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Functional gut microbiome signatures underlying interindividual variability in metabolic responses to red raspberry consumption

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Red raspberries have been shown to exert beneficial effects on immunometabolic health in numerous studies; however, these effects are subject to interindividual variability. Building on a previous transcriptomic-based clustering analysis from an 8-week randomized controlled trial in which 24 individuals consumed 280 g of red raspberries daily, we investigated whether functional metagenomic profiling may enhance our understanding of the observed interindividual variability in metabolic responses. Participants were classified as responders ($n = 13$) or non-responders ($n = 11$) based on prior clustering approaches, which identified significant reductions in plasma levels of C-reactive protein (CRP), triglycerides, and total cholesterol in responders. Microbial DNA extracted from fecal samples collected before and after the intervention was sequenced, and carbohydrate-active enzyme (CAZyme) counts were generated using a bioinformatics pipeline. Differential analysis revealed distinct functional metagenomic profiles between responders and non-responders. Multiple linear regressions identified potential associations between baseline CAZyme levels and changes in CRP, with contrasting trends observed between responders and non-responders. CBM8 and CBM49 were among the highlighted CAZymes. GH5 and several GH5 subfamilies were also identified as candidate CAZymes associated with interindividual variability observed in metabolic responses. These findings support the integration of microbiome-derived functional data alongside other omics to improve precision nutrition strategies.

Keywords Raspberry, CAZymes, Gut microbiome, Metabolic health, Metagenomics, Precision nutrition

Red raspberries are widely consumed, and numerous randomized controlled trials have demonstrated their positive effects on cardiometabolic and immune health in both healthy and unhealthy individuals[1–5]. These benefits are largely attributed to bioactive compounds present in raspberries, including polyphenols[3,6], particularly anthocyanins[7] and ellagitannins[8], as well as dietary fiber[9]. However, these beneficial effects exhibit considerable interindividual variability, in the metabolic responses to raspberry consumption[10–12]. Depending on the analytical approach and dataset used to classify responders and non-responders, such as transcriptomics[10] or metabolomics[13], different response groups may be identified. Using the data from an 8-week randomized controlled trial on red raspberry consumption[14], two different groups of responders were found using transcriptomics[10] and metabolomics[13]. These findings underscore the importance of applying comprehensive, multi-omics approaches to the analysis of nutritional intervention responses, to adequately capture the multiple factors contributing to interindividual variability. Among these factors, the gut microbiome, particularly its composition and functional metabolic capacity, has emerged as a key contributor[15–17]. However, to date, most studies have focused primarily on microbial composition, with relatively few exploring microbial functionality in depth[18,19].

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Carbohydrate-active enzymes (CAZymes) are primarily produced by gut bacteria, as humans lack the endogenous capacity to synthesize most of these enzymes[20]. Their primary role is to degrade carbohydrates, including dietary fiber and other complex polysaccharides, thereby enabling microbial utilization leading to the production of short-chain fatty acids (SCFAs). CAZymes can be classified based on their function: glycoside hydrolases (GH), carbohydrate esterases (CE), polysaccharide lyases (PL) and in some cases, auxiliary activity enzymes (AA) contribute to carbohydrate degradation, while glycosyltransferases (GT) are involved in carbohydrate synthesis[21]. Carbohydrate binding modules (CBM) further facilitate degradation by targeting specific substrates[22]. Only in recent years have CAZymes gained attention for their functional roles in linking gut microbiome activity to host health[23–26]. In this study, we aimed to investigate how functional metagenomic profiles, particularly CAZyme-related activity, may contribute to the interindividual variability observed in response to an 8-week red raspberry consumption, and whether this layer of analysis may provide some insights into potential functional pathways underlying this interindividual variability.

Methods

Study design and response groups

The data used in this study were obtained from a two-arm, parallel-group, randomized controlled trial (RCT) conducted at the Institute of Nutrition and Functional Foods (INAF), Université Laval, between January 2018 and August 2019[14]. The trial evaluated the effects of daily red raspberry consumption on metabolic and immune parameters in individuals at risk of metabolic syndrome residing in the Québec City metropolitan area. The trial was registered on 08/01/2018 at clinicaltrials.gov (NCT03620617) and was approved by the Université Laval Ethics Committee (CER-Université Laval RAMI 2017–218). All methods were carried out in compliance with applicable local regulations. A total of 59 individuals aged between 18 and 60 years, with excess weight or abdominal obesity (body mass index (BMI) ranging from 25 to 40 kg/m^2 or waist circumference ≥ 80 cm for women and ≥ 94 cm for men), and presenting mild hyperinsulinemia or hypertriglyceridemia, were randomized into two groups. Informed written consent to participate in the study was obtained from all individuals. One group ($n = 24$) consumed 280 g of frozen red raspberries daily (equivalent to roughly two cups), while the other group ($n = 25$) was instructed to maintain their usual diets over an 8-week period[14]. Blood, fecal and fasting plasma samples were collected at baseline (week 0) and post-intervention (week 8). Participants also completed medical history questionnaires and underwent physical examinations to collect anthropometric data at both time points. For the present study, only pre- and post-intervention data from the 24 individuals in the raspberry intervention group were analyzed. The full details of the RCT have been previously published[14]. Dietary intake data were collected at weeks 0, 4 and 8 using validated web-based food frequency questionnaires (FFQs)[27], with nutrient data values derived from the Canadian Nutrient File (CNF)[28], including estimates of fiber intake.

