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Whole Grain Wheat Consumption Affects Postprandial Inflammatory Response in a Randomized Controlled Trial in Overweight and Obese Adults with Mild Hypercholesterolemia in the Graandioos Study

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ABSTRACT

Background: Whole grain wheat (WGW) consumption is associated with health benefits in observational studies. However, WGW randomized controlled trial (RCT) studies show mixed effects.

Objectives: The health impact of WGW consumption was investigated by quantification of the body's resilience, which was defined as the "ability to adapt to a standardized challenge."

Methods: A double-blind RCT was performed with overweight and obese (BMI: 25–35 kg/m^2) men (n = 19) and postmenopausal women (n = 31) aged 45–70 y, with mildly elevated plasma total cholesterol (>5 mmol/L), who were randomly assigned to either 12-wk WGW (98 g/d) or refined wheat (RW). Before and after the intervention a standardized mixed-meal challenge was performed. Plasma samples were taken after overnight fasting and postprandially (30, 60, 120, and 240 min). Thirty-one biomarkers were quantified focusing on metabolism, liver, cardiovascular health, and inflammation. Linear mixed-models evaluated fasting compared with postprandial intervention effects. Health space models were used to evaluate intervention effects as composite markers representing resilience of inflammation, liver, and metabolism.

Results: Postprandial biomarker changes related to liver showed decreased alanine aminotransferase by WGW (P = 0.03) and increased β-hydroxybutyrate (P = 0.001) response in RW. Postprandial changes related to inflammation showed increased C-reactive protein (P = 0.001), IL-6 (P = 0.02), IL-8 (P = 0.007), and decreased IL-1B (P = 0.0002) in RW and decreased C-reactive protein (P < 0.0001), serum amyloid A (P < 0.0001), IL-8 (P = 0.02), and IL-10 (P < 0.0001) in WGW. Health space visualization demonstrated diminished inflammatory (P < 0.01) and liver resilience (P < 0.01) by RW, whereas liver resilience was rejuvenated by WGW (P < 0.05).

Conclusions: Twelve-week 98 g/d WGW consumption can promote liver and inflammatory resilience in overweight and obese subjects with mildly elevated plasma cholesterol. The health space approach appeared appropriate to evaluate intervention effects as composite markers. This trial was registered at www.clinicaltrials.gov as NCT02385149. *J Nutr* 2019;149:2133–2144.

Keywords: whole grain wheat, phenotypic flexibility, composite biomarkers, challenge test, metabolic health, inflammation, liver, resilience, (compromised) healthy subjects

Introduction

In meta-analyses of prospective studies, high whole grain intake has been acknowledged for its potential role in lowering risk of type 2 diabetes, cancer, respiratory system disorders, and cardiovascular disease (1–3). It remains unclear, however, how these effects are mediated. Refined flours in contrast to whole

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grains mainly contain the grain kernel's endosperm as they lose their bran and germ fractions by milling and sifting processes. The bran and germ contain many micronutrients, bioactive compounds, and dietary fiber, which may contribute to lowering the risk for the aforementioned related diseases (4).

Although epidemiological studies indicate beneficial effects of whole grain intake on the risk of total mortality, incident coronary artery disease, and metabolic risk factors (BMI, waist-hip ratio, total and LDL cholesterol, and insulin sensitivity) (5–7), some studies with whole grains as an intervention have shown no effect at all (8–11), or have reported inconsistent results for improvement of glucose (12–15) and/or lipid metabolism (15–17). The discrepancy between observational and intervention studies could be a result of a small effect (lack of power), large inherent differences in intervention response, a rather healthy sample population (more difficult to find improvements), or a short intervention duration (small and heterogeneous effects of intervention). Therefore, it is of importance to develop more sensitive measures to quantify health effects of specific foods such as whole grain.

Traditionally, health effects are measured by a few single biomarkers measured after overnight fasting before and after a nutritional intervention. Lately "health" has been redefined as "the ability to adapt or cope with ever changing environmental conditions," instead of merely the absence of disease or infirmity (18). In the context of metabolic health, the ability to adapt homeostasis to external stressors has been termed phenotypic flexibility or "resilience" (19). Resilience has been quantified by measuring the response in time of a set of biomarkers to a homeostatic stressor such as a standardized meal, temperature change, or physical activity (19–22). For this purpose a standardized nutritional challenge test, called the PhenFlex challenge test (PFT) has been developed. This high caloric mixed-meal challenge test induces a subtle systemic metabolic response for evaluation of health status (20, 23), which allows a sensitive assessment of (individual) health and nutritional intervention effects based on the integrated panel of biomarkers belonging to the same biological process (23). The creation of such a composite biomarker can be accomplished by applying a "health space" model as proposed by Bouwman et al. (24). The health space model can be used to visualize the integrated responses of multiple biomarkers representing a specific health domain such as "inflammation," "liver," and "metabolism" according to a reference population. Subjects of the reference population typically represent the extremes within the healthy range of the population, such as an optimal healthy population (young and lean) and a compromised health group (old and overweight) defined according to the health aspect of interest (23). This allows an objective evaluation of the intervention effect, which is considered beneficial or detrimental when, after intervention, the participants' scores are closer situated to the optimal healthy reference group or closer to the compromised group, respectively. Complementary to our recent work where it was shown that whole grain wheat (WGW) protects against hepatic fat accumulation (25), we now aim to expand our analysis in the Graandioos study by investigating the postprandial response more in depth to deliver a first proof of concept that the application of the PFT (20, 23) in conjunction with a health space approach is able to substantiate subtle beneficial health effects from WGW, as opposed to evaluating traditional single biomarkers in overnight fasting conditions.

Methods

Subjects

A total of 50 middle-aged (45–70 y) overweight and obese (BMI between 25 and 35 kg/m^2) men (n = 19) and postmenopausal women (n = 31) with mildly elevated concentrations of total cholesterol (>5 mmol/L) were recruited. The inclusion of participants with an elevated risk profile of cardiovascular disease increases the chance of finding nutrition-induced beneficial health effects. Having a history of medical or surgical events that may affect study outcome, smoking, use of cholesterol-lowering medication, having an aversion or intolerance to gluten, whole wheat, or other items in the intervention were used as exclusion criteria. The experimental protocol and procedures were approved by the Medical Ethical Committee of Wageningen University and in accordance with the Helsinki declaration of 1975 as revised in 1983. The study was registered at clinicaltrials.gov as NCT02385149. All participants gave their written consent before participation.

Study design

The Graandioos study was a randomized double-blind parallel trial. Subjects were aligned on a 4-wk refined wheat (RW) run in diet as whole grain consumption in the Netherlands is relatively high [for details (25)]. Next, participants were randomly assigned to a 12-wk (colored) RW or WGW intervention. The WGW intervention contained 98 g of whole grain or refined wheat per day in the form of bread and ready to eat cereals [for details (25)]. Subjects were stratified among the intervention groups based on age, gender, and plasma total cholesterol concentration. Furthermore, subjects maintained their body weight during the 12-wk intervention. The primary outcome of this study was to investigate the health benefits of WGW on cardiometabolic health by means of applying a mixed-meal challenge with plasma total cholesterol, glucose and insulin concentrations, plasma markers of cardiovascular health, glucose metabolism, and liver and adipose tissue health as main study endpoints. For more details on the study design see (25), **Supplemental Methods**, **Supplemental Figure 1**, and **Supplemental Table 1**.

