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A Whole-Grain–Rich Diet Reduces Urinary Excretion of Markers of Protein Catabolism and Gut Microbiota Metabolism in Healthy Men after One Week[1,2]

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Abstract

Epidemiological studies consistently find that diets rich in whole-grain (WG) cereals lead to decreased risk of disease compared with refined grain (RG)-based diets. Aside from a greater amount of fiber and micronutrients, possible mechanisms for why WGs may be beneficial for health remain speculative. In an exploratory, randomized, researcher-blinded, crossover trial, we measured metabolic profile differences between healthy participants eating a diet based on WGs compared with a diet based on RGs. Seventeen healthy adult participants (11 female, 6 male) consumed a controlled diet based on either WG-rich or RG-rich foods for 2 wk, followed by the other diet after a 5-wk washout period. Both diets were the same except for the use of WG (150 g/d) or RG foods. The metabolic profiles of plasma, urine, and fecal water were measured using ^{1}H-nuclear magnetic resonance spectroscopy and gas chromatography-mass spectrometry (plasma only). After 1 wk of intervention, the WG diet led to decreases in urinary excretion of metabolites related to protein catabolism (urea, methylguanadine), lipid (carnitine and acylcarnitines) and gut microbial (4-hydroxyphenylacetate, trimethylacetate, dimethylacetate) metabolism in men compared with the same time point during the RG intervention. There were no differences between the interventions after 2 wk. Urinary urea, carnitine, and acylcarnitine were lower at wk 1 of the WG intervention relative to the RG intervention in all participants. Fecal water short-chain fatty acids acetate and butyrate were relatively greater after the WG diet compared to the RG diet. Although based on a small population and for a short time period, these observations suggest that a WG diet may affect protein metabolism. J. Nutr. doi: 10.3945/jn.112.172197.

Introduction

Intake of whole-grain (WG)[4] foods has consistently been associated with a decreased risk of cardiovascular diseases (1,2), diabetes (3–5), and some cancers (6,7) as well as lower body fat (8) in both males and females and across diverse populations. Whereas many studies support the health benefits of WGs, especially for cardiovascular disease risk markers (9–15), others report inconclusive results (16,17), creating a degree of uncertainty around both the consistency of WG health benefits and what mechanisms may lie behind any observed benefits.

The proposed mechanisms of action behind potential WG health benefits have centered around dietary fiber and its effects on absorption, continence, and possible prebiotic effects and the greater amount of vitamins, minerals, and phytochemicals present in WG compared with refined grain (RG) foods (18–21). This picture, although plausible, is confounded by the wide variation in the composition of different WGs (22), though epidemiological studies support both mixed WG diets and specific grains as having a disease reduction effect (23) and fewer disease biomarkers can be observed in studies based on several different grain types (11,13). However, the possible mechanisms for the health benefits that could be common among cereal grains remain elusive.

[1] Author disclosures: All authors are employees of the Nestlé Research Center, part of the Nestlé company that produces a wide range of food and beverage products, including those containing whole grains.
[2] Supplemental Table 1, Supplemental Method Information 1, and Supplemental Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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[4] Abbreviations used: OPLS-DA, orthogonal projections on latent structures-discriminant analysis; RG, refined grain; TCA, tricarboxylic acid; TOFMS, time of flight mass spectrometry; WG, whole grain.
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To explore potential mechanisms of action of commercially available WG cereal foods on healthy human metabolism, we performed a cross-over study comparing a diet rich in WGs with the same diet but with WG foods replaced with RG foods. The metabolic profile of plasma, urine, and fecal water extracts was analyzed using a combination of proton ^{1}H-NMR spectroscopy and GC-time of flight MS (TOFMS) to obtain a global view of metabolic changes due to the WG and RG diets.

