Nuclear Magnetic Resonance–Based Metabolomics Enable Detection of the Effects of a Whole Grain Rye and Rye Bran Diet on the Metabolic Profile of Plasma in Prostate Cancer Patients

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Abstract

Prostate cancer (PC) is the most common cancer in the Western world and the second most important cancer causing male deaths, after lung cancer, in the United States and Britain. Lifestyle and dietary changes are recommended for men diagnosed with early-stage PC. It has been shown that a diet rich in whole grain (WG) rye reduces the progression of early-stage PC, but the underlying mechanism is not clear. This study sought to identify changes in the metabolic signature of plasma in patients with early-stage PC following intervention with a diet rich in WG rye and rye bran product (RP) compared with refined white wheat product (WP) as a tool for mechanistic investigation of the beneficial health effects of RP on PC progression. Seventeen PC patients received 485 g RP or WP in a randomized, controlled, crossover design during a period of 6 wk with a 2-wk washout period. At the end of each intervention period, plasma was collected after fasting and used for 1H NMR-based metabolomics. Multilevel partial least squares discriminant analysis was used for paired comparisons of multivariate data. A metabolomics analysis of plasma showed an increase in 5 metabolites, including 3-hydroxybutyric acid, acetone, betaine, N,N-dimethylglycine, and dimethyl sulfone, after RP. To understand these metabolic changes, fasting plasma homocysteine, leptin, adiponectin, and glucagon were measured separately. The plasma homocysteine concentration was lower (\(P = 0.017\)) and that of leptin tended to be lower (\(P = 0.07\)) after RP intake compared to WP intake. The increase in plasma 3-hydroxybutyric acid and acetone after RP suggests a shift in energy metabolism from anabolic to catabolic status, which could explain some of the beneficial health effects of WG rye, i.e., reduction in prostate-specific antigen and reduced 24-h insulin secretion. In addition, the increase in betaine and N,N-dimethylglycine and the decrease in homocysteine show a favorable shift in homocysteine metabolism after RP intake.

Introduction

PC is the most commonly diagnosed malignancy in men and the second most frequent cause of cancer-related death in the Western world (1). As the life expectancy of Western populations increases, medical experts anticipate that PC will become an even greater health problem because of the increasing incidence of prostate carcinoma with age (1).

Active surveillance (a strategy of closely monitoring newly diagnosed patients, including regular PSA measurements, digital rectal examinations, and biopsies; treatment with disease progression or patient choice) and lifestyle and dietary changes are recommended for men diagnosed with early-stage PC (2,3). With regard to the dietary changes, the effects of WG and especially WG rye have been investigated. A 3-wk intervention with WG rye was reported to increase tumor apoptotic index in men with conservatively treated PC (4). In a previous part of the present study, consumption of a diet rich in WG rye and rye bran products by patients \((n = 17)\) with early-stage PC reduced the blood concentration of PSA, indicating reduced tumor activity...
and progression (5). In comparison with a refined wheat diet, a diet rich in rye has been shown to reduce PSA concentration and tumor volume in mice with human LNCaP xenografts (6) and early tumor growth in rats with prostate adenocarcinoma implants (7) and to increase epithelial cell apoptosis in transgenic mice designed to develop PC (8). Therefore, previous human and animal experiments suggest that WG rye can potentially be used together with other recommended lifestyle and dietary changes to suppress the progression of early-stage PC during active surveillance. However, the mechanism by which WG rye reduces PSA has not yet been identified. A mechanistic understanding of the effect of WG rye on PC can support and validate the observed effect of WG rye on prostate PC progression and help in developing more potent WG rye products.