Nutritional PFT

Responses to the PFT were measured at the beginning and end of the 12-wk intervention period. The PFT was a high-fat, high-glucose, high-caloric drink (400 mL, 950 kcal) consisting of 320 mL tap water, 60 g palm oleine, 75 g dextrose, 20 g protein supplement, and 0.5 g artificial vanilla aroma as previously described (20, 23, 25, 26). The participants were instructed to consume the PFT within 5 min.

Plasma samples were taken before (t = 0, under fasting conditions) and after consumption of the drink (t = 30, 60, 120, 240 min). No food or beverages were allowed during the 4-h time course except for water.

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Supplemental Methods, Supplemental Figures 1 and 2, and Supplemental Tables 1–7 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: AUCt, total AUC; CRP, C-reactive protein; GGT, γ-glutamyltransferase; NEFA, nonesterified fatty acid; PFT, PhenFlex test or standardized mixed-meal tolerance test; RCT, randomized controlled trial; RW, refined wheat; SAA, serum amyloid A; sICAM, secreted intercellular adhesion molecule-1; sVCAM, secreted vascular adhesion molecule-1; TG, triglyceride; WGW, whole grain wheat.

TABLE 1 Formulas for calculating indices of insulin sensitivity[1]

Index	Formula	Reference
Matsuda index	$\frac{10000}{\sqrt{(fasting\ G * fasting\ I)(mean\ G * mean\ I)}}$	(27)
Insulinogenic index	$\frac{30\ \text{min}\ insulin - fasting\ insulin}{30\ \text{min}\ glucose - fasting\ glucose}$	(28)
HOMA-IR	$\frac{I0 * G0}{22.5}$	(29)
HOMA-B	$\frac{(20 * FPI)}{(FPG - 3.5)}$	(30, 31)
Disposition index	$[AUC_{30\text{min insulin}}/AUC_{30\text{min glucose}}] \times$ Matsuda	(32)
Hepatic insulin resistance index	fasting glucose × fasting insulin	(27)
Muscle insulin sensitivity index	$\frac{(\frac{\Delta G}{\Delta t})}{mean\ plasma\ insulin}$	(33)

[1] FPG, fasting plasma glucose; FPI, fasting plasma insulin; G, glucose; I, insulin; t, time.

On the evening before each test day, subjects consumed a standardized low-fat meal and were asked to refrain from alcohol or exercise.

Clinical chemistry and inflammatory marker measurements

The following biomarkers were assayed with use of an Atellica CH analyzer in combination with Atellica Solution tests (Siemens Healthcare Diagnostics Products) in all plasma samples: alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), glucose, insulin, total cholesterol, HDL cholesterol, and triglycerides (TGs). Plasma nonesterified fatty acids (NEFAs) were determined with use of an enzymatic method (Instruchemie). Plasma concentrations of β-hydroxybutyrate were analyzed with use of a colorimetric assay (Stichting Huisartsenlaboratorium Oost Velp).

Multiplexed immunoassays were used for quantification of 5 inflammatory proteins in plasma: IL-1B, IL-6, IL-8, IL-10, and TNFα (custom made Multiplex Panel Human Proinflammatory; Meso Scale Discovery) and of 8 vascular proteins: C-reactive protein (CRP), secreted intercellular adhesion molecule 1 (sICAM1), secreted vascular adhesion molecule 1 (sVCAM1), and serum amyloid A (SAA) (Multiplex Panel Human Vascular Injury II; Meso Scale Discovery), and E-selectin, P-selectin, ICAM3, and thrombomodulin (Multiplex Panel Human Vascular Injury I; Meso Scale Discovery). Finally, a custom made multiplex was used for 3 glucose-related proteins: glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1, and glucagon (Meso Scale Discovery).

Postprandial blood pressure (systolic and diastolic) and augmentation index

All vascular measurements were performed after 10 min of rest. Brachial systolic blood pressure, diastolic blood pressure, and heart rate were assessed automatically (DINAMAP PRO 100) for 10 min with a 3-min interval, heart rate corrected augmentation index, a measure of wave reflection and arterial stiffness, were assessed by pulse wave analysis of the radial artery (SphygmoCor CP system, ATcor Medical) as described previously (34).

Calculations of insulin indices

We used several indices derived from fasting state and PFT to evaluate insulin sensitivity, formulas are shown in **Table 1** (27–33).

Statistical analysis

AUCs were calculated for all PFT measurements with use of the trapezoidal method with a subtraction correction for fasted measurements in all subsequent measures. Missing data points were excluded. The absolute sum of the areas below and above the fasted value was defined as total AUC (AUCt). The following features were analyzed by linear mixed models:

1) Overnight fasting concentrations of biomarkers ($t = 0$) with "treatment" (WGW/RW), "week" (0 compared with 12) and their interactions as fixed effects, and "subject" as random factor;
2) Glucose-related and insulin-related indices with "treatment" (WGW/RW), "week" (0 compared with 12) and their interactions as fixed effects, and "subject" as random factor;
3) AUCt for the PFT measurements with "treatment" (WGW/RW), "week" (0 compared with 12) and their interactions as fixed effects, and "subject" as random factor;
4) PFT response curves were made by linear mixed models for repeated measures with "treatment," "week," and "postprandial measurement timepoints" ($T = 0$ min, $T = 30$ min etc.) and their interactions as fixed factors, and "subject" as a random factor.

For all linear mixed models, statistical outliers, defined as an observation having an absolute residual >3 times root mean square error of the model, were removed. Plots of residuals compared with the corresponding fitted values were inspected. If these plots revealed a residual variation that increases with the fitted value, the data were transformed by taking their natural logarithm on the original data set. Only interaction effects are reported where a 2-tailed value of $P < 0.05$ was considered significant; main effects of the fasting values, indices, AUCt, and PFT response curves are presented in **Supplemental Tables 2–5**, respectively. To further identify if the observed interaction effect was induced by the WGW and/or RW intervention, post hoc analysis (2-sided Student t test) was performed where a 2-tailed value of $P < 0.05$ was considered significant.

Statistical analyses were performed with the software package SAS, version 8.2 (SAS Institute). Means ± SDs were calculated for plasma variables.

Data integration into a health space

A health space model was developed, adapted from the original principle of Bouwman et al. (24). In short, we used 2 reference groups based on data from a previous study (23) from which we selected the overlapping biomarkers and time points ($t = 0$, 30, 60, 120, and 240 min) from 20 men and 20 women in response to PFT to be included in the health space. The first reference group represents the optimal healthy group and included 20 subjects of young age (20–29 y) with a low to normal fat percentage (<20% for men; <30% for women). The second reference group represents a compromised health group and included 20 subjects of older age (60–70 y) with normal to high fat percentage (>20% for men; >30% for women). In the health space model, 3 aspects of resilience were represented by 3 separate axes; the "liver" axis (ALT, AST, β-hydroxybutyrate, and GGT), the "metabolism" axis (glucose, HDL cholesterol, insulin, NEFAs, total cholesterol, and TGs), and the "inflammation" axis (IL-10, IL-6, IL-8, TNFα). Here, a biomarker–time point combination was included as a separate feature in the construction of the health space model. An elastic net regression model with leave-1-out cross-validation was fit to describe the data from the 2 reference groups along the liver, metabolism, and inflammation axes, respectively. The root mean squared error on the cross-validation (RMSECV) data was 0.42, 0.27, and 0.32 for the liver, metabolism, and inflammation axes, respectively. **Supplemental Table 6** shows the normalized regression coefficients for each biomarker–time point combination for separation of the young compared with the old reference group. Outcomes before and after the intervention from participants from the current study were