A transcriptomics-based clustering analysis was performed on whole blood RNAseq data[10] collected before and after the red raspberry consumption intervention. This analysis involved a Partial Least Squares Discriminant Analysis (PLSDA), followed by Hierarchical Clustering Analysis (HCA). Based on this approach, two distinct subgroups were identified: responders ($n = 13$) and non-responders ($n = 11$)[10]. In the present study, we used these predefined response groups to further investigate baseline microbiome profiles, specifically CAZymes.

Shotgun metagenomic data analysis

DNA was extracted from the fecal samples using Qiagen QIAamp Fast DNA Stool Mini Kits (cat. no. 51604) with ASL buffer (cat. no. 19082). DNA samples were sequenced at the *Centre d'expertise et de services Génome Québec* using the Illumina NovaSeq 6000, generating 60 to 70 million reads per sample. Low-quality bases were trimmed from the raw reads using Trim Galore v0.6.10, a wrapper tool based on Cutadapt and FastQC[29]. Host sequences were removed using bowtie2[30] and the GRCh38 human reference genome[31]. Clean reads were assembled into contigs using MEGAHIT v1.2.9[32] for all 48 samples (corresponding to 24 individuals at both pre- and post-raspberry consumption time points), with default parameters. Protein-coding sequences were predicted from the contigs using Prodigal v2.6.3[33], a fast gene prediction tool for prokaryotic genomes. Functional annotation was performed using dbCAN3, a database for the identification of CAZymes[34]. Annotations were generated using hmmscan in HMMER v3.4 with recommended parameters. Taxonomic classification of the contigs, down to the genus and species levels, was carried out using MMseqs2 v13-45111[35], with the Genome Taxonomy Database (GTDB, v220)[36] as the reference. Final CAZyme counts and gene coverage were calculated by counting mapped reads per gene using HTSeq[37].

Statistical analyses

R v4.2.3 was used for data processing and visualization. Statistical significance was tested at $\alpha = 0.05$. T-tests were conducted to compare characteristics between the response groups, including pre- and post-intervention red raspberry consumption and categorization of CAZymes throughout the different families. Differential analysis of CAZyme abundance between pre- and post-intervention samples was performed within each response group using the DEseq2 R package v1.38.3[38] through a two-way ANOVA with interaction model. The model accounted for group and timepoint. CAZymes with an adjusted p-value (False Discovery Rate, FDR) below 0.05 and an absolute log2 fold change (|log2 FC|) greater than 1 were considered significantly differentially expressed. Additionally, multiple linear regression analyses were performed between pre-intervention CAZyme raw counts and changes in plasma CRP, triglyceride, and total cholesterol levels (delta values: post-intervention minus pre-intervention levels) for both responders and non-responders. All models were adjusted for age, sex and pre-intervention BMI. Only CAZymes detected in at least five individuals per response group in pre-intervention samples were included in the analysis. S-layer homology (SLH), cohesin (COH) and dockerin (DOC) domains were not considered in the analyses as they do not possess catalytic activity and are not part of the main CAZyme families. While they play important structural roles in microbial systems, their presence does not directly reflect

carbohydrate-active enzymatic functions. Multiple testing correction was not applied to the regression analyses; therefore, these analyses should be considered exploratory and their results interpreted with caution. CAZymes identified as significant in the differential analysis and in the linear regressions were further compared between the two response subgroups using the Wilcoxon rank-sum test, for pre- and post-intervention levels.