Materials and Methods

Study design

A randomized, researcher-blinded, cross-over study was designed to compare the global metabolic effects of a diet rich in WG with a diet rich in RG, as previously described in detail (13). The study design is outlined in **Figure 1**. This study was conducted in Lausanne, Switzerland according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants/patients were approved by the Ethical Committee of the Lausanne Region, Vaud Canton, Switzerland (Protocol 178/07). The study was carried out from February 2008 to December 2008. This was an exploratory trial with metabolic profiling as the primary outcome, so no power calculations were possible. The target number of participants to recruit was 15–25 based on previous cross-over intervention trials on WGs (10,11).

Subjects

A total of 22 participants were recruited into the study. Written informed consent was obtained from each participant and selection took place following a medical questionnaire and confirmation of adherence to inclusion/exclusion criteria [healthy (normal blood lipid panel, BMI 19–28 kg/m^2, no chronic or recent illness, no recent use of antibiotics or medication, nonsmokers), between 20 and 50 y old, and low habitual WG intake (<30 g/d, as determined by FFQ (24)].

Study foods

Foods used in the study were all commercially available from Nestlé SA worldwide or purchased from supermarkets (Lausanne, Switzerland and

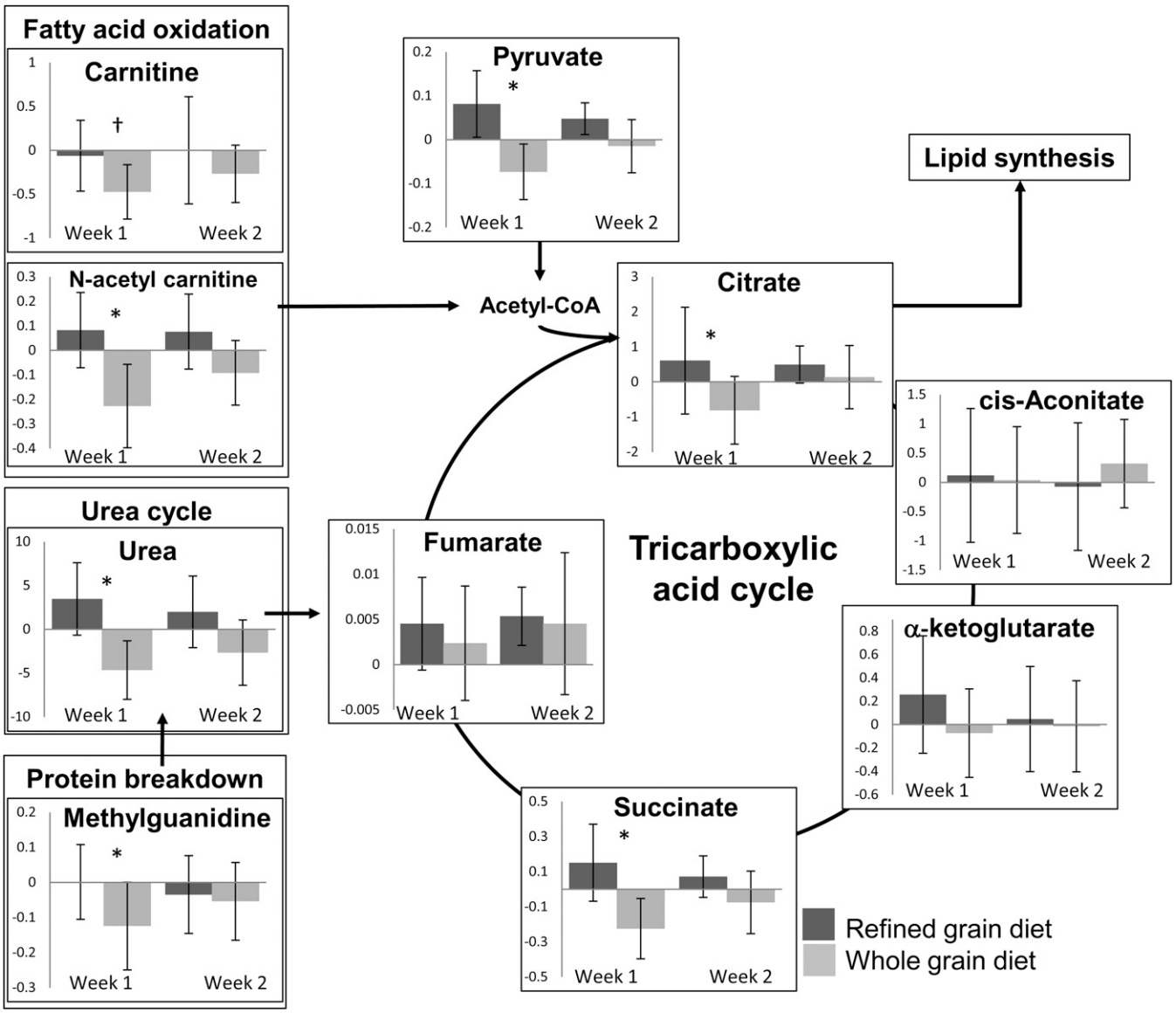

FIGURE 1 Overview of changes to central energy metabolism in healthy men consuming WG- and RG-rich diets for 2 wk in a randomized crossover trial. Data are based on urinary metabolite excretion measured by NMR. Significant differences were determined using the General Linear Model including diet and diet order as factors and subject and baseline values as covariates. Bars are the mean change from baseline for each diet ± SD of the NMR peak height/creatinine peak height, $n = 6$. Symbols indicate that interventions differ at that time: $^{\dagger}0.05 \leq P \leq 0.10$; $^{*}P < 0.05$. RG, refined grain; WG, whole grain.