Rye bread is associated with a reduced postprandial insulin level without any difference in glucose level compared with refined wheat bread, an effect known as RF (9–11). In a PC-WGR intervention, a reduction in C-peptide was also observed, which might account for the reduced PSA. Recent evidence from population studies indicates that hyperinsulinemia is related to adverse outcomes and higher mortality in PC (12,13). The mitogenic effects of human insulin have been noted in vitro and in animal and human studies and have been proposed as an explanation for increased PC progression and mortality (14). However, previous hypothesis-driven studies have not provided a mechanistic explanation for RF (9–11). Notably, WG rye is a rich source of fiber and bioactive compounds with diverse biological effects, which might also be associated with the observed effects on PC.

To understand the mechanism(s) of action of WG rye, it is important to determine changes in the metabolic profile following a WG rye intervention. Recent progress in high-throughput analytical technologies, particularly metabolomics, allows the simultaneous analysis of many metabolites constituting the metabolome (pool of metabolites) in biofluids and tissues (15). These analyses can potentially reveal a complex metabolic fingerprint characteristic of a given stimulus, e.g., intervention with a diet rich in WG rye. They therefore describe the effects of diet on metabolism and may help identify the interaction between different foods and the human body or disease status.

This study sought to utilize the power of NMR-based metabolomics to determine the effects of a diet rich in WG rye products on the profile of metabolites in the plasma of PC patients from the PC-WGR intervention. Being independent of prior assumptions, metabolomics approaches allow hypothesis generation, in this case about how intervention with WG rye can reduce PSA. In addition, metabolite profiling can detect any metabolic changes associated with RF.

**Materials and Methods**

**Human intervention.** This study is part of the PC-WGR intervention project, which was set up to investigate the effects of a diet rich in WG rye and rye bran products on the progression of PC (5). Twenty-four men with early-stage, previously untreated, histologically confirmed PC were enrolled in this study (Table 1). They had chosen not to undergo any conventional treatment and had low risk PC, as defined by the Gleason score and tumor stage. The study was carried out using a randomized, controlled, crossover design with two 6-wk intervention periods and a 2-wk washout period. The participants were not smokers or users of dietary supplements or medications. The treatments were a diet rich in WG rye and rye bran products (RP) or a diet of refined WG products with added cellulose (WP) as the control treatment (5,9,10,16,17). The participants were not informed about the order of treatments, but they may have guessed which treatment they received, because products appeared somewhat differently. For logistical reasons, the study was performed on three occasions between April and July 2005, with the treatment order being separately randomized on each occasion. Seven participants dropped out of the study, mostly due to constipation problems, which might be related to the inability to adapt to the high-dietary fiber content of the diets. Seventeen men completed the entire study and provided completed data records from the three occasions. On the first occasion, 2 men started with the RP diet and 3 men started with the WP diet; on the second occasion, 5 men started with RP and 3 started with WP; and on the third occasion, 2 men started with RP and 2 with WP.

Fasting blood (heparin) samples collected at the end of each intervention period were used for the metabolomics study. The human intervention was performed in accordance with the standards of Örebro University Hospital, Sweden, and approval was obtained from the regional ethics committee.

Because energy intake may affect the progression of PC, the intervention foods were designed to contain almost the same energy content and sufficient energy was provided to prevent any decrease in weight. Participants were instructed by a dietician to keep their habitual diet except for bread, porridge, muesli, and other fiber- or lignin-rich foods and table spreads, which were replaced by products provided by the study (intervention foods) (Table 2). During each intervention period, participants were asked to include the intervention foods as part of their breakfast, lunch, dinner, and 2–3 snacks/d. The intervention foods included 300 g/d bread (3 pieces), 100 g/d crisp bread (10 pieces), 50 g/d breakfast cereals, 33 g/d porridge (uncooked), and 58 g/d table spread, to make a total of 485 g/d of RP or WP, corresponding to almost 50% of daily energy intake. Participants were not allowed to consume any other cereal-based products, such as bread, baked goods, porridge, or table spread and were instructed to consume all intervention foods and keep leftovers, if any. Cereal products for the respective treatments were provided by WasaBrod and Lantmännen (Sweden) and were specially designed for the purpose of the present study, with the aim of providing the same macronutrient intake. To balance the dietary fiber content and energy density in the WP and RP diets, purified wheat cellulose (Vitacel) was added to the refined wheat products used as the control diet (WP) in this study. This provided a similar weight of daily recommended intervention foods when they were adjusted for their digestible energy content. In addition, a table spread was provided to avoid differences in fat quality between treatments.