Results

We previously applied a transcriptomics-based clustering approach to whole blood RNAseq data collected before and after the 8-week red raspberry intervention (2). This analysis identified two distinct subgroups: responders ($n=13$) and non-responders ($n=11$). The two response groups exhibited significantly different responses following the 8-week raspberry intervention. After 8 weeks of raspberry consumption, plasma triglyceride, total cholesterol, and C-reactive protein (CRP) levels decreased significantly in responders compared to non-responders[10]. Baseline values in responders and non-responders are shown in Table 1. At baseline, the two response groups were comparable for the proportion of men and women, age, BMI, plasma total cholesterol, triglyceride and CRP levels. In addition, fiber intake did not differ significantly between groups either at baseline (week 0; $p=0.66$) or post-intervention (week 8; $p=0.13$). Both groups showed a significant increase in fiber intake during the intervention, with similar magnitudes of change[10]. No abnormally high values of plasma CRP concentration (>50 mg/L) were noted in study participants. Only one participant showed CRP levels above 10 mg/L in pre- and post-intervention samples (11.8 mg/L and 13.5 mg/L respectively), but these values were retained in the analyses as this elevation is not considered severe[39]. Furthermore, this cutoff value has been the subject of reassessment in the recent years[40]. We further used these predefined response groups to investigate whether baseline microbiome profiles, specifically through the analysis of CAZymes, could help explain the interindividual variability observed in metabolic responses.

General features of the metagenome

Functional annotations of genes using dbCAN3 identified a total of 785 272 CAZyme counts across all 48 samples, including pre- and post-intervention timepoints. These CAZymes spanned all six major functional classes: 17 018 AAs, 75 025 CBMs, 91 646 CEs, 351 760 GHs, 197 773 GTs and 15 600 PLs. Additional domains associated with protein complexes such as COH, DOC and S-layer homology SLH accounted for 36 450 counts. The most abundant CAZyme families were GHs and GTs, representing 44.8% and 25.2% of total counts, respectively. In contrast, PLs and AAs were the least represented, contributing 2% and 2.2% of the total counts, respectively. Across all samples, a total of 685 unique CAZymes were identified: 17 AAs, 95 CBMs, 18 CEs, 342 GHs, 92 GTs and 92 PLs.

Response group profiling by baseline CAZymes

One participant was excluded from the analyses due to missing clinical data at the post-intervention timepoint, resulting in 13 individuals in the responder groups and 10 in the non-responder group. No significant differences in CAZyme family proportions were observed between the two groups. In terms of composition, responders expressed 15 distinct AAs, 95 CBMs, 18 CEs, 335 GHs, 90 GTs and 87 PLs, while non-responders expressed 15 AAs, 94 CBMs, 18 CEs, 326 GHs, 87 GTs and 87 PLs. The mean number of unique CAZymes detected was 453 in responders and 456 in non-responders. Abundances of individual CAZyme families for each participant are shown in Fig. 1. After filtering out CAZymes present in fewer than five samples per group, as well as SLH, COH and DOC domains, 464 out of the original 685 remained for analysis.

Differential analysis of CAZyme profiles across response groups and timepoints

Differential analysis between responders and non-responders identified 52 CAZymes with a $p<0.05$ (Supplementary Table 1): however, after adjusting for multiple comparisons (FDR), only 4 remained statistically significant. CBM8 (FDR < 0.0005, $p<0.00005$) was absent from post intervention samples in responders, while CBM63 (FDR < 0.0005, $p<0.00005$) was absent in non-responder post-intervention samples. PL42 (FDR = 0.01, $p<0.0005$) increased in responders but decreased in non-responders over the course of the intervention. CBM51 (FDR = 0.05, $p=0.0004$) increased in both groups. Notably, Wilcoxon rank-sum tests revealed that GH130_3 levels were significantly lower in non-responders at baseline ($p=0.004$), while GH63 levels were higher in non-responders post-intervention ($p=0.008$). These CAZymes are displayed in Fig. 2A, while those with $p>0.05$ are shown in Fig. 2B. As further detailed below, baseline CBM8, one of the most differentially expressed CAZymes,

Characteristics	Responders (n = 13)	Non-responders (n = 10)	P-value
Sex (women/men)	3.333	1.500	0.680
Age (years)	32.6 (10.59)	33.40 (9.82)	0.860
BMI (kg/m2)	29.3 (3.94)	32.39 (5.82)	0.140
Total cholesterol (mmol/L)	4.70 (0.96)	4.47 (0.86)	0.570
Triglycerides (mmol/L)	1.49 (0.91)	1.44 (0.71)	0.890
CRP (mg/L)	3.22 (3.37)	2.35 (1.99)	0.480
Dietary fiber intake (g/day)	23.9 (10.32)	21.23 (8.42)	0.550

Table 1. Anthropometric and metabolic characteristics of responders and non-responders at week 0. Data are means ± standard deviation. Sex is presented as counts. BMI, body mass index; CRP, C-reactive protein.