Huskvarna, Sweden). WG, energy, protein, fat, carbohydrate, and fiber content were determined from the declared ingredient and nutrient compositions. The WG component of foods was assumed to have all 3 major anatomical fractions of the cereal grain (bran, germ, and endosperm) in the same proportions found naturally as per the American Association of Cereal Chemists definition (25). These diets have been described in detail in a previous publication (13).

Analytical methods

Sample collection. Plasma for metabolomics analysis was collected on lithium heparin-coated tubes and separated from blood by centrifugation at 1000 × *g* for 10 min at 4°C. Then 24-h urine collections were made and aliquots were stored at −80°C until analysis. Fecal water was produced according to the method of Klindler et al. (26). Fecal water is the liquid component of feces and is rich in gut microbiota-related metabolites as well as nonabsorbed food components. All samples were stored at −80°C.

Metabolic profiling. Baseline (after the initial 1-wk non-WG diet washout period prior to each intervention period), intervention (after 1 wk and 2 wk), and postintervention non-WG diet (wk 1 and 2 after the end of the intervention) plasma and urine samples were analyzed by NMR. GC-MS was used to analyze the same time points for plasma only. Fecal water samples from baseline and at the end of each 2 wk intervention periods were analyzed by NMR.

^{1}H-NMR spectroscopy. Plasma, urine, and fecal water were analyzed using standard methods (27) on a Bruker Avance III 600 MHz spectrometer equipped with a 5-mm inverse probe (Bruker Biospin). NMR profiling generates a spectrum with 22,000 data points, from which ~50, 80, and 100 metabolites can be identified for plasma, urine, and fecal water, respectively. Only those peaks highlighted as explaining differences in multivariate models were investigated for identification. Peak height for these metabolites was used for further data analysis. Details of sample preparation and NMR settings are provided in the Online Supporting Material (Supplemental method information).

GC-TOFMS. GC-TOFMS profiling of plasma samples was carried out using the method of A et al. (28) adapted for GC-TOFMS (Leco Pegasus III) (29). This method separates 303 individual metabolites, with positive identification of 70 metabolites.