A dietician or nurse had regular telephone contact with the participants to ensure compliance. Before the first treatment period and at the end of each treatment period, participants completed 4-d weighed food records. These were used to check compliance and to calculate nutrient and total energy intake (Table 3). The difference

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**Table 1** Baseline characteristics of participants with PC according to treatment sequence (RP-WP or WP-RP) at entry to the study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RP-WP, n = 9</th>
<th>WP-RP, n = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>74.8 ± 3.6 (74)</td>
<td>74.4 ± 5.8 (75)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>89.3 ± 18.7 (79)</td>
<td>80.9 ± 13.7 (81.5)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.7 ± 5.1 (28.8)</td>
<td>26.5 ± 4.1 (26.2)</td>
</tr>
<tr>
<td>Gleason score</td>
<td>5.3 ± 2.2</td>
<td>5.1 ± 2.3</td>
</tr>
<tr>
<td>Total energy intake, kJ/d</td>
<td>8190 ± 1180 (8110)</td>
<td>8400 ± 1900 (8520)</td>
</tr>
<tr>
<td>Total fat intake, g/d</td>
<td>76.1 ± 17.5</td>
<td>61.0 ± 12.3</td>
</tr>
<tr>
<td>SFA intake, g/d</td>
<td>32.0 ± 7.2</td>
<td>24.5 ± 6.4</td>
</tr>
<tr>
<td>Unsaturated fatty acid intake, g/d</td>
<td>38 ± 9.5</td>
<td>32.1 ± 5.5</td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>86.8 ± 16.7</td>
<td>88 ± 16.1</td>
</tr>
<tr>
<td>Dietary fiber intake, g/d</td>
<td>24.5 ± 8.0 (23.1)</td>
<td>23.0 ± 10.2 (24.0)</td>
</tr>
</tbody>
</table>

1 Data are mean ± SD (median). Data are from (5). PC, prostate cancer; RP, whole grain rye and rye bran product; WP, refined white wheat product.
2 Fatty acids from habitual diet + table spread.
between distributed and consumed intervention foods at the end of each treatment and plasma total alkylresorciol concentration as a biomarker of WG and bran intake (18) were also used as additional checks of compliance. Nutrient and energy intake were calculated from intervention product labels and by using the food database at the Swedish National Food Administration (PC-Kost 1.99, SLV) and the software program MATs 4.05 (Rudans Lättdata).

**NMR analysis.** NMR analysis of plasma samples collected at the end of each intervention period was performed using previously described methods (19,20). The Nanosep centrifugal filters with 3-kDa cutoff (Pall Life Science) were washed 8 times with 0.5 mL water at 4000 unit/kg fresh product.

**Biochemical and bioinformatics analysis.** To further examine the metabolic changes detected in metabolomics analysis, adiponectin and leptin were analyzed separately in plasma using standard ELISA, glucagon was analyzed using standard RIA, and homocysteine was analyzed using a published method (21). In addition, Internet-based bioinformatics tools, i.e., Kyoto Encyclopedia of Gene and Genomes and Human Protein Atlas, were used for the interpretation of metabolomics and biochemical findings.

**Statistical analysis.** The spectral data were processed using Bruker Topspin 1.3 software and were Fourier-transformed after multiplication by a line broadening of 0.3 Hz and referenced to TSP at 0.0 ppm. Spectral phase and baseline were manually corrected. Each spectrum was integrated using Amix 3.7.3 (Bruker BioSpin) into 0.01-ppm integral regions (buckets) between 0.5 and 8.55 ppm, in which areas between 4.25 and 6.75 ppm containing area were removed. Each spectral region was then normalized to the intensity of internal standard (TSP).