TABLE 2 Overnight fasting plasma biomarkers in overweight and obese adults with mild hypercholesterolemia before (Week 0) and after (Week 12) the intervention period with RW or WGW products[1]

	RW		WGW		
	Week 0	Week 12	Week 0	Week 12	*P*
Metabolic health					
Glucose, mmol/L	5.42 ± 0.54	5.41 ± 0.52	5.55 ± 0.69	5.55 ± 0.52	0.69
Insulin, mU/L	8.86 ± 7.82	9.24 ± 8.83	7.92 ± 4.06	7.84 ± 4.53	0.60
GIP, pg/mL	97.5 ± 125	118 ± 176	59.1 ± 24.3	72.6 ± 44.5	0.49
GLP-1, pg/mL	9.34 ± 6.60	10.9 ± 7.20	8.09 ± 5.00	10.1 ± 6.40	0.70
Glucagon, pg/mL	95.1 ± 75.3	91.1 ± 65.6	82.2 ± 48.9	109 ± 75.7	0.10
NEFAs, mmol/L	0.46 ± 0.15	0.44 ± 0.16	0.43 ± 0.12	0.44 ± 0.16	0.60
Total cholesterol, mmol/L	5.80 ± 0.89	5.80 ± 0.76	5.80 ± 0.68	5.70 ± 0.61	0.81
LDL cholesterol, mmol/L	3.76 ± 0.74	3.60 ± 0.62	3.75 ± 0.64	3.54 ± 0.76	0.75
HDL cholesterol, mmol/L	1.34 ± 0.33	1.38 ± 0.41	1.31 ± 0.36	1.33 ± 0.38	0.87
TGs, mmol/L	1.65 ± 0.68	1.74 ± 0.90	1.73 ± 0.85	1.66 ± 0.94	0.41
Ratio total/HDL cholesterol	4.6 ± 1.1	4.5 ± 1.2	4.7 ± 1.1	4.6 ± 1.2	0.93
Liver health					
ALT, U/L	30.4 ± 9.50	30.0 ± 8.60	34.2 ± 11.9	32.1 ± 9.40	0.49
AST, U/L	19.0 ± 6.30	18.6 ± 4.01	19.0 ± 4.60	18.7 ± 3.70	0.79
GGT, U/L	23.7 ± 19.9	23.6 ± 17.6	19.5 ± 12.3	19.2 ± 12.5	0.54
β-hydroxybutyrate, mmol/L	0.30 ± 0.21	0.29 ± 0.32	0.19 ± 0.14	0.27 ± 0.26	0.07
Vascular health					
Diastolic BP, mmHg	76.0 ± 8.60	75.9 ± 7.57	79.6 ± 1.00	77.6 ± 10.0	0.16
Systolic BP, mmHg	127 ± 14.1	125 ± 10.0	133 ± 18.0	127 ± 18.0	0.09
Augmentation index	23.0 ± 9.39	22.8 ± 9.43	21.3 ± 10.3	22.6 ± 9.64	0.12
E-selectin, ng/mL	3.15 ± 2.10	3.10 ± 2.28	2.65 ± 2.04	2.91 ± 2.06	0.42
P-selectin, ng/mL	35.9 ± 10.9	36.5 ± 14.1	34.2 ± 12.3	35.2 ± 14.3	0.87
VCAM1, ng/mL	607 ± 153	601 ± 175	600 ± 125	597 ± 128	0.82
ICAM1, ng/mL	522 ± 141	528 ± 140	500 ± 149	491 ± 137	0.53
ICAM3, ng/mL	0.20 ± 0.10[a]	0.20 ± 0.18[a]	0.18 ± 0.09[a]	0.20 ± 0.10[a]	0.03
Thrombomodulin, ng/mL	0.97 ± 0.33	0.95 ± 0.33	1.05 ± 0.36	1.08 ± 0.38	0.09
Inflammatory status					
CRP, μg/mL	2.58 ± 2.70[a]	5.24 ± 14.1[a]	5.29 ± 8.14[a]	2.16 ± 1.82[a]	0.03
SAA, μg/mL	2.28 ± 2.08	2.76 ± 2.80	9.15 ± 19.7	1.96 ± 1.94	0.06
TNFα, pg/mL	2.26 ± 1.43	2.29 ± 1.38	3.07 ± 1.85	2.90 ± 1.89	0.26
IL-10, pg/mL	0.40 ± 0.30	0.40 ± 0.36	0.73 ± 1.40	0.30 ± 0.10	0.08
IL-1B, pg/mL	0.14 ± 0.15	0.15 ± 0.17	0.14 ± 0.09	0.14 ± 0.21	0.24
IL-6, pg/mL	1.09 ± 0.81	1.46 ± 1.58	1.17 ± 1.26	1.13 ± 0.89	0.73
IL-8, pg/mL	4.87 ± 1.28	4.55 ± 1.66	5.21 ± 1.64	4.84 ± 1.47	0.70

[1]Data are presented as means ± SDs. RW: $n = 25$, WGW: $n = 25$. *P* values represent the interaction effect between time and treatment; the main effects are displayed in Supplemental Table 2. [a]Similar letters indicate statistically similar values after post hoc analysis. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BP, blood pressure; CRP, C-reactive protein; GGT, γ-glutamyltransferase; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; ICAM1, intercellular adhesion molecule 1; ICAM3, intercellular adhesion molecule 3; NEFA, nonesterified fatty acid; RW, refined wheat; SAA, serum amyloid A; TG, triglyceride; VCAM1, vascular cell adhesion molecule-1; WGW, whole grain wheat.

projected into the health space and resulting 2-dimensional spaces were visualized and the difference between RW and WGW was statistically evaluated after an ANOVA test. Outcomes from the interventions were compared to both reference groups to allow for interpretation of both interventions. Development of the health space models and visualization of the data was performed in R statistical software, version 3.4.3 (www.r-project.org).

Results

Effect of WGW or RW intervention on overnight fasting biomarkers

After overnight fasting no significant differences were found between the RW and WGW intervention groups (Table 2, Supplemental Table 2) for most of the 31 measured biomarkers. Only CRP ($P = 0.03$) and sICAM3 ($P = 0.03$) showed a significant different intervention effect for WGW compared with RW. CRP increased from 2.5 to 5.2 μg/mL in RW and decreased from 5.2 to 2.1 μg/mL in WGW, a change which was not significant within either intervention arm (post hoc test RW0 compared with RW12 $P = 0.25$ and WGW0 compared with WGW12 $P = 0.05$). sICAM3 remained at 0.20 ng/mL in RW and increased from 0.18 to 0.20 ng/mL in WGW, a change which was not significant within either 1 of the intervention arms (post hoc test RW0 compared with RW12, $P = 0.14$, and WGW0 compared with WGW12, $P = 0.10$).

Effects of WGW or RW intervention on postprandial challenge biomarkers

Metabolic health.