Statistical analyses. Differences in metabolites between diet periods were analyzed using multivariate statistical analysis to guide further univariate statistical analysis, which was used to determine the outcomes of this study. This approach has the advantage of using multivariate statistics to pick out potential metabolite changes from the hundreds of metabolites/features measured and then using the well-defined paradigms of univariate statistical methods that are more easily comparable with nonmetabolomic studies. Both multivariate and univariate modeling included all time points available for each biofluid, though reported comparisons focused on differences between the diets at the 2 intervention time points, as the goal was to compare WG and RG diets rather than changes over time for either diet period.

Multivariate statistical analyses were performed using Principal Components Analysis and Orthogonal Projections on Latent Structures-Discriminant Analysis (OPLS-DA) with SIMCA software (Umetrics). OPLS-DA is a modification of partial least squares modeling where the systematic variation in the *x* matrix not correlated to the *y* matrix is removed (30). Random Forests analysis (31) was performed on GC-TOFMS datasets using the package RandomForest (32) in R (33). In-house Matlab (The Mathworks) routines were used for data importation and preprocessing. Discriminant and quantitative models controlled for over-fitting and rejected if this was evident. The robustness of the models tested was determined using the Q^2_Y value from SIMCA. Further modeling of potentially discriminating features OPLS-DA modeling was used to guide further univariate analysis, and potentially discriminating features were then quantified and analyzed using the general linear model (Minitab 15.0). Gender, diet, and diet order were included as factors in the model and subject and baseline values included as covariates. Where gender was a significant covariate, both genders were analyzed using the same model (excluding gender). Differences between groups for diet were determined using Tukey's pairwise comparisons and these *P* values are reported. Only differences between diets at the same time point (i.e., wk 1 or 2) are reported. Data are presented as normalized values (either to internal standard for GC-MS, total spectral intensity for NMR profiling of fecal water, or value relative to creatinine peak height for NMR profiling of urine).

Values from metabolic profiling are reported as mean (95% CI) based on normalized peak areas (GC-TOFMS) or spectral peak heights (NMR). Unadjusted P values < 0.05 are reported as indicating potentially interesting results, based on the International Conference of Harmonization E9 guidelines (34). As this type of exploratory trial was designed to be hypothesis generating rather than aiming to prove a previous hypothesis, no correction for multiplicity was applied during statistical analyses. Trends (P = 0.05–0.1) are also reported. All metabolic changes reported are comparisons between WG and RG interventions at the same time point, after correction for baseline values prior to each intervention period.

Results

Of the 22 participants recruited into the study, 17 completed the study. Only samples from participants who completed the study were included in subsequent analyses. Participant dropouts during the study were due to an inability to comply with the controlled diet (1 participant), conflicts with work commitments (3 participants), and unrelated illness (1 participant) (13). The clinical chemistry results of this study were previously reported (13) and this work focuses on differences in the metabolite profiles of systemic biofluids and feces. There was a small difference in carbohydrate intake (P < 0.05) due to the lower relative amount of carbohydrate in WGs. Substitution of RGs for WGs was done a weight for weight basis, so this relative difference is reflected in overall intake. Dietary fiber also differed between the WG and RG interventions (**Supplemental Table 1**).

Plasma. Based on Principal Components Analysis and OPLS-DA modeling, ^{1}H-NMR–based metabolic profiles of plasma did not differ between the 2 diet periods. GC-TOFMS plasma profiling detected that at wk 2 only, the plasma urea concentration was greater during the WG intervention than during the RG intervention (**Table 1**).