PCA was performed using SIMCA-P 12.0.1 software (UMETRICS) after centering and pareto-scaling of the data as previously described (22). In addition, MLPLS-DA was performed using Matlab (version 2009a, MathWorks) and in-house written Matlab routines, which were kindly made available for public use via Internet by the Department of Biosystems Analysis, University of Amsterdam (23,24). MLPLS-DA was used to account for the crossover design and the possibility of pairwise comparison of participants after RP and WP treatments. MLPLS-DA can be considered a multivariate extension of a paired t test that generates different multivariate submodels for the between-subject and within-subject variation in the data (23,24). The advantage of this variation splitting is

### TABLE 2 Nutrient composition of the intervention food products

<table>
<thead>
<tr>
<th>Intervention food</th>
<th>Soft bread</th>
<th>Crisp bread</th>
<th>Muesli</th>
<th>Porridge</th>
<th>Soft bread</th>
<th>Crisp bread</th>
<th>Muesli</th>
<th>Porridge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g</td>
<td>83</td>
<td>107</td>
<td>59</td>
<td>113</td>
<td>81</td>
<td>93</td>
<td>72</td>
<td>67</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>357</td>
<td>596</td>
<td>632</td>
<td>502</td>
<td>364</td>
<td>608</td>
<td>637</td>
<td>537</td>
</tr>
<tr>
<td>Fat, g</td>
<td>27</td>
<td>35</td>
<td>47</td>
<td>30</td>
<td>24</td>
<td>43</td>
<td>26</td>
<td>14</td>
</tr>
<tr>
<td>Dietary fiber, g</td>
<td>100</td>
<td>182</td>
<td>165</td>
<td>243</td>
<td>96</td>
<td>184</td>
<td>182</td>
<td>295</td>
</tr>
</tbody>
</table>

1. Data are from (5). RP, whole grain rye and rye bran product; WP, refined white wheat product.

### TABLE 3 Advised and estimated intakes of intervention foods and macronutrients by PC patients during the WG, RP, and WP periods

<table>
<thead>
<tr>
<th>Intervention food</th>
<th>Advised intake (g/d)</th>
<th>Estimated intake2 (g/d)</th>
<th>Advised intake (g/d)</th>
<th>Estimated intake2 (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intervention food</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft bread</td>
<td>300</td>
<td>247 ± 34</td>
<td>300</td>
<td>246 ± 39</td>
</tr>
<tr>
<td>Crisp bread</td>
<td>100</td>
<td>89 ± 17</td>
<td>100</td>
<td>96 ± 19</td>
</tr>
<tr>
<td>Muesli</td>
<td>50</td>
<td>50 ± 9</td>
<td>50</td>
<td>39 ± 15</td>
</tr>
<tr>
<td>Porridge</td>
<td>35</td>
<td>35 ± 10</td>
<td>35</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>Table spread</td>
<td>58</td>
<td>52 ± 26</td>
<td>58</td>
<td>49 ± 20</td>
</tr>
<tr>
<td><strong>Intervention food macronutrient (total macronutrient)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>43</td>
<td>37 ± 5 (10 ± 15)</td>
<td>40</td>
<td>34 ± 6 (9 ± 17)</td>
</tr>
<tr>
<td>Fat</td>
<td>15</td>
<td>13 ± 2 (65 ± 14)</td>
<td>13</td>
<td>11 ± 2 (63 ± 17)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>216</td>
<td>190 ± 22 (212 ± 58)</td>
<td>221</td>
<td>189 ± 32 (319 ± 59)</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>65</td>
<td>58 ± 7 (66 ± 15)</td>
<td>67</td>
<td>57 ± 9 (67 ± 14)</td>
</tr>
<tr>
<td>Ash</td>
<td>14</td>
<td>13 ± 2 (25 ± 5)</td>
<td>6</td>
<td>5 ± 1 (20 ± 3)</td>
</tr>
<tr>
<td>Energy intake, MJ/d</td>
<td>5.47</td>
<td>4.81 ± 0.57 (10.1 ± 1.4)</td>
<td>5.45</td>
<td>4.66 ± 0.79 (10.0 ± 1.7)</td>
</tr>
</tbody>
</table>