The PFT response curves of glucose, glucagon, insulin, and the incretin hormones glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide showed no significant

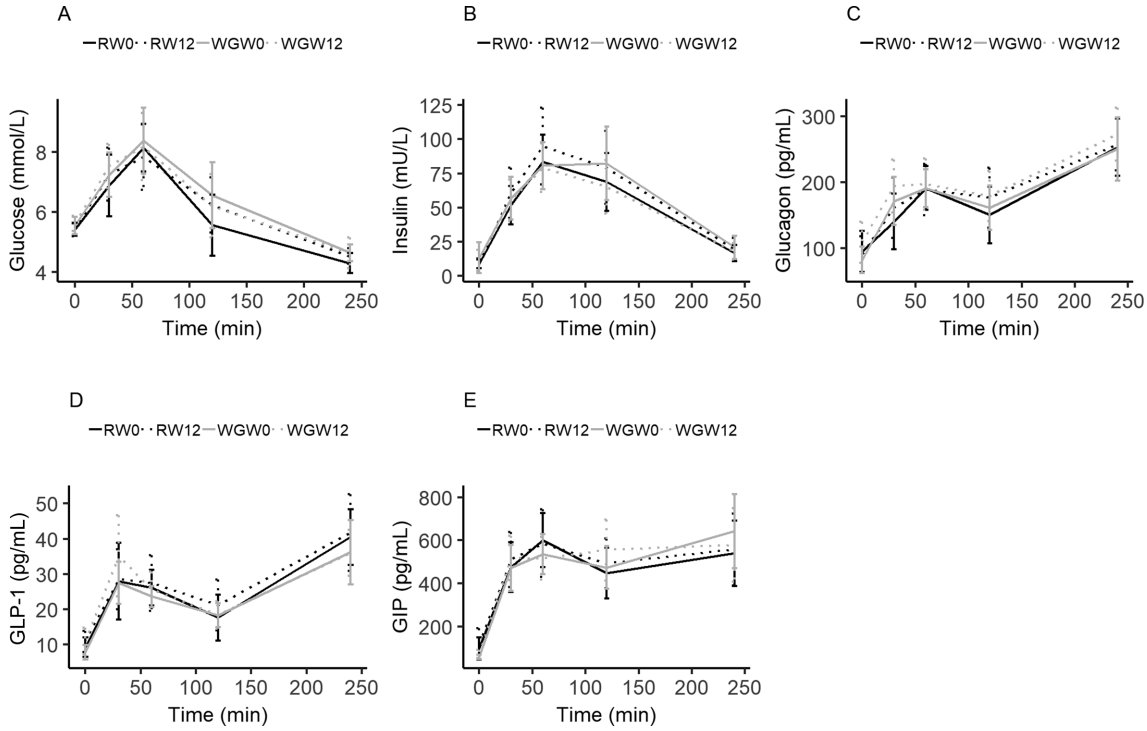

FIGURE 1 Glucose metabolism in overweight and obese adults with mild hypercholesterolemia before and after a 12-wk intervention period with RW (RW0, RW12) or WGW (WGW0, WGW12) products. Glucose (A), insulin (B), glucagon (C), GLP-1 (D), and GIP (E) concentrations in plasma before (Week 0) and after a 12-wk (Week 12) intervention of RW or WGW are shown in response to the PhenFlex challenge test (76.3 g carbohydrates, 17.6 g protein, 60.0 g fat). Data are means ± 95% CI. RW: $n = 25$, WGW: $n = 25$, statistical evaluation in Supplemental Tables 4 and 5. GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; RW, refined wheat; WGW, whole grain wheat.

week × treatment interaction for WGW and the RW intervention between week 0 and week 12 (**Figure 1**, Supplemental Tables 4 and 5). This is in accordance with the nonsignificant interaction effects between week and treatment for glucose-related and insulin-related indices (**Table 3**, Supplemental Table 3). Postprandial TG was significantly different between the RW and WGW intervention (TG AUCt $P < 0.05$, Supplemental Table 4), because of a significant increase in AUCt within the WGW group (418 to 431 min × mmol/L; $P = 0.001$). Postprandial HDL cholesterol showed a significant interaction effect ($P = 0.01$, Supplemental Table 5), because of increased HDL cholesterol concentrations within the RW intervention arm (1.29–1.35 mmol/L; $P < 0.0001$, **Figure 2**). Postprandial NEFA and total cholesterol, as well as the postprandial ratio of HDL cholesterol to total cholesterol were not significantly affected by either intervention (Figure 2, Supplemental Tables 4 and 5).

Liver health.

Liver enzyme ALT showed a significant postprandial change to PFT between WGW and RW ($P = 0.03$, Supplemental Table 5), because of decreased postprandial ALT concentrations within WGW intervention (27.8 to 25.7 U/L; $P = 0.03$, **Figure 3**). AST and GGT responses remained similar to baseline PFT after 12 wk of either intervention (Figure 3). The ketone body β-hydroxybutyrate showed a significant change in response to the PFT (AUCt, $P = 0.0007$; 3-way interaction, $P = 0.0003$), because of increased postprandial AUCt (74.1 to 74.6 min × mmol/L; $P = 0.001$) and concentrations ($t = 120$ min; 0.33–0.39 mmol/L; $P = 0.039$) within the RW intervention group,

TABLE 3 Glucose and insulin related indices before (Week 0) and after (Week 12) intervention period with RW or WGW in overweight and obese adults with mild hypercholesterolemia[1]

	RW		WGW		
	Week 0	Week 12	Week 0	Week 12	*P*
HOMA-B[2]	95.4 ± 75.7	96.7 ± 82.8	79.6 ± 36.7	73.3 ± 34.7	0.29
HOMA-IR[2]	2.17 ± 2.01	2.27 ± 2.27	2.02 ± 1.24	2.01 ± 1.26	0.77
Hepatic insulin resistance index	878 ± 816	921 ± 922	819 ± 500	816 ± 509	0.76
Disposition index	7.22 ± 3.81	7.32 ± 4.59	10.4 ± 12.6	9.95 ± 8.90	0.50
Insulinogenic index	1.29 ± 0.63	1.41 ± 0.94	1.71 ± 1.63	1.58 ± 1.18	0.72
Matsuda index	6.23 ± 3.77	6.41 ± 4.70	6.09 ± 4.14	6.40 ± 4.67	0.39
Muscle insulin sensitivity index	−2.72 ± 1.33	−3.05 ± 1.91	−3.26 ± 2.36	−3.61 ± 2.72	0.33

[1] Data are presented as means ± SDs. RW: $n = 25$, WGW: $n = 25$. *P* values represent the interaction effect between time and treatment; the main effects are displayed in Supplemental Table 3. HOMA-B, homeostatic model assessment of β cell function; RW, refined wheat; WGW, whole grain wheat.

[2] Only $t = 0$ measurement taken into account from PhenFlex challenge test.