Urine. Multivariate modeling of urine ^{1}H-NMR analyses indicated that gender was a key determinant of the models obtained, so male and female participants were analyzed separately. Two cross-validated OPLS-DA models for the WG compared with the RG intervention periods were generated for ^{1}H-NMR analysis of urine from men at 1 wk and at 1 and 2 wk combined (Q^2_Y = 0.28 and 0.3, respectively). These Q^2_Y values are within the range normally found in nutrition studies. In all participants, there was less excretion of carnitine, acetylcarnitine, urea, and taurine at wk 1 and no differences observed at 2 wk (Table 1). The only additional diet-related metabolic change in women was an increase in fumarate at wk 1, a difference not observed in men. In men, the WG diet led to less excretion of 4-hydroxyphenylacetate (gut microbiota metabolite of aromatic amino acids), dimethylamine and trimethylamine (gut microbiota processing of choline and related precursors), and methylguanadine (a metabolite of protein catabolism), pyruvate, citrate, succinate (central carbon metabolism intermediates), 3-hydroxyisovalerate (metabolite of leucine metabolism and indicator of biotin status), and N-acetyl-glycoproteins (unclear

TABLE 1 Metabolite concentrations in plasma and 24-h urine and fecal water samples from healthy adults consuming WG- and RG-rich diets each for 2 wk[1]

Metabolite	Analytical method	Gender, *n*	RG	WG	*P*
Plasma					
Wk 2					
Protein metabolism/kidney function					
Urea	GC-MS	B (17)	0.41 (0.36, 0.46)	0.58 (0.45, 0.71)	0.05
Urine					
Wk 1					
Mitochondrial β-oxidation and breakdown of carnitine; transport of fatty acids across the mitochondrial membrane					
Carnitine	NMR	B (17)	1.02 (0.86, 1.18)	0.80 (0.66, 0.94)	0.06
N-acetylcarnitine	NMR	B (17)	0.75 (0.64, 0.81)	0.63 (0.56, 0.71)	0.03
Bile acid synthesis					
Taurine	NMR	B (17)	2.46 (1.8, 3.12)	1.78 (1.48, 2.08)	0.01
Gut microbiota metabolism of aromatic amino acids					
4-hydroxyphenylacetate	NMR	B (17)	0.31 (0.27, 0.34)	0.34 (0.28, 0.40)	0.93[2]
		F (11)	0.34 (0.29, 0.40)	0.40 (0.33, 0.48)	0.42
		M (6)	0.27 (0.18, 0.35)	0.22 (0.19, 0.25)	0.05
Gut microbial metabolism of dietary precursors (e.g., choline, carnitine)					
Dimethylamine	NMR	B (17)	1.08 (0.96, 1.20)	0.95 (0.85, 1.05)	0.06[2]
		F (11)	1.09 (0.93, 1.26)	1.04 (0.93, 1.16)	0.81
		M (6)	1.06 (0.81, 1.30)	0.78 (0.71, 0.84)	0.02
Trimethylamine	NMR	B (17)	0.45 (0.36, 0.55)	0.40 (0.32, 0.48)	0.60[2]
		F (11)	0.50 (0.40, 0.60)	0.47 (0.39, 0.56)	1.00
		M (6)	0.37 (0.15, 0.59)	0.26 (0.20, 0.33)	0.03
Protein metabolism/nitrogen balance, increased degradation of branched chain amino acids and/or increased flux through the urea cycle					
Urea	NMR	B (17)	18.9 (15.7, 22.1)	13.9 (11.8, 16.0)	<0.01[2]
		F (11)	21.0 (16.5, 25.5)	16.1 (14.0, 18.2)	0.05
		M (6)	14.9 (12.4, 17.5)	9.86 (7.49, 12.2)	0.01
Product of protein catabolism					
Methylguanadine	NMR	B (17)	0.53 (0.46, 0.59)	0.49 (0.43, 0.55)	0.60[2]
		F (11)	0.55 (0.47, 0.63)	0.55 (0.50, 0.61)	1.00
		M (6)	0.48 (0.35, 0.62)	0.37 (0.35, 0.39)	0.05
Muscle energy metabolism (conversion of creatine to creatinine)					
Creatinine	NMR	B (17)	9.67 (9.64, 9.71)	9.72 (9.69, 9.74)	0.02[2]
		F (11)	9.67 (9.63, 9.71)	9.71 (9.68, 9.74)	0.72
		M (6)	9.68 (9.60, 9.75)	9.73 (9.70, 9.77)	0.01
Central energy metabolism (TCA cycle)					
Pyruvate	NMR	B (17)	0.45 (0.39, 0.51)	0.40 (0.35, 0.46)	0.38[2]
		F (11)	0.47 (0.39, 0.55)	0.38 (0.33, 0.43)	1.00
		M (6)	0.41 (0.30, 0.52)	0.30 (0.28, 0.33)	<0.01
Citrate	NMR	B (17)	4.89 (4.14, 5.64)	4.66 (3.53, 5.79)	0.89[2]
		F (11)	5.25 (4.29, 6.23)	5.51 (4.00, 7.02)	0.98
		M (6)	4.21 (2.81, 5.61)	3.10 (2.17, 4.03)	0.04
Succinate	NMR	B (17)	0.72 (0.59, 0.85)	0.68 (0.53, 0.82)	0.78
		F (11)	0.74 (0.58, 0.91)	0.82 (0.65, 0.99)	0.92
		M (6)	0.68 (0.42, 0.94)	0.41 (0.36, 0.46)	0.03
Fumarate	NMR	B (17)	0.25 (0.20, 0.30)	0.31 (0.26, 0.37)	0.13[2]
		F (11)	0.28 (0.22, 0.34)	0.37 (0.31, 0.43)	0.02
		M (6)	0.20 (0.12, 0.29)	0.21 (0.17, 0.24)	0.98
Diverse range of glycoproteins with excretion possibly resulting from differences in kidney function					
N-acetyl-glycoproteins	NMR	B (17)	0.97 (0.84, 1.10)	0.86 (0.74, 0.98)	0.21[2]
		F (11)	1.03 (0.87, 1.18)	0.99 (0.87, 1.11)	1.00
		M (6)	0.87 (0.59, 1.15)	0.61 (0.56, 0.67)	<0.01