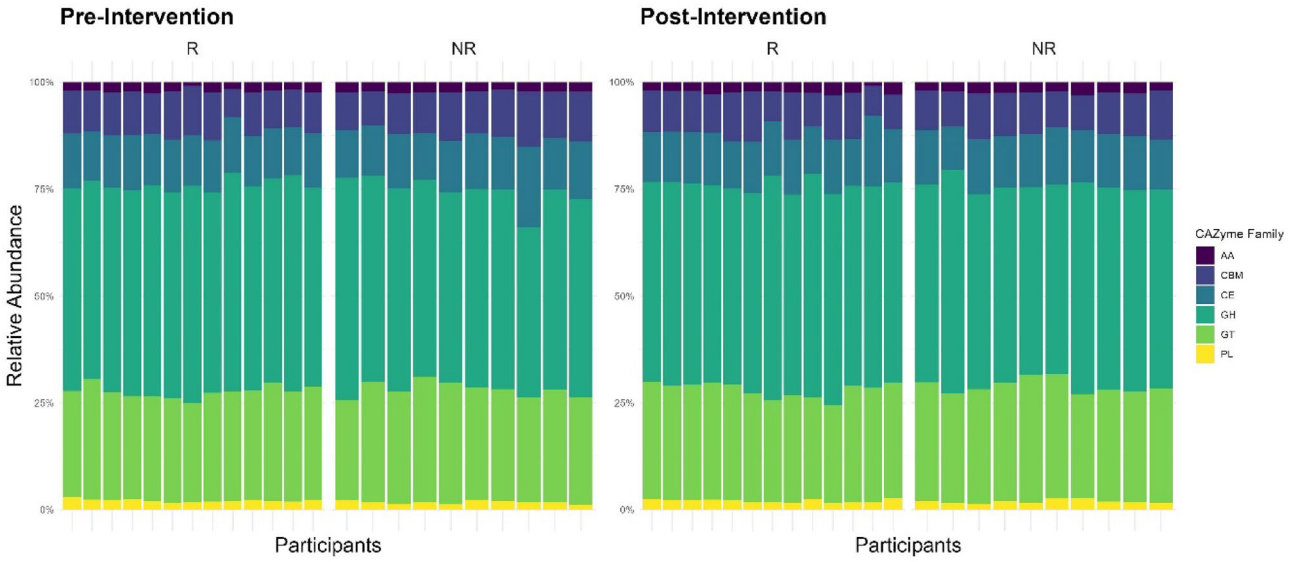

Fig. 1. CAZyme family abundance. CAZyme family abundance for all individuals ($n = 23$) grouped by response group (responders (R) and non-responders (NR)). Pre-intervention samples are shown on the left, and post-intervention samples are shown on the right.

was also significantly associated with changes in plasma CRP levels in linear regression analysis. Additionally, two CAZymes: baseline GH30_8 (FDR = 0.4, $p = 0.04$) and baseline GH5_5 (FDR = 0.3, $p = 0.008$), showed a significant association with CRP despite not passing the FDR threshold in the differential analysis. According to the Wilcoxon rank-sum test, the levels of these CAZymes did not differ significantly between responders and non-responders at either the pre- or post-interventions time points (Fig. 3).

Associations between baseline CAZymes and clinical parameters

Multiple linear regressions adjusted for age, sex and baseline BMI identified 22 interactions between baseline CAZymes and CRP changes which showed opposite directions between the two responder groups. Specifically, CRP decreased in responders while the baseline CAZymes increased, and CRP increased in non-responders while baseline CAZymes increased. These interactions were statistically significant. They show that the associations between CAZyme abundance and changes in CRP differ significantly between responders and non-responders. The most statistically significant association was observed with CBM49. Specifically, higher pre-intervention levels of this CAZyme were linked to a decrease in CRP among responders, while CRP increased among non-responders. This contrast in responses between the two groups is what the analysis highlights. All 22 significant interactions were linked to changes in CRP, and none were statistically significant for total cholesterol and triglyceride changes. Among these CAZymes, 12 were GHs (GH11, GH117, GH12, GH13_41, GH148, GH30, GH30_8, GH5, GH5_18, GH5_19, GH5_5), 5 were CBMs (CBM38, CBM49, CBM76, CBM8, CBM85), 3 were GTs (GT73, GT78, GT84), and 1 was a PL (PL8_3). All interactions are shown in Fig. 4A, while the eight regressions with the highest absolute beta coefficients (|β|) are highlighted in Fig. 4B. Detailed regression results are presented in Table 2. Comparison of these CAZyme levels between groups using the Wilcoxon rank-sum test revealed no significant differences at baseline. However, three CAZymes showed statistically significant differences at the post-intervention timepoint: CBM8 ($p = 0.01$), GH12 ($p = 0.04$) and PL8_3 ($p = 0.01$). As highlighted previously, CBM8 was absent from post-intervention responder samples. At week 8, GH12 levels were significantly higher in non-responders, while PL8_3 levels were significantly higher in responders. Pre- and post-intervention levels of these CAZymes are presented in Fig. 3. CAZymes with significant differences between groups are detailed in Supplementary Fig. 1.