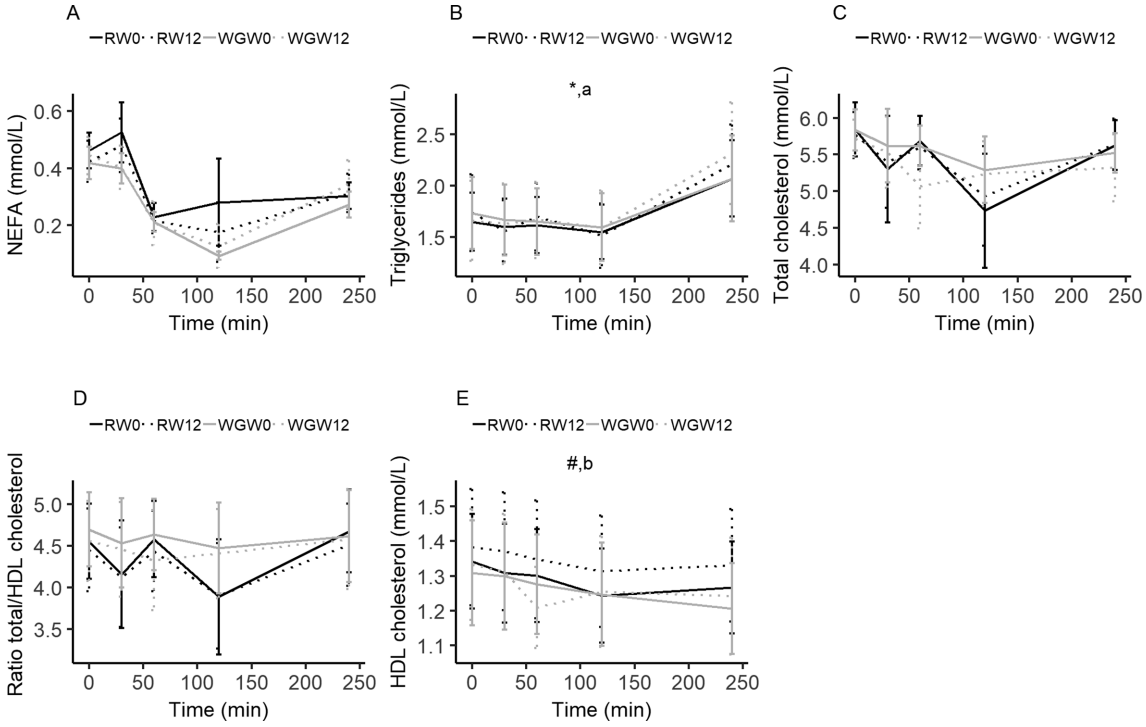

FIGURE 2 Lipid metabolism in overweight and obese adults with mild hypercholesterolemia before and after a 12-wk intervention period with RW (RW0, RW12) or WGW (WGW0, WGW12) products. NEFA (A), TG (B), total cholesterol (C) concentration, and ratio cholesterol to HDL cholesterol (D), and HDL cholesterol concentration (E) in plasma before (Week 0) and after a 12-wk (Week 12) intervention upon RW or WGW are shown in response to the PFT (76.3 g carbohydrates, 17.6 g protein, 60.0 g fat). Data are means ± 95% CI. RW: $n = 25$, WGW: $n = 25$. $^{*}P < 0.05$ on the basis of 2-way interaction of the AUCt between week and treatment, $^{\#}P < 0.05$ on the basis of a 2-way interaction of the PFT patterns between week and treatment. [a]Post hoc difference between week 0 and week 12 in the WGW group, [b]post hoc difference between week 0 and week 12 in the RW group (full statistical evaluation in Supplemental Tables 4 and 5). AUCt, total AUC; NEFA, nonesterified fatty acid; PFT, PhenFlex challenge test; RW, refined wheat; WGW, whole grain wheat.

and reduced concentrations ($t = 120$ min, 0.27–0.21 mmol/L; $P = 0.026$) after 12 wk of WGW (Figure 3, Supplemental Tables 4 and 5).

Vascular health.

Postprandial blood pressure (systolic and diastolic), augmentation index, and vascular inflammation markers did not show significant interaction effects (**Figure 4**).

Inflammatory status.

A significant change in response to PFT between RW and WGW was found for CRP ($P < 0.0001$), SAA ($P < 0.0001$), IL-10 ($P < 0.0001$), IL-1B ($P = 0.01$), TNFα ($P = 0.03$), IL-6 ($P = 0.02$), and IL-8 ($P = 0.0003$), all shown in **Figure 5** and Supplemental Tables 4 and 5. RW intervention increased CRP (2.21 to 4.76 pmol/L; $P = 0.001$), TNFα ($t = 120$, 1.74 to 2.11 pmol/L; $P = 0.04$), IL-6 ($P = 0.02$; 1.15 to 1.51 pmol/L), and IL-8 (4.27 to 4.70 pmol/L; $P = 0.007$), and decreased IL-1B (0.201 to 0.197 pmol/L; $P = 0.0002$) plasma concentrations in response to PFT, while WGW intervention decreased plasma concentrations of CRP (5.06 to 1.89 pmol/L; $P < 0.0001$), SAA ($P < 0.0001$), TNFα ($t = 0$; 3.07 to 2.90 pmol/L; $P = 0.046$; $t = 60$, 2.94 to 2.72 pmol/L; $P = 0.004$), IL-8 (4.96 to 4.74 pmol/L; $P = 0.02$), and IL-10 (0.839 to 0.322 pmol/L; $P = 0.0001$) in response to PFT (Figure 5).

Effect of WGW or RW intervention on resilience as measured by the health space approach

In Figure 6A and B, visualization of individual participant resilience reveals that on average the study population reflected the old age reference group. Similar average metabolic and liver resilience was seen at baseline, while inflammatory baseline resilience was higher in WGW subjects compared to RW subjects ($P = 0.02$). After 12 wk of intervention, average resilience of the RW subjects shifted further into the old age reference group, while the WGW subjects moved in the opposite direction towards the young reference group (Figure 6A and B). To obtain insight into which health domain contributed to the movement of the WGW subjects, we zoomed in on the separate axes. Significant interaction effects between week and treatment were found for liver ($P = 0.0001$) and inflammation ($P = 0.002$, Figure 6, **Supplemental Table 7**). Post hoc analysis revealed a significant increase in liver score ($P = 0.002$, Figure 6E) and inflammation score ($P = 0.006$, Figure 6C) within the RW group, indicating a reduction of liver and inflammatory resilience. A significant decrease in liver score ($P = 0.014$, Figure 6E) and a trend in inflammation score ($P = 0.09$, Figure 6C) was observed within the WGW group, indicating an increase of liver and inflammatory resilience.

An integrative physiological summary of adaptive responses to the WGW intervention

Supplemental Figure 2 summarizes the effect of the 12-wk WGW intervention compared to the RW intervention and shows how different processes adapted from a metabolically stressed state towards an improved resilience as represented by the young reference group. It is hypothesized that homeostatic control was improved in subjects with elevated plasma concentrations of total cholesterol after 12 wk of WGW intervention compared to subjects on RW shown by enhanced resilience.