(Continued)

TABLE 1 *Continued*

Metabolite	Analytical method	Gender, *n*	RG	WG	*P*
Metabolic product of leucine and biotin metabolism					
3-hydroxyisovalerate	NMR	B (17)	0.48 (0.40, 0.57)	0.43 (0.35, 0.51)	0.30[2]
	NMR	F (11)	0.51 (0.39, 0.64)	0.49 (0.39, 0.59)	0.98
	NMR	M (6)	0.43 (0.33, 0.53)	0.32 (0.26, 0.38)	0.03
Fecal water					
Microbial fermentation of carbohydrates					
Acetate	NMR	B (17)	660.2 (559.4, 761.0)	738.3 (619.4, 857.1)	0.02
Butyrate	NMR	B (17)	56.5 (42.4, 70.6)	66.6 (48.3, 84.9)	0.05
Metabolism of nicotinic acid (niacin)					
Nicotinurate	NMR	B (17)	0.94 (0.83, 1.06)	1.18 (1.02, 1.34)	0.02
Microbial fermentation of peptides and amino acids					
Isovalerate	NMR	B (17)	42.8 (37.4, 48.3)	37.7 (34.2, 41.2)	0.04
Microbial fermentation of diverse compounds (e.g., amino acids and SCFAs)					
Succinate	NMR	B (17)[2]	0.49 (0.46, 0.53)	0.43 (0.39, 0.48)	0.01[2]
		F (11)	0.51 (0.47, 0.55)	0.47 (0.43, 0.50)	0.04
		M (6)	0.46 (0.39, 0.53)	0.37 (0.28, 0.46)	0.11

[1] Mean values (95% CI) based on peak area (GC-MS) or peak height (^{1}H-NMR). GC-MS values are peak areas normalized to internal standard (methyl stearate). NMR values for fecal water are peak heights normalized to total spectral intensity and multiplied by 1×10^7; and for urine are peak heights normalized to the creatinine peak height, except for creatinine, which is based on absolute peak height. *P* values are for the metabolite differences between the two diets accounting for gender, diet order, baseline values, and subject. 95% CI values may overlap for significant differences as the model accounts for the crossover design (based on intra-individual differences in response to the diets, rather than mean differences). Where gender was a significant covariate, both genders were analyzed separately using the same model (excluding gender) and results were reported separately. B, both genders; F, females only; M, males only; RG, refined grain; TCA, tricarboxylic acid; WG, whole grain.
[2] Gender also a significant covariate.

what these may indicate in healthy participants) and an increase in creatinine (muscle metabolism).