1. Data are mean ± SD, n = 17. PC, prostate cancer; RP, whole grain rye and rye bran product; WG, whole grain; WP, refined white wheat product.
2. Estimated intake of intervention food was calculated by subtracting the amount of intervention food left after treatment from the amount provided.
3. Intervention foods + habitual diet.
4. Energy intake (kJ/d) reported as the sum of energy from fat (37 kJ/g), protein (17 kJ/g), carbohydrates (17 kJ/g), and dietary fiber (8 kJ/g).
that each submodel can be separately analyzed without being confounded by the other variation source. Rank product provided by MLPLS-DA analysis was then used to determine the most important NMR signals in the multilevel classification model (23,24). The spectral variable with the highest PLS regression coefficient and possesses the largest discriminative power (23,24). The NMR signals that were identified as a discriminating response variable based on their low rank product in MLPLS-DA were further investigated by paired t test to provide a more widely recognizable value beside rank product. The presence of outliers was investigated using PCA-Hotelling T² Ellipse (95% CI) and the normality of multivariate data using the normal probability plot of PCA model. The multivariate data were normally distributed. The individual discriminating NMR signals were log-transformed before the paired t test when the distribution was skewed (Kolmogrov-Smirnov test, $P < 0.05$). The values from targeted analysis of homocysteine, leptin, adiponectin, and glucagon were also log-transformed before the paired t-test. In addition, one of the participants had distinct homocysteine values in both RP and WP treatments, so these values were removed before further statistical analysis. Values in the text are mean ± SD.

## Results

Overall, the participants complied well with the high dose of advised food (5,18) even though they did not fully consume the advised amounts of breads and crisp breads, as indicated by 4-d weighed food records and by the differences between provided and remaining food products (Table 3). However, the differences were consistent and similar between control and treatment groups. Therefore, total energy intakes did not differ between the groups. Body weights of control and treatment groups also did not differ after 2, 4, and 6 wk of intervention. A 2% mean weight loss ($P = 0.04$) occurred for the RP group ($n = 8$) during period 2, but a similar effect was not observed during period 1 or in the control during periods 1 or 2. Total fat and protein intakes from the baseline diet and the intervention diets did not differ. However, dietary fiber intakes were higher from both intervention diets than from the baseline diet ($P < 0.05$) (5).

Of the 24 participants initially enrolled, 17 participants completed the study according to the protocol. Nine completed the RP-WP sequence and 8 completed the WP-RP sequence. Therefore, total energy intakes did not differ between the groups. Body weights of control and treatment groups also did not differ after 2, 4, and 6 wk of intervention. A 2% mean weight loss ($P = 0.04$) occurred for the RP group ($n = 8$) during period 2, but a similar effect was not observed during period 1 or in the control during periods 1 or 2. Total fat and protein intakes from the baseline diet and the intervention diets did not differ. However, dietary fiber intakes were higher from both intervention diets than from the baseline diet ($P < 0.05$) (5).

The plasma alkylresorcinol concentration, which is a biomarker of rye and wheat WG and bran intake, was analyzed as an independent measure of compliance after 2, 4, and 6 wk within each treatment and remained stable within individuals during the two treatment periods (18). Plasma alkylresorcinol increased during the RP period but came back to the baseline value after the 2-wk washout period and there was no difference in baseline plasma alkylresorcinol concentrations between the RP and WP periods (18).