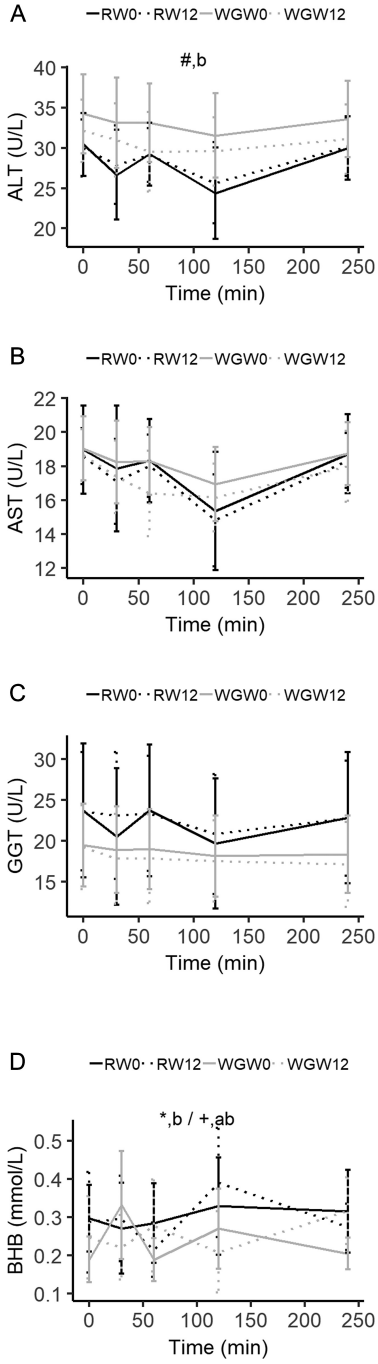

FIGURE 3 Liver metabolism in overweight and obese adults with mild hypercholesterolemia before and after a 12-wk intervention period with RW (RW0, RW12) or WGW (WGW0, WGW12) products. ALT (A), AST (B), GGT (C), and BH B (D) concentrations in plasma before (Week 0) and after a 12-wk (Week 12) intervention upon RW or WGW are shown in response to the PFT (76.3 g carbohydrates, 17.6 g protein, 60.0 g fat). Data are means ± 95% CI. RW: $n = 25$, WGW: $n = 25$. $^{*}P < 0.05$ on the basis of 2-way interaction of the AUCt between week and treatment, $^{\#}P < 0.05$ on the basis of a 2-way interaction of the PFT patterns between week and treatment, $^{+}P < 0.05$ on the basis of a 3-way interaction of the PFT patterns between week, treatment, and postprandial time point. [a]Post hoc difference between week 0 and week 12 in the WGW group, [b]post hoc difference between week 0 and week 12 in the RW group (full statistical evaluation in Supplemental Tables 4 and 5). ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUCt, total AUC; BHB, β-hydroxybutyrate; GGT, γ-glutamyl transferase; PFT, PhenFlex challenge test; RW, refined wheat; WGW, whole grain wheat.

The WGW intervention exerted a pleiotropic effect on liver (ALT, AST, β-hydroxybutyrate, and GGT), and inflammatory (IL-10, IL-6, IL-8, TNFα) resilience resembling a phenotype of younger age compared to RW. Furthermore, WGW protected against increased intrahepatic lipid content (as measured by MRI), which might be related to better lipid disposal capacity as postprandial TG response was increased in the WGW group (25).

Discussion

The current study results suggest possible mechanisms which support data from observational studies in which WGW consumption is associated with health benefits. Here, we examined to what extent a series of biomarkers representing physiological processes involved in resilience are modulated by a nutritional challenge test in comparison to traditional overnight fasting measurements by the consumption of WGW compared with RW. In this study we focused on biomarkers of cardiometabolic health, including metabolic health, liver health, vascular health, and inflammatory status.

Traditional overnight fasting measurements so far have shown no or few small effects upon WGW interventions. For example, nearly all randomized controlled trials (RCTs) which used only wheat products did not show effects on metabolic health measurements such as glucose and insulin (35–38). Meta-analysis indicates that mainly whole grain oats appear to exhibit a hypocholesterolemic effect, while an effect is absent for WGW on plasma lipids and glucose (39, 40). Furthermore, Vitaglione et al. found a significant reduction in plasma TNFα concentrations after 8 wk of WGW consumption (41). Interestingly, similar to our study, Vitaglione et al. included volunteers with a compromised health state (overweight subjects with hyperglycemia and mildly elevated total cholesterol with a low fruit, vegetable, and WGW intake and a sedentary lifestyle). This could explain why our study as well as the study from Vitaglione et al. were able to show an effect of WGW intake, in contrast to other studies that included healthier subjects in whom there may be little room for health improvement.

The challenge response curves showed significant health effects on several biomarkers when comparing the effect of WGW and RW. This included liver health biomarkers ALT and β-hydroxybutyrate, metabolic health biomarkers TGs and HDL cholesterol, and inflammatory biomarkers CRP, IL-8, SAA, IL-10, IL-6, IL-1B, and TNFα. Protection against an increased inflammatory status caused by WGW consumption could provide a beneficial health effect in persons at risk for the development of lifestyle-related disorders, as inflammation has been postulated as the main cause for metabolic disease (42, 43). In this intervention study measurement of resilience showcases that a dietary product was able to induce subtle systemic responses, which are difficult to identify with only fasting measurements. This is in accordance with our previous studies, which showed subtle effects of an anti-inflammatory supplement and overfeeding through use of challenge tests (21, 44).

A next step into understanding the subtle changes of physiology to maintain homeostasis is to combine the challenge response data. Here, when combined into a health space model, significant changes in inflammatory and liver health were observed, as well as a trend in metabolic health status. The health space model appears applicable to our WGW