These results suggest that changes after 1 wk of the WG diet were the key drivers for the combined wk 1 and 2 model. Univariate analysis of key ^{1}H-NMR measured urinary metabolites from women confirmed the multivariate analysis result that there were limited differences in the urinary metabolic profile due to a greater inter-subject variability, possibly related to different phases of the menstrual cycle (35) (not controlled in this study).

Fecal water. Metabolites in fecal water were profiled using ^{1}H-NMR spectroscopy and a significant OPLS-DA model was obtained for a difference between the two dietary periods for all subjects (Q^2_Y = 0.35) (Table 1). The WG diet was associated with a greater excretion of nicotinurate, acetate, and butyrate and a lower excretion of isovalerate. The succinate concentration of fecal water was lower during the WG diet period only in women.

Discussion

The metabolic changes due to a WG diet compared with a RG diet suggest that a WG diet may affect a variety of different pathways, including protein, lipid, and microbial metabolism. The urinary excretion of tricarboxylic acid (TCA) cycle intermediates suggests a reduction of energy flux through the TCA cycle from glycolysis (lower concentrations of pyruvate indicate lower conversion of pyruvate to acetyl-CoA). Cis-aconitate and α-ketoglutarate behaved similarly, though not different, between diet periods, possibly due to greater variability. Succinate was different at wk 1 as were pyruvate and citrate. One reason for an overall lower flux through the TCA cycle could be the slightly lower amount of carbohydrate consumed during the WG diet. Replacement of RGs for WGs was done on a weight for weight basis, as would be likely under everyday conditions, rather than matching for macronutrients. This led to a 6 and 7% lower carbohydrate intake during the WG diet intervention in men and women, respectively, corresponding to the difference in carbohydrate composition between RGs and WGs, with no difference in fat or protein intake. Differences in food structure may have also played a role in energy utilization, though all foods were processed in a similar manner, reducing the chance for major differences in food structure between similar RG and WG foods.

The increase in urinary excretion of creatinine after consuming the WG diet may support the change to protein metabolism being reflected in lean mass, given that excretion is normally stable; however, there were no anthropometric data collected in this study to back this up. Three studies have indicated that a WG diet can alter body composition (reduce body fat or hip circumference) without greater weight loss than a RG diet (36–38), suggesting that lean mass may be altered by a WG diet. This apparent change in protein metabolism cannot be explained by gross differences in protein intake, as this was the same during both diet periods and most protein was from meat and dairy intake rather than cereals. Prebiotic fiber sources have been found to reduce urinary N excretion during a 24-h period after ingestion (39), and rats fed WG millet had a lower 24-h urinary urea excretion compared with controls (40), pointing to other fermentable foods having a possible effect of N balance in humans. This potential effect of WGs on protein metabolism needs to be assessed using stable isotope methods for measuring protein turnover before clear conclusions can be made.

Fumarate was the only urinary metabolite that significantly differed between diet periods for women, but not for men. Fumarate has been previously found to differ between genders in metabolic profiling (41), suggesting that enzymes/metabolites close to fumarate respond differently to the 2 diets due to gender. The differing response compared with other TCA-cycle metabolites may be due to a difference in flux through gluconeogenesis

(malate and oxaloacetate are key initial intermediaries in this pathway), tyrosine metabolism, or an increased flux through the urea cycle.