NMR-based metabolomics analysis of plasma showed an increase in five metabolites, including 3-hydroxybutyric acid, acetone, betaine, N,N-dimethylglycine, and dimethyl sulfone, after RP intake (Fig. 1; Table 4). The discriminative metabolites were identified based on their rank products in MLPLS-DA and further investigated by paired t test. (Table 4). The fasting plasma homocysteine concentration after RP intake (11.4 ± 2.41 μmol/L) was less ($P = 0.017$) than after WP intake (12.2 ± 2.31 μmol/L) and that of leptin tended to be lower after RP (7.7 ± 8.12) compared to WP (8.5 ± 8.30) intake ($P = 0.07$).

## Discussion

The effects of RP intake on the progression of PC have been investigated in a number of animal and human experiments (4–8). In another part of this study (PC-WGR intervention), a reduction in PSA and 24-h urinary excretion of C-peptide was observed after intervention with RP for 6 wk (5). Numerous hypothesis-driven animal and human trials have been performed to investigate the mechanistic link between WG cereal consumption and their beneficial health effects (25). Some of these have shown that consumption of WG rye bread is associated with reduced postprandial insulin level without any difference in glucose response compared with refined wheat bread, an effect known as the RF (9–11). In the present study, we used metabolomics to study metabolic effects of RP in humans. The determination of metabolic changes may help us understand the beneficial health effects of rye, including those observed for

### Table 4 Results of NMR-based metabolomics analysis of plasma from PC-WGR

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>NMR signal, ppm</th>
<th>Rank product</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hydroxybutyrate</td>
<td>1.20, 2.29, 4.17</td>
<td>34.8, 22.0, 17.5</td>
<td>0.037, 0.018, 0.026</td>
</tr>
<tr>
<td>Acetone</td>
<td>2.24</td>
<td>23.6</td>
<td>0.026</td>
</tr>
<tr>
<td>Betaine</td>
<td>3.91</td>
<td>2.6</td>
<td>0.005</td>
</tr>
<tr>
<td>N,N-dimethylglycine</td>
<td>2.93</td>
<td>34.8</td>
<td>0.048</td>
</tr>
<tr>
<td>Dimethyl sulfone</td>
<td>3.16</td>
<td>19.5</td>
<td>0.026</td>
</tr>
</tbody>
</table>

1 All discriminating metabolites were greater after the RP period compared to the WP period. PC-WGR, prostate cancer-whole grain rye intervention; RP, whole grain rye and rye bran product; WP, refined white wheat product.

2 The values are reported on a logarithmic scale (23).

3 The P value was calculated using log-transformed data ($n = 17$).

4 Three NMR signals were independently detected as discriminating metabolites, strengthening the validity of the findings.
Body weight was found between control and treatment, but a

and plasma lipids (34).

health effects of WG rye consumption, e.g., reducing cholesterol

fatty acid biosynthesis. This may explain some of the beneficial

carboxylase, which are rate-limiting enzymes in cholesterol and

Activation of AMPK can cause phosphorylation and inhibition

of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acetyl-CoA

metabolism indicated by an increase in plasma ketone bodies in

ybutyric acid in plasma (32,33). Therefore, the shifts in energy

acid oxidation (30,31) and consequently increases 3-hydrox-

balance (30,31). Activation of AMPK increases hepatic fatty

and similar postprandial blood levels shown by previous studies

reduced delivery and intestinal absorption of glucose in WG rye

compared with refined wheat bread (9,29). Therefore, the

supporting the reduced intestinal absorption of glucose after WG

bread (28), is reduced by intake of WG rye bread, concentration, which is correlated with the rate of intestinal

postprandial insulin response and thereby produces an equal rise

in plasma glucose levels after WG rye bread compared with refined

wheat bread, as shown in RF studies (9–11). In addition, the

postprandial plasma glucose-dependent insulinotropic polypeptide

concentration, which is correlated with the rate of intestinal glucose absorption (28), is reduced by intake of WG rye bread,
supporting the reduced intestinal absorption of glucose after WG

rye compared with refined wheat bread (9,29). Therefore, the

reduced delivery and intestinal absorption of glucose in WG rye

and similar postprandial blood levels shown by previous studies
could mean reduced mass flow of glucose from intestine to

periphery, which is consistent with our findings in the present

study regarding a shift in energy metabolism to catabolic status

after consumption of RP.