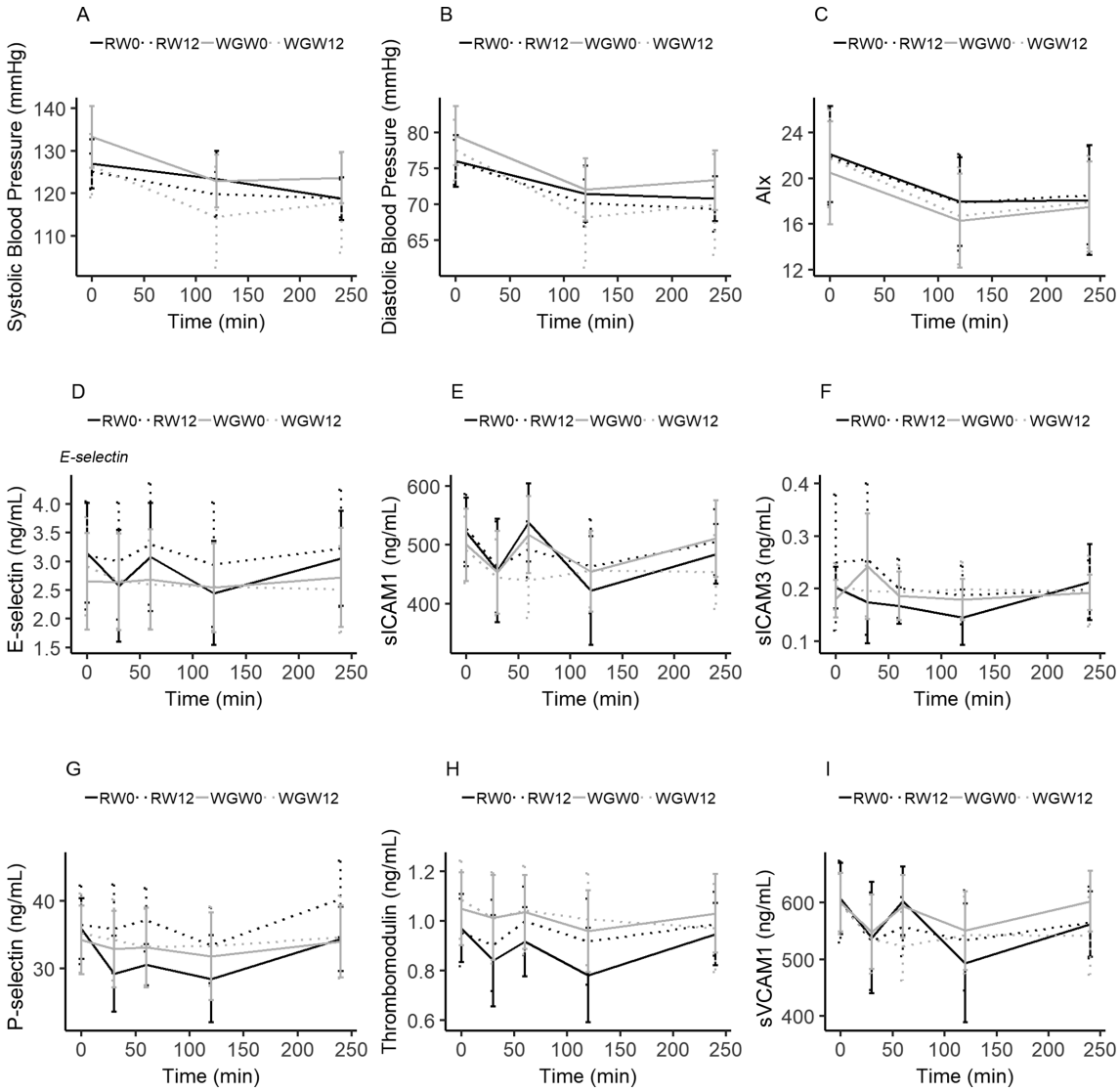

FIGURE 4 Vascular health markers in overweight and obese adults with mild hypercholesterolemia before and after a 12-wk intervention period with RW (RW0, RW12) or WGW (WGW0, WGW12) products. Systolic blood pressure (A), diastolic blood pressure (B), augmentation index (C), E-selectin (D), sICAM1 (E), sICAM3 (F), P-selectin (G), thrombomodulin (H), and sVCAM1 (I) concentrations in plasma before (Week 0) and after a 12-wk (Week 12) intervention upon RW or WGW are shown in response to the PhenFlex challenge test (76.3 g carbohydrates, 17.6 g protein, 60.0 g fat). Data are means ± 95% CI. RW: $n = 25$, WGW: $n = 25$, full statistical evaluation in Supplemental Tables 4 and 5. RW, refined wheat; sICAM1, intercellular adhesion molecule 1; sICAM3, secreted intercellular adhesion molecule 3; sVCAM1, secreted vascular cell adhesion molecule-1; WGW, whole grain wheat.

intervention study. First of all, the position of our relatively old and metabolically compromised subjects in the "old" range of the health space confirms the plausibility of the model with regard to quantification of health status. Secondly, as effects were observed on inflammatory and liver health, but only trends in metabolic health, this is in line with the observations on the individual biomarker concentrations which show inflammatory and liver effects, but few effects on metabolic markers [Figures 2, 4 and 5, Supplemental Tables 4 and 5; HDL cholesterol and TGs (25)]. This confirms the plausibility of the model for the evaluation of WGW intervention effects. Interestingly, the health space is mainly driven by postprandial biomarker responses upon the PFT, underlining the idea that health is defined by someone's ability to respond to a challenge (i.e., resilience), rather than the fasting status of an individual (18, 19). Indeed, the top 50% of liver health effects was driven by AST (1 h, 2 h; 26%), ALT (30 min; 11%), β-hydroxybutyrate (30 min; 9%), and GGT (30 min; 9%) (Supplemental Table 6). Similarly, the top 50% of explained variance in the inflammatory health axis was determined by IL-8 (30 min, 1 h, 4 h; 25%), IL-6 (*t* 0, 4 h; 15%), IL-10 (30 min, 8%), and TNFα (4 h, 7%), based on the reference groups (Supplemental Table 6). The 50% main contributors to the metabolic health were total cholesterol (30 min, 1 h, 2 h, 4 h; 35%), NEFAs (0 s, 4 h; 14%), and glucose (30 min; 6%) (Supplemental Table 6). The added value of representing resilience as composite markers via a health space, where the intervention effect is evaluated against 2 reference groups representing the upper and lower ranges within the healthy range of the population, is that it allows an objective evaluation of the intervention effect on specific health domains, in our case metabolism, liver, and inflammation. Results showed that RW intervention significantly reduced liver and inflammatory resilience, whereas WGW improved liver resilience. Taken together, our results

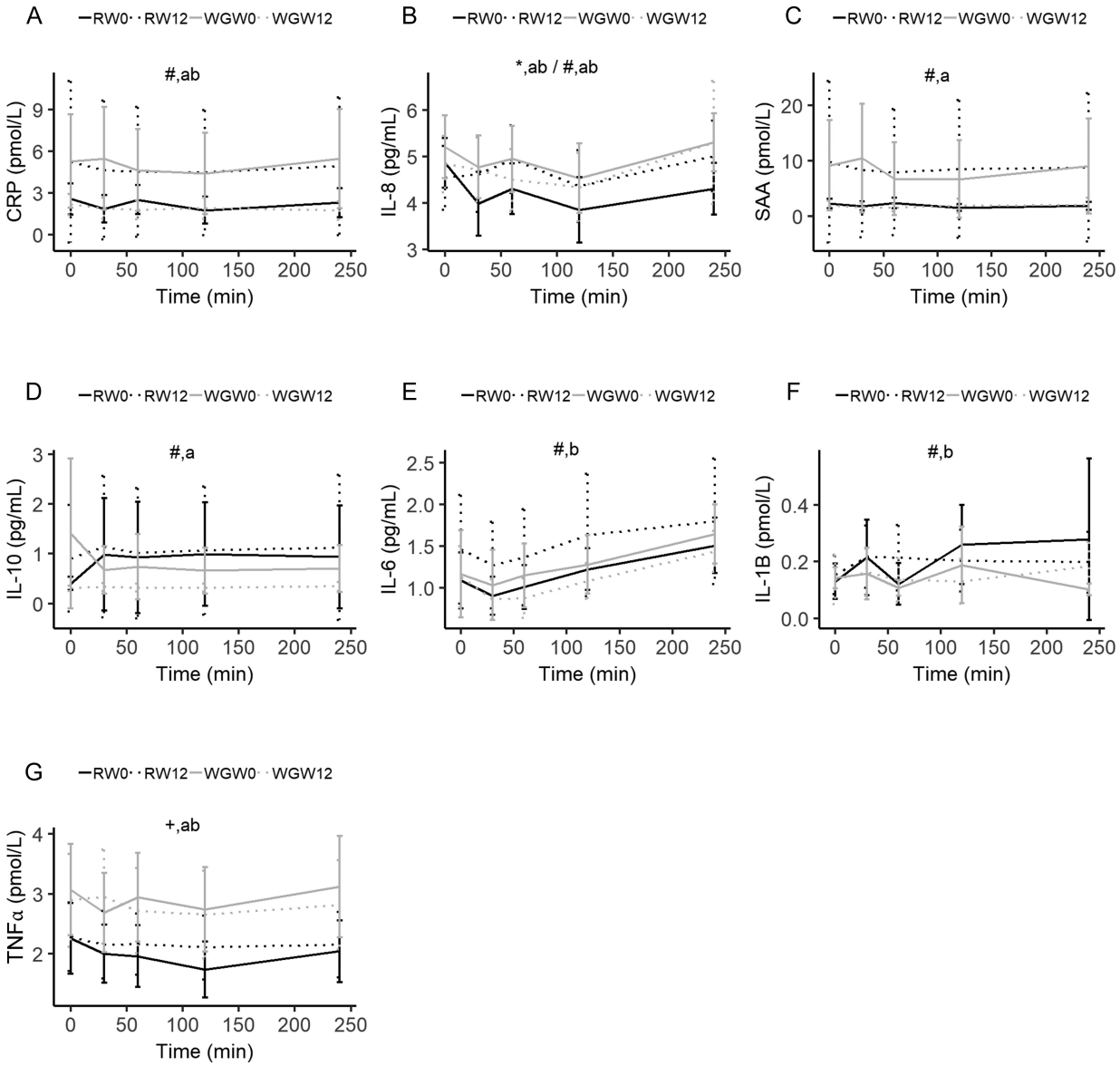

FIGURE 5 Inflammation markers in overweight and obese adults with mild hypercholesterolemia before and after a 12-wk intervention period with RW (RW0, RW12) or WGW (WGW0, WGW12) products. CRP (A), IL-8 (B), SAA (C), IL-10 (D), IL-6 (E), IL-1B (F), and TNFα (G) concentration in plasma before (Week 0) and after a 12-wk (Week 12) intervention upon RW or WGW are shown in response to the PFT (76.3 g carbohydrates, 17.6 g protein, 60.0 g fat). Data are means ± 95% CI. RW: $n = 25$, WGW: $n = 25$ $^{*}P < 0.05$ on the basis of 2-way interaction of the AUCt between week and treatment, $^{\#}P < 0.05$ on the basis of a 2-way interaction of the PFT patterns between week and treatment, $^{+}P < 0.05$ on the basis of a 3-way interaction of the PFT patterns between week, treatment, and postprandial time point. [a]Post hoc difference between week 0 and week 12 in the WGW group, [b]post hoc difference between week 0 and week 12 in the RW group (full statistical evaluation in Supplemental Tables 4 and 5). AUCt, total AUC; CRP, C-reactive protein; PFT, PhenFlex challenge test; RW, refined wheat; SAA, serum amyloid A; WGW, whole grain wheat.