Discussion

In the present study, we observed interindividual variability not only in the clinical response to the 8-week raspberry intervention, but also in gut microbiome composition at both baseline and post-intervention, suggesting that microbiome characteristics may be associated with differential responsiveness. While baseline levels of most key CAZymes did not differ significantly between groups, some displayed non-significant trends toward higher abundances in responders compared to non-responders prior to the intervention. These levels tended to converge during the intervention, which suggests that baseline differences in gut microbiome functional potential may be associated with differential responsiveness to raspberry consumption[41]. These CAZymes include GH175, GH43_18 and PL10_2, identified through the differential analysis. A similar trend was observed for CAZymes associated with changes in CRP, including CBM76 and CBM49, which showed the strongest association and were highly expressed in responders before the intervention. However, because most of the other CAZymes linked to clinical outcomes showed comparable levels in both groups, these findings should not be interpreted as evidence that relative CAZyme abundance alone explains differences in

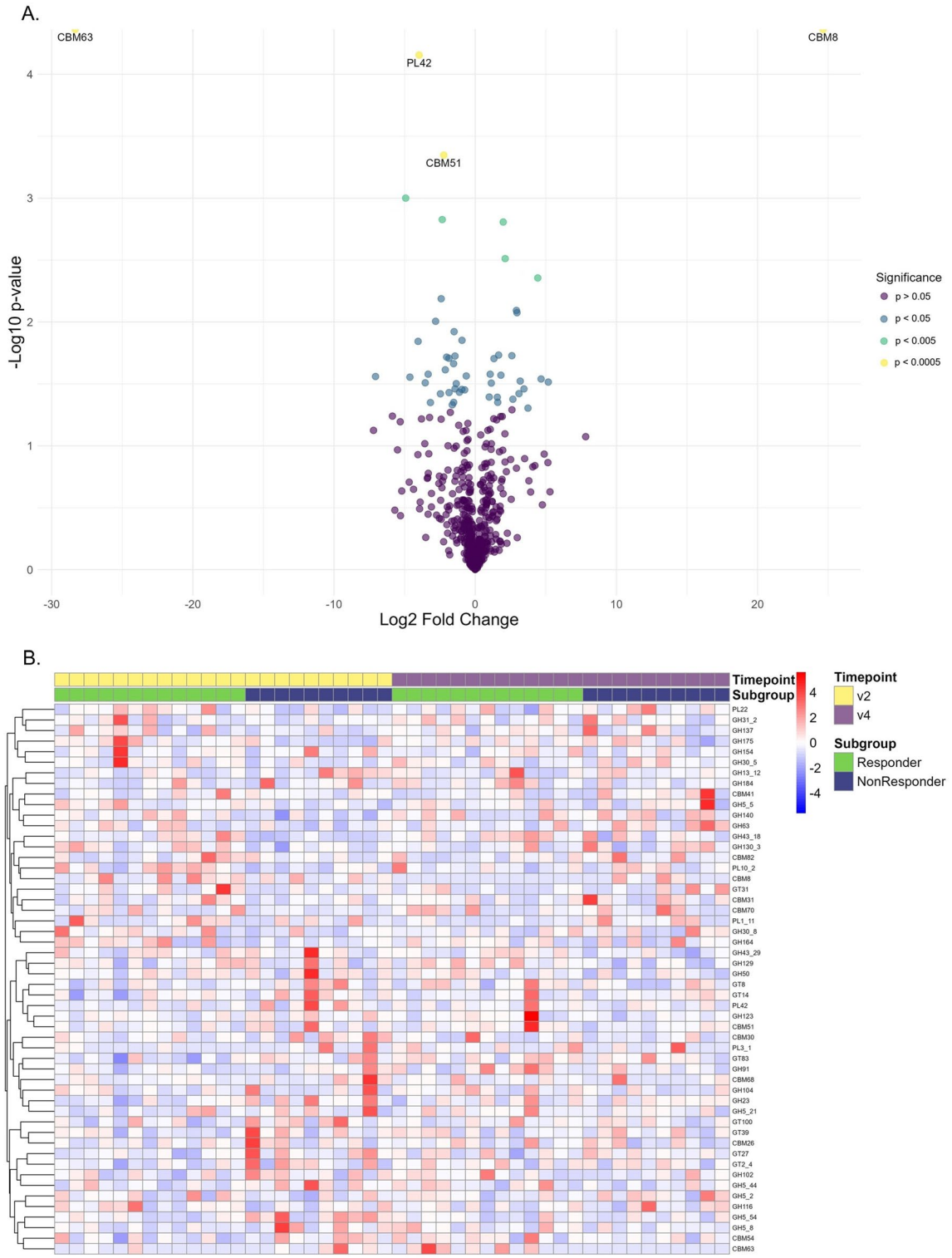

Fig. 2. Differential analysis of CAZymes. (**A**) Volcano plot for differential CAZymes. Significantly differentially expressed CAZymes between groups determined by fold change and FDR-adjusted p-value (|log2 FC| > 1, FDR < 0.05). Colors represent the level of significance (yellow, $p < 0.005$; green, $p < 0.05$; blue, $p < 0.05$; purple, not significant). (**B**) Heatmap of all CAZymes with $p < 0.05$ separated by response group (responders and non-responders) and timepoint (week 0, pre and week 8, post).