AMPK is a master energy sensor that regulates cellular

metabolism (30). Once activated by a deficit in energy and an

increase in the AMP:ATP ratio, or by pharmaceuticals (30), it

triggers catabolic pathways that produce ATP while turning off

anabolic energy-consuming pathways to restore the energy

balance (30,31). Activation of AMPK increases hepatic fatty

acid oxidation (30,31) and consequently increases 3-hydroxy-
ybutyric acid in plasma (32,33). Therefore, the shifts in energy

metabolism indicated by an increase in plasma ketone bodies in

the present study suggest the activation of AMPK after RP.

Activation of AMPK can cause phosphorylation and inhibition

of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acetyl-CoA

carboxylase, which are rate-limiting enzymes in cholesterol and

fatty acid biosynthesis. This may explain some of the beneficial

health effects of WG rye consumption, e.g., reducing cholesterol

and plasma lipids (34).

In the present study, no significant difference in participant

body weight was found between control and treatment, but a

2% reduction in body weight was observed for RP participants
during period 2 (P = 0.04; n = 8). However, no similar effect was

observed during period 1 or for the control during periods 1 and

2. This may partially explain the tendency for lower fasting

plasma leptin (P = 0.07) after intake of RP compared with WP. A

reduction in weight gain, adiposity, and fasting plasma leptin has

also been observed in C57BL/6J mice after intervention with

WG rye compared with WG wheat (34), which is consistent with

the findings in the present study. The reduction in adipocyte size

reported in previous animal (34) and human (35) experiments

after intervention with a WG rye diet is also consistent with our

findings suggesting increased fatty acid oxidation and a shift in

metabolism to catabolic status under RP treatment.

**Effect of rye intervention on ketone bodies.** Compared with

WP, RP caused an increase in the ketone bodies 3-hydroxybutyric

acid and acetone, which are known to increase under energy

starvation and fasting (26). We have found these compounds to be

the most discriminative metabolites between fasting and non-

fasting humans (data not shown). During fasting, FFA are released

from the adipose tissue and metabolized in the liver to the ketone

bodies (acetone, acetoacetate, and 3-hydroxybutyrate) through

ketogenesis (26). The increase in ketone bodies indicates a shift

from anabolic to catabolic status during energy starvation and

fasting, where the ketone bodies can act as energy sources (26).

The reduction in secretion of insulin (the most potent anabolic

hormone) observed in the present study after RP (5), and

previously in RF studies (9–11), is consistent with, and may

explain, the observed shift in energy metabolism. Juntunen et al.

(10) suggested that the lower postprandial insulin response to rye

bread could be explained by its mechanically firmer structure,

leading to obstructed amylolysis and therefore a slower rate of

glucose delivery and intestinal absorption. In insulin-dependent

diabetic patients (type 1) whose blood glucose level is not regulated

by insulin, WG rye bread consistently causes a lower glucose

response compared with mixed WG rye/wheat flour (50:50),

indicating a lower rate of intestinal glucose absorption after rye

bread (27). It is plausible that in healthy nondiabetic participants,

lower glucose absorption in rye is effectively regulated by lower

postprandial insulin response and thereby produces an equal rise

in plasma glucose levels after WG rye bread compared with refined

wheat bread, as shown in RF studies (9–11). In addition, the

postprandial plasma glucose-dependent insulinotropic polypeptide

concentration, which is correlated with the rate of intestinal glucose absorption (28), is reduced by intake of WG rye bread, supporting the reduced intestinal absorption of glucose after WG rye compared with refined wheat bread (9,29). Therefore, the reduced delivery and intestinal absorption of glucose in WG rye and similar postprandial blood levels shown by previous studies could mean reduced mass flow of glucose from intestine to periphery, which is consistent with our findings in the present study regarding a shift in energy metabolism to catabolic status after consumption of RP.