have shown that liver and inflammatory resilience, and to some extent lipid metabolism (TGs and HDL cholesterol) of men and women with mildly elevated plasma cholesterol is changed by a daily intake of 98 g WGW for 12 wk, whereas vascular health remained unchanged. Quantification of resilience as measured by the response of a set of biomarkers to the standardized and validated PFT was more sensitive in identifying health effects compared to traditional fasting measures. By integrating a subset of biomarkers into the health space we were able to show a rejuvenative effect on inflammatory and liver health. Subjects moved on average in the direction of the "young" reference population, suggesting recovery of youthful characteristics by WGW consumption. As most separate postchallenge effects are small, it is not surprising that only a multitude of small effects on many related biomarkers leads to a visible improvement of the health status. To our knowledge, this is the first RCT study that shows direct health modification, a change in resilience, of WGW compared with RW intake. An interesting future step would be to investigate to what extent such change in resilience has on long-term health implications (45), as there is already substantial evidence for instance that foods modulate inflammation both acutely and chronically (46–48).

A limitation of our study is the offset difference in health status between the RW and WGW intervention groups, as the

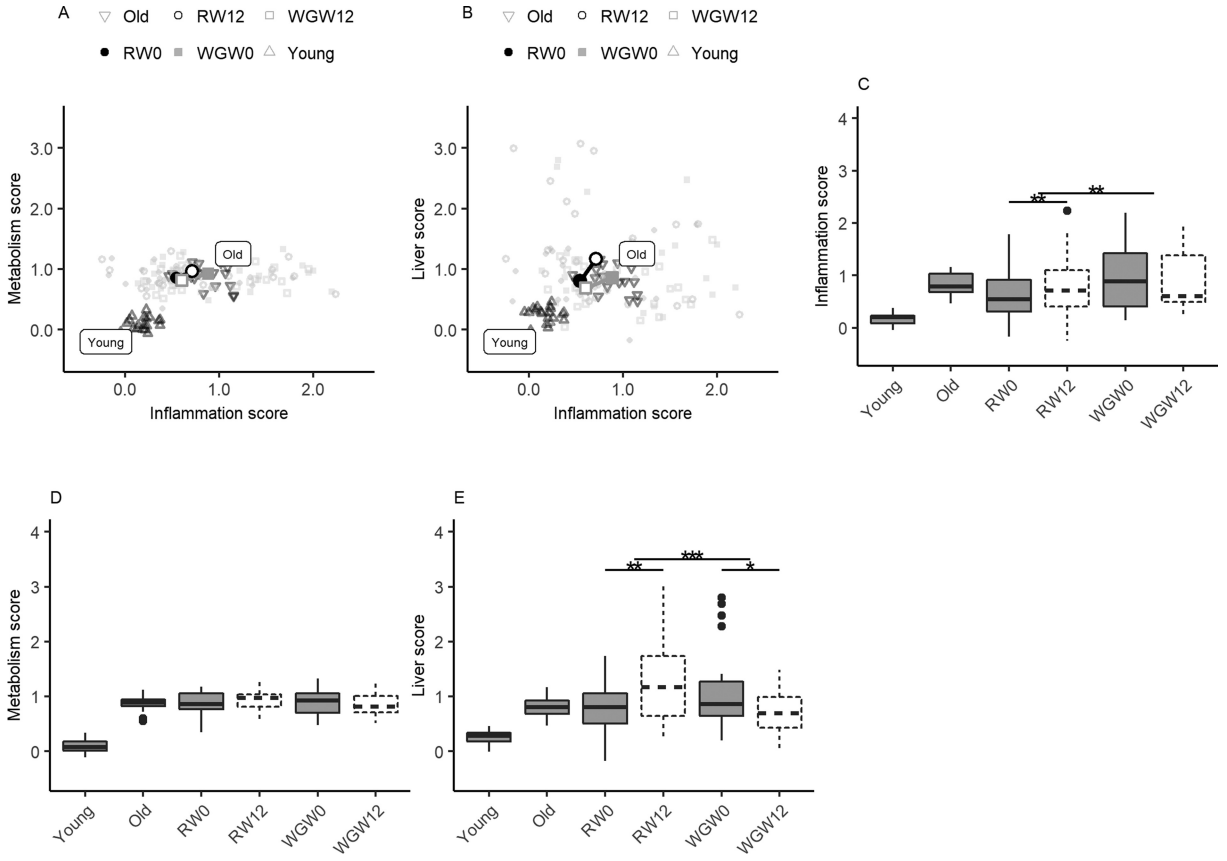

FIGURE 6 Health space resembling the individual resilience before and after 12-wk RW (RW0, RW12) or WGW (WGW0, WGW12) intervention in overweight and obese adults with mild hypercholesterolemia. Data were projected on inflammation and metabolism axes (A), and inflammation and liver axes (B) between young and old reference populations to show the individual and average health scores of the RW and the WGW groups, respectively. One-dimensional visualization in boxplots of the inflammation (C), metabolism (D), and liver (E) axis for RW and WGW relative to the reference population (23). RW: $n = 25$, WGW: $n = 25$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. RW, refined wheat; WGW, whole grain wheat.

participants were not stratified to these criteria but to traditional stratification vectors. Awaiting further development of the health space, however, our study has shown the potential of this approach and the need to stratify accordingly in future studies. Also, although the acute phase proteins CRP and SAA were measured in the current study, these data were not available for the reference groups, limiting the full inflammatory evaluation with the health space approach. Future development of the inflammatory axis is envisioned to provide even further insight into the relation between inflammation, health, and whole grain interventions.

The strengths of our study include the integration of multiple biomarkers and multiple data sets from different studies into 1 model, allowing demonstration of health effects upon WGW consumption while preserving biological relevance. Furthermore, this study carefully selected a multitude of biomarkers representing different organs and health-related processes involved in cardiometabolic health. This allowed us to shape a thorough picture of the subtle health effect of WGW (Supplemental Figure 2) on long-term reduction of cardiometabolic diseases, such as cardiovascular disease and type 2 diabetes mellitus. Such metabolic diseases are systems diseases, which require a systems diagnosis. Finally, the same set of biomarkers was evaluated both after an overnight fast and in response to PFT, allowing a side-to-side evaluation of the classical overnight fasting approach compared with resilience methodology that may result in the next generation of biomarkers and health claims.

In conclusion, no clear beneficial effects were found in the WGW intervention when investigating traditional overnight fasting markers. However, for the first time we were able to show in an RCT that substituting RW products with WGW products can improve liver health, and protect against induced inflammatory status as measured by integrated composite biomarkers of resilience.

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