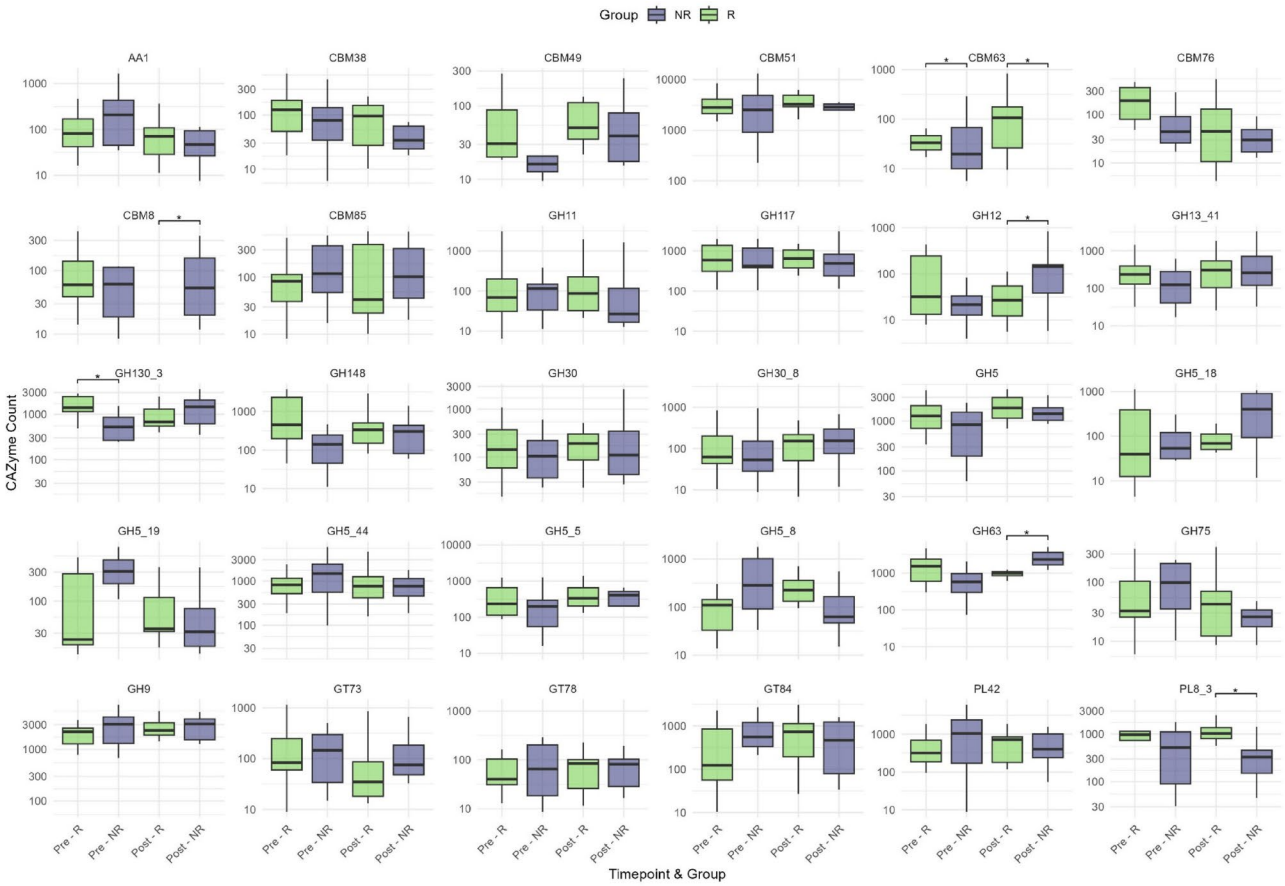

Fig. 3. CAZymes counts differences between groups and timepoints. Differences in CAZymes counts (log_{10}-transformed) between response groups, responders (R) and non-responders (NR), and timepoint, week 0 (pre) and week 8 (post). Comparisons were made between groups at each timepoint using the Wilcoxon rank-sum test. Statistically significant differences are indicated by asterisks ($p < 0.05$).