The lower urinary excretion of carnitine and acetylcarnitine during the WG diet points to an effect on lipid metabolism, possibly associated with the decrease in circulating LDL cholesterol (13). Increased urinary excretion of acetylcarnitine is associated with extended fasting in healthy humans as well as type 2 diabetics (42), implying that the WG diet may decrease fat oxidation during fasting periods.

The observed changes in fecal microbiota composition (13) appeared to be reflected in the urinary metabolic profile via a lower urinary excretion of 4-hydroxyphenylacetate, a colonic metabolite of tyrosine and phenylalanine breakdown by enterobacteria *Clostridium perfringens* and *C. difficile* respectively (43,44). Changes to trimethylamine and dimethylamine excretion in urine are also likely to be of microbial origin, possibly due to a difference in substrate availability between the 2 diets (45) *Clostridium leptum*, a preferential carbohydrate-fermenting bacteria, increased during the WG diet (13) at the same time as protein-derived gut microbiota metabolites decreased, suggesting that more carbohydrate was available in the distal colon, reducing the use of protein as a substrate (46). The WG diet lowered fecal excretion of isovalerate, a product of metabolism of amino acids by microbiota and previously found to decrease during diets high in fermentable carbohydrates (47). These changes support the hypothesis that the WG diet leads to a shift toward carbohydrate as the main fermentation substrate. Previous studies have found that a WG diet may modulate fecal microbiota in humans (21,48), though this is yet to be linked to functional changes outside of increased fecal content of SCFAs.

Previous studies using metabolomics to understand global metabolic changes due to WG-based diets have generally found only limited changes to plasma metabolites [phosphatidylcholines (49), DHA (50), TGs, and ribose metabolites (51)] and 3-hydroxybutyric acid and acetone (52). Our study did not replicate these findings and 2 wk may also have been too short a time to observe major metabolic changes due to the intervention diets. However, 48 h was sufficient to reestablish metabolic equilibrium after switching from a RG to a WG diet in rats (53) and some differences between gut microbiota populations were discernible after 2 wk (13), indicating that some global metabolic changes start to be apparent within a relatively short time frame. These previous WG metabolomics studies used WG diets based on rye, 3 used unhealthy populations (prostate cancer and metabolic syndrome), the other in postmenopausal women, whereas the present study was based on mixed WG sources (predominantly wheat) in healthy adults.

The present study was designed as an exploratory study, and hence a relatively small population was used. Possibly due to this fact, most results were only found after 1 wk in men, which limits their generalization across a wider population. The results do suggest that in healthy individuals, metabolic equilibrium is reestablished within 14 d of the dietary change, which matches some earlier results from this study (e.g., plasma betaine concentration no longer significantly differed after 2 wk) (13), though not all results (trend for greater cholesterol reduction after 2 wk). Study adaptation or fatigue may be another explanation for different results between wk 1 and 2, though compliance measured by diaries, food weighbacks, and plasma alkylresorcinols did not suggest any difference between wk 1 or 2 during either dietary period. The small sample size would also make our study underpowered for finding smaller metabolic changes, though we can have some confidence in those that we have observed, as more than one metabolite in related pathways were similarly altered (e.g., for protein, central energy, and microbial metabolism). A follow-up study would need to address these weaknesses by having a larger sample size and equal gender distribution and preferably be carried out over a longer time period.

In conclusion, a diet based on WG cereal products appears to affect protein catabolism in men, a possible mechanism of action for WG health effects that hitherto has not been suggested. Other areas of metabolism that were apparently different between a WG and RG diet include central energy metabolism, lipid oxidation, and microbial metabolism. It is possible that improved regulation of energy metabolism plays a key role in the observed changes, possibly mediated by the inherently lower carbohydrate content of the WG diet. Further work using fully quantitative analyses of the biomarkers highlighted here and stable isotope studies to investigate underlying changes to energy flux are needed to confirm these metabolomics-generated hypotheses for WG benefits on health.

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