**Effect of RP on the metabolism of homocysteine and dimethyl sulfone.** WG and bran fractions are rich sources of betaine (36), which may explain the increased betaine level observed after RP in our study. An increase in betaine was previously reported in postprandial plasma collected from pigs fed a WG rye diet (37), but our study shows that it is measurable even in overnight fasting plasma. Betaine acts as a methyl donor in the betaine-homocysteine methyl transferase reaction, which converts homocysteine and betaine to methionine and N,N-
dimethylglycine (38). We also observed a reduction in homocysteine and an increase in plasma N,N-dimethylglycine after RP, which indicates a favorable shift in homocysteine metabolism. Elevated circulating homocysteine levels are an independent risk factor for cardiovascular diseases (39–41). Insulin suppresses the expression of betaine homocysteine methyl transferase and consequently reduces the rate of the betaine-
homocysteine methyl transferase reaction (42). Therefore, the favorable shift in homocysteine metabolism in our study could be explained by reduced insulin secretion (5) as well as higher bioavailability of betaine as reaction precursor after RP treatment.

We also observed an increase in the organic sulfur compound dimethyl sulfone after RP treatment. Dimethyl sulfone is a metabolite occurring in the plasma and cerebrospinal fluid of normal humans (43). It derives from dietary sources, from intestinal bacterial metabolism and human endogenous methanethiol metabolism (43). It is plausible that higher dimethyl sulfone is associated with a higher rate of intestinal fermentation after RP intake (16). SCFA are also the products of intestinal fermentation (44), but they were not detected in our NMR analysis, possibly because of their limit of detection in plasma.

**WG rye and PC.** Energy intake and metabolic status have been shown to affect prostate tumor growth (45), which is consistent with our findings in the present study. Shifting energy metabolism by targeting AMPK and fatty acid synthesis has been the strategic basis of recent pharmaceuticals developed for the treatment of PC (46). We also observed slightly lower fasting plasma leptin after RP compared to WP intake (P = 0.07). Our search on the human protein atlas indicated that prostate and PC tissues possess a higher interaction with leptin receptor anti-

bodies compared with other tissues in the body. The role of leptin in promoting PC progression has been investigated (47,48) and might be another mechanistic link between RP and reduced PSA.

A diet high in refined carbohydrates is associated with concomitant increases in insulin secretion, tumor growth, and activation of signaling pathways related to the insulin receptor in a murine model of PC (49). A reduction in signaling pathways related to the insulin receptor has been observed after modification of the diet with rye and pasta products (35). Therefore,
reduced insulin response in RP (5) could be one possible mechanism by which PC progression is alleviated.

In conclusion, this metabolomics study indicated a shift in energy metabolism toward catabolic status following intervention with RP compared with WP as a possible explanatory mechanism for RP functionality. The metabolic change observed might also occur in healthy humans and might partly explain the observed protective effects of WG products against lifestyle-associated diseases, i.e., cardiovascular disease, cancer, and diabetes. Our results also suggest a hypothetical explanatory mechanism for the RF based on changes in glucose mass flow from the intestine to peripheral tissues. The shift in energy metabolism observed in our study indicates changes in post-translation regulation of AMPK, 3-hydroxy-3-methyl-glutaryl-CoA reductase, and acetyl-CoA carboxylase, which have been individually associated with the development of chronic disease. The results obtained justify further investigation of the post-translational modulation of these proteins. Our findings concerning the shift in energy metabolism after RP should be validated in future studies. A similar metabolic shift happens when PC patients reduce their body weight as recommended by clinical routine (1). Our results also highlight the effects of RP on homocysteine metabolism as an important pathway associated with the development of chronic disease.

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