CRP responses. Rather, they may reflect broader differences in gut microbiome functional potential or other unmeasured factors. Importantly, the present study assessed baseline CAZyme profiles rather than enzymatic activity or metabolite production and therefore does not allow direct inference regarding downstream metabolic mechanisms. Because multiple-testing correction was not applied to the regression analyses, and given the small sample size, these associations should be interpreted as exploratory rather than mechanistic. Overall, our findings support further study of gut microbiome functional features in relation to interindividual variability in metabolic responses to dietary interventions. Furthermore, the minimal differences and lack of significant associations between baseline CAZyme levels and CRP suggest that short-term dietary variations are unlikely to account for the divergent CAZyme–CRP relationships observed over the 8-week period: significant changes to the functional microbiome may take longer to be reflected in samples[24]. Importantly, the 2-week run-in phase effectively standardized participants diets, and no differences in fiber intake were detected between responders and non-responders at baseline or during the intervention[14].

Gut microbiome composition is strongly influenced by numerous factors, including both short- and long-term dietary intake as well as environmental exposures[42]. Among these, dietary fiber plays a central role as a key substrate for gut microbes, with the type and amount of fiber consumed capable of significantly shaping the microbiome and related systems, such as the metabolome[43], and may affect microbial function, including SCFA production[44]. Differences in CAZyme levels observed in the present study as well as CAZyme utilization and function in the gut may influence downstream stages of fiber metabolization. Degradation and fermentation of dietary fiber in the gut represents the first step of SCFA production[45], meaning that variability at this enzymatic stage can impact the entire production process and consequently, SCFA availability and utilization. Low fiber intake has been associated with reduced CAZyme abundance in the gut, which in turn may lower SCFA production and diminish their health benefits. The CAZymes showing higher levels in responders before the intervention such as CBM49, CBM76, GH130_3, GH175, GH43_18 and PL10_2, may reflect differences in gut microbiome functional potential relevant to these processes. Others, including CBM8 and CBM63, absent from post-intervention samples for responders and non-responders respectively, also suggest that their functional activity and role in metabolic health needs to be further investigated. However, the present study assesses baseline CAZyme profiles rather than enzymatic activity or metabolite production and therefore cannot directly infer metabolic activity through these pathways.

