0.038)\). For total amino acids, a significant treatment effect \((P < 0.001)\) but no time \(\times\) treatment interaction was found.

There was a significant correlation \((P < 0.001)\) between insulin and leucine concentrations at 30, 45, and 60 min \((R^2 = 38.8\%, 44.2\%, \text{and} 24.0\%, \text{respectively})\). There were significant but weak correlations between insulin and glucose at 30 and 60 min \((R^2 = 17.4\% \text{ and} 11.6\%, \text{respectively}; P < 0.01)\).

**Identification of metabolic subgroups.** The OPLS model separated the 19 women into 2 metabolic subgroups along the predictive component comprising 6 and 13 women, respectively, based on the association between the fasting concentration of 189 metabolites \((0 \text{ min}, \text{before RWB intake}, \text{X variable})\) and the postprandial insulin concentration at 30 min \((\text{after eating RWB}, \text{Y variable}; \text{model parameters} R^2 = 0.53, Q^2 = 0.31, \text{and} \text{cross-validated ANOVA,} P = 0.048)\) (Fig. 3). HCA also separated the 19 women into 2 main clusters \(\text{(subgroups) comprising} 13 \text{ and} 6 \text{ women, respectively} \text{(RWB baseline)}\). The metabolic subgroups identified by OPLS and HCA were identical and included the same participants. In addition, the metabolic subgroups were clearly separated in the PCA score plot as shown in Supplemental Figure 1 \(\text{(model parameter} R^2 = 0.38, \text{model parameter} R^2 = 0.17)\) in which they were separated along the second component. The women belonging to the 2 subgroups identified in the previous steps \((\text{using RWB})\) consistently clustered into the same subgroups after fitting a PCA model including all 3 baseline metabolic profiles.
collected at each visit (model parameter $R^2 = 0.38, R^2$ component $= 0.19$) (Supplemental Fig. 2). An OPLS-DA model was fitted to identify the discriminative metabolites between the 2 subgroups of women using the baseline values of the RWB trial (Supplemental Fig. 3). The model parameters were $R^2 = 0.82$ and $Q^2 = 0.69$ (cross-validated ANOVA, $P = 3.48 \times 10^{-6}$). The discriminative metabolites between the 2 groups of participants are presented in Table 2.

After incorporating all 3 baseline values for each woman, no significant differences were found in the baseline values of glucose and insulin between the 2 metabolic subgroups. The serum insulin and glucose responses to treatments (breads) in the metabolic subgroups were investigated at different time points (Fig. 4). For insulin, significant treatment ($P < 0.001$), metabolic subgroup ($P = 0.045$), time $\times$ treatment ($P < 0.001$), and time $\times$ metabolic subgroup ($P < 0.001$) effects were found, but no treatment $\times$ metabolic subgroup effect was found. For glucose, no significant treatment, metabolic subgroup, time $\times$ treatment, time $\times$ metabolic subgroup, or treatment $\times$ metabolic subgroup effects were found.

For serum sphingomyelins and PCs in response to the different treatments in the 2 metabolic subgroups, no significant time $\times$ treatment, time $\times$ metabolic subgroup, or treatment $\times$ metabolic subgroup effects were observed (Supplemental Figs. 4 and 5).

**Glucagon responses.** There was no significant treatment effect or time $\times$ treatment effect in the postprandial responses of glucagon. There were no differences in postprandial glucagon response between the breads at 30, 45, 60, and 180 min. Glucagon concentration was higher (ANOVA and multiple comparison test, $P < 0.05$) in WRB consumption compared with RWB consumption at 90 min. There was no significant difference between RRB and the other breads.

### TABLE 2  Metabolites that were found to be different in the fasting metabolic profile between metabolic subgroups A and B identified among postmenopausal women

<table>
<thead>
<tr>
<th>Metabolite*</th>
<th>Subgroup A</th>
<th>Subgroup B</th>
<th>VIP (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting concentration</strong></td>
<td></td>
<td></td>
<td>$\mu$mol/l</td>
<td></td>
</tr>
<tr>
<td>PC aa C34:2</td>
<td>667 ± 134</td>
<td>454 ± 81.2</td>
<td>5.2 (1.8)</td>
<td>0.003</td>
</tr>
<tr>
<td>SM C24:1</td>
<td>188 ± 42.5</td>
<td>69.7 ± 11.5</td>
<td>4.7 (0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SM C16:0</td>
<td>229 ± 34.0</td>
<td>131 ± 23.0</td>
<td>4.2 (0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PC aa C36:2</td>
<td>371 ± 67.4</td>
<td>258 ± 45.5</td>
<td>4.0 (1.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>PC aa C36:3</td>
<td>227 ± 45.0</td>
<td>149 ± 34.8</td>
<td>3.3 (1.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>PC aa C36:4</td>
<td>272 ± 51.6</td>
<td>187 ± 64.0</td>
<td>3.3 (1.6)</td>
<td>0.007</td>
</tr>
<tr>
<td>SM C24:0</td>
<td>61.4 ± 10.4</td>
<td>22.2 ± 4.5</td>
<td>2.7 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glutamine</td>
<td>85.8 ± 23.0</td>
<td>139 ± 51.8</td>
<td>2.6 (2.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>Leucine</td>
<td>179 ± 33.4</td>
<td>228 ± 43.6</td>
<td>2.4 (1.8)</td>
<td>0.014</td>
</tr>
<tr>
<td>SM C18:0</td>
<td>58.2 ± 11.2</td>
<td>30.0 ± 6.3</td>
<td>2.2 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>80.4 ± 14.5</td>
<td>110 ± 14.4</td>
<td>2.1 (0.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>SM (OH) C22:2</td>
<td>40.7 ± 8.40</td>
<td>16.5 ± 3.5</td>
<td>2.1 (0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SM (OH) C22:1</td>
<td>37.2 ± 6.20</td>
<td>14.4 ± 3.7</td>
<td>2.1 (0.4)</td>
<td>0.007</td>
</tr>
<tr>
<td>SM C18:1</td>
<td>29.5 ± 5.60</td>
<td>16.9 ± 3.5</td>
<td>1.4 (0.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SM C16:1</td>
<td>35.1 ± 6.80</td>
<td>22.1 ± 4.20</td>
<td>1.4 (0.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Fasting concentration values are means ± SDs; subgroup A, $n = 13$ and subgroup B, $n = 6$. The orthogonal partial least-squares–discriminant analysis model parameters for 1 predictive component fitted were as follows: $R^2 = 0.82$, $Q^2 = 0.69$, and cross-validated ANOVA, $P = 3.48 \times 10^{-6}$. C, total number of carbon atoms in FAs; total number of double bonds in FAs; PC aa, phosphatidylcholine with diacyl residue; SM, sphingomyelin with acyl residue; SM (OH), hydroxysphingomyelin with acyl residue; VIP, variable influences on projection.

2 Metabolites were measured using targeted liquid chromatography–MS.

### Discussion

A lower postprandial insulin response to RBs compared with RWB despite similar glucose responses is a characteristic of RBs that has been shown consistently in different trials (2–5). This is known as the rye bread factor. The lower insulin response after RB is not explained by the amount of fiber or enhanced liver extraction of insulin (4). In the present study, distinct differences were observed in the postprandial responses of amino acids between different breads using a metabolomics approach (Supplemental Tables 1 and 2). The serum concentrations of BCAA, i.e., leucine and isoleucine, were higher after RWB intake compared with the 2 RBs at 45–60 min (Fig. 1). Intriguingly, the differences in insulin responses between RWB and RBs at 30–60 min (4) resemble those of leucine. Insulinotropic properties of some amino acids and proteins were presented in several in vitro and in vivo studies (19–26). When protein, protein hydrolysate, and/or free amino acids are ingested in combination with carbohydrate, a synergistic postprandial insulin response is triggered (23–28). The plasma concentration of insulin after the simultaneous ingestion of carbohydrate with leucine, phenylalanine, and protein hydrolysate was shown to be highly correlated with the plasma concentration of leucine, isoleucine, valine, lysine, and phenylalanine (23,24,29,30). Consistently, a significant correlation between insulin and leucine (30–60 min; $P < 0.001$), which is the most potent insulinogenic amino acid, was observed in the present study. These findings suggest that the higher postprandial insulin response after consumption of WB compared with RBs (2–4) might at least partly be explained by higher postprandial responses in insulinogenic BCAAs, i.e., leucine and isoleucine after WB (Fig. 1). Leucine is converted to 2-oxo-isocapropate by amino transferases as the first step in the leucine catabolic pathway (31). Intriguingly, there were higher postprandial responses of 2-oxo-isocapropate after eating RWB compared with RBs (Fig. 2), which can be explained by higher leucine responses after intake of RWB vs. RBs. Recent human studies showed strong associations between elevated plasma BCAA and their metabolites with increased incidence, progression, and remission of insulin resistance and type 2 diabetes (T2D) (31,32). We showed previously a higher concentration of BCAA after 8 wk of intervention with RWB compared with WRB (14). Therefore, the findings in the present study highlight the importance of considering the role of amino acids, especially BCAAs, when the beneficial health effects of different breads are investigated.

Plasma insulin response increases in parallel with plasma glucose when the duodenal glucose load remains <2 kcal/min (i.e., 30 g of glucose over a period of 60 min) (33,34). However, above this threshold, substantially greater glucose-dependent insulinotropic polypeptide and insulin responses reduce the glucose response to the level produced by a duodenal glucose load of 2 kcal/min (33,34). In the present study, all breads contained 50 g of starch, thus delivering a glucose load of 3.3 kcal/min over a period of 60 min. Thus, it is possible that the high duodenal glucose load associated with faster starch digestibility of RWB compared with RBs (4) resulted in similar glucose concentrations after consumption of RWB and RBs despite increased insulin response after RWB consumption. High duodenal glucose load above the threshold can also mask the glucose-lowering effects of insulinotropic amino acids. Van Postprandial metabolic responses in humans 5 of 8
Loom et al. (26) showed that a high glucose load (50 g) can mask amino acid augmented insulin response when a mixture of glucose and protein are ingested. This may explain why higher insulinogenic amino acids and insulin after intake of RWB did not reduce glucose concentration compared with RBs. No differences in glucagon concentration were observed between the breads at 30–60 min, which indicates that hepatic glucose output after the breads was similar and could not explain the lack of a plasma glucose–lowering effect of higher insulin concentration after RWB consumption (35).

After adjusting for total starch intake (50 g), RRB, WRB, and RWB provided 4.9, 11.1, and 9.0 g of protein per bread portion, respectively. The differences in protein content between RRB and WRB are attributed to the presence of germ and bran proteins in WRB. Germ constitutes only a small proportion of the whole kernel and its proteins are easily digested, whereas the bran proteins are enclosed in thick-walled aleurone cells and therefore less bioaccessible. Chick et al. (36) reviewed a number of studies showing that, when whole-meal bread is consumed, between two-thirds and three-quarters of the proteins contained in the bran are used by humans despite that the digestion of bran proteins continues until ingested bran reaches the cecum. Consistently, although WRB contained almost twice as much protein as RRB, total amino acid responses (including 21 amino acids) were not different between these breads at any postprandial time point. In addition, our findings suggest that bran proteins do not contribute to postprandial insulin responses, because there were no differences in leucine and isoleucine responses between the RBs. Higher serum leucine and isoleucine after consumption of RWB vs. WRB may be explained by differences in the bioaccessibility and/or amino acid profile between wheat and rye proteins. To account for the differences in total protein content between the breads, a comparison was undertaken of leucine-to–total amino acids and isoleucine-to–total amino acids ratios in serum between the breads. Consistent with the concentrations, the ratios were also higher after intake of RWB compared with RBs (45–60 min). The present study suggests that the inherent differences in composition and the bioaccessibility of proteins can be translated into differences in postprandial concentration of amino acids after eating RRB, WRB, and RWB.

In the present study, 2 metabolic subgroups were identified based on the association between the fasting metabolic profile and the postprandial insulin concentration (Fig. 3, Table 2; Supplemental Figs. 1–3). These metabolic subgroups were consistently identified using a supervised model, i.e., OPLS, and unsupervised models, i.e., HCA and PCA. Their metabolic differences remained constant over 3 different sampling days (Supplemental Fig. 2). In addition, the multivariate regression models (OPLS and OPLS–DA) were highly robust and reliable as indicated by their cross-validated ANOVA $P$ values. Compared with metabolic subgroup B ($n = 6$), metabolic subgroup A ($n = 13$) had higher serum concentrations of sphingomyelins and diacyl-PCs with 2 or more double bonds and lower concentrations of BCAAs, i.e., leucine and isoleucine (Table 2). Higher concentrations of BCAAs and lower concentrations of sphingomyelin and unsaturated diacyl-PCs have been associated with the increased risk of developing T2D (32,37), which suggests that subgroup A may be in a lower T2D risk category than subgroup B. Intriguingly, none of the women in the present study had impaired glucose tolerance or impaired fasting plasma glucose at baseline. In addition, no significant differences in fasting glucose, insulin, BMI, and HOMA-IR index (data not shown) were found between the 2 subgroups. There were no differences in the postprandial glucose responses, but subgroup A had consistently lower insulin responses after all breads compared with subgroup B (Fig. 4). Similar glucose and lower insulin responses in subgroup A compared with subgroup B suggest higher insulin sensitivity in subgroup A. Subgroup B,
Despite having lower insulin sensitivity (higher resistance), still possessed enough insulin secretion capacity to control the glucose concentration, and therefore no difference in glucose response was found between the subgroups and their glucose concentration stayed in a healthy range. Our findings indicate that a heterogeneous metabolic fingerprint is associated with altered metabolic response, i.e., high insulin response. This can be used for early identification of humans with reduced insulin sensitivity and at risk of developing additional metabolic complications. The characteristic metabolic fingerprint can be used for stratification of humans as a step toward targeted personalized nutritional interventions.

In conclusion, in the present study, differences were observed in the postprandial responses of amino acids, i.e., insulinoenic BCAAs, between RWB and RBs. These findings suggest that the postprandial responses of amino acids should be considered when the associations between postprandial glucose and insulin responses or beneficial health effects of cereal products are investigated. In addition, by using the metabolomics approach, 2 metabolic subgroups were identified that responded with different concentrations of insulin for controlling the postprandial glucose concentration. However, our observations were in a small cohort of participants, and it is important to test the validity of these findings in larger independent cohorts. This would involve an experiment in which the participants are categorized into the subgroups a priori rather than discovering these subgroups as a posteriori, as in this study. The metabolic classification, if further confirmed in independent cohorts, can in principle be used as a basis for personalized nutrition by identifying and targeting individuals with emerging insulin resistance and at risk of developing metabolic complications.

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Literature Cited