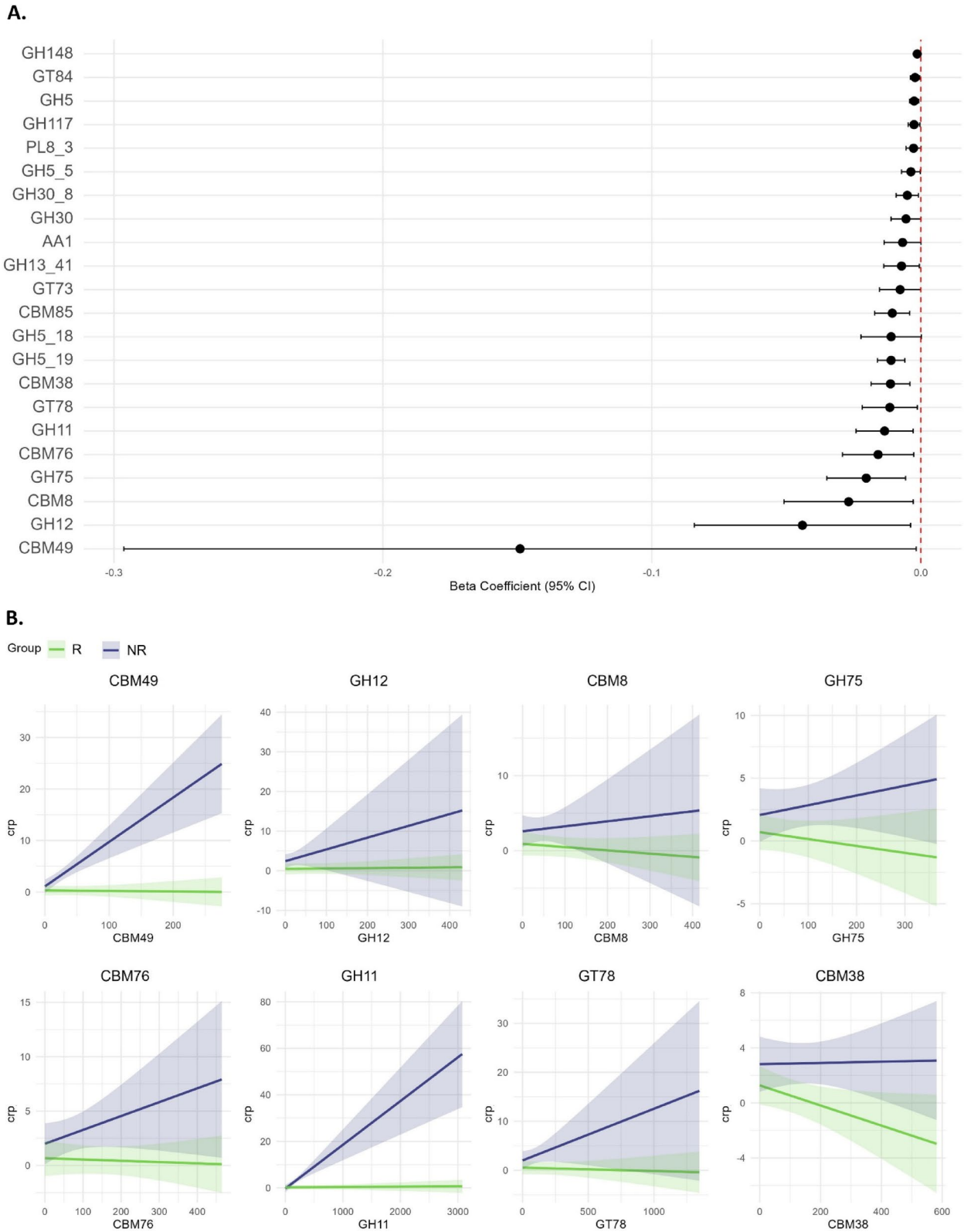

Fig. 4. Multiple linear regressions results. (**A**) Forest plot showing significant ($p < 0.05$) multiple linear regressions beta coefficients for the interaction between baseline CAZyme levels and C-reactive protein (CRP) changes during the intervention (post-intervention minus pre-intervention), with confidence intervals. All interactions are adjusted for age, sex and baseline BMI. (**B**) Eight multiple linear regression with the highest beta coefficients represented individually with confidence intervals.

Independent variable (CAZyme)	Dependent variable (Clinical parameter)	Beta coefficient	Standard error	p-value
CBM49	CRP	-0.149	0.069	0.040
GH12	CRP	-0.044	0.019	0.030
CBM8	CRP	-0.027	0.011	0.030
GH75	CRP	-0.020	0.007	0.0090
CBM76	CRP	-0.016	0.006	0.020
GH11	CRP	-0.013	0.005	0.010
GT78	CRP	-0.012	0.005	0.030
CBM38	CRP	-0.011	0.003	0.004
GH5_19	CRP	-0.011	0.002	0.000
GH5_18	CRP	-0.011	0.005	0.050
CBM85	CRP	-0.011	0.003	0.003
GT73	CRP	-0.008	0.004	0.040
GH13_41	CRP	-0.007	0.003	0.030
AA1	CRP	-0.007	0.003	0.050
GH30	CRP	-0.006	0.003	0.050
GH30_8	CRP	-0.005	0.002	0.020
GH5_5	CRP	-0.004	0.002	0.030
PL8_3	CRP	-0.003	0.001	0.050
GH117	CRP	-0.003	0.001	0.020
GH5	CRP	-0.002	0.001	0.007
GT84	CRP	-0.002	0.001	0.010
GH148	CRP	-0.001	0.001	0.030

Table 2. Multiple linear regressions results. Multiple linear regression results between baseline CAZyme levels and CRP changes during the intervention (post-intervention minus pre-intervention) with beta coefficients, standard error and p value. All interactions are adjusted for age, sex and baseline BMI. Only results showing a significant difference between responders and non-responders at $p < 0.05$ are shown.

CBM49 is a plant endoglucanase associated module from the GH9 family, specifically part of the Class C subclass, which is characterized by a distinct C-terminal domain. Its primary function is to hydrolyze artificial cellulosic polymers, cellulose oligosaccharides, and various plant cell wall polysaccharides in vitro[46]. CBM8, also found appended to GH9 enzymes, exhibits broad binding specificity, likely helping to position the catalytic domain near substrates rather than targeting specific recognition motifs. It binds to xyloglucan, glucomannan, β-glucan, hydroxyethyl cellulose (HEC), and insoluble forms of cellulose, but not to xylan[47]. These two CBMs are related to GH9, suggesting that this glycoside hydrolase may be a candidate functional feature relevant to the variability observed in response to this intervention, even though it was not statistically associated with the metabolic outcomes. GH9 is involved in the degradation of β-glucans[48], which may present some beneficial effects on human and animal health, including enhancing immune function, a hypocholesterolemic effect. Plasma LDL-cholesterol and total cholesterol levels have been shown to decrease following beta-glucan consumption[49], the latter reflecting changes seen in responders in the present study. In mice models, they have been linked to improvements in markers of metabolic syndrome, specifically by lowering plasma triglyceride and total cholesterol levels, and to reduce pro-inflammatory cytokine levels[50]. In the present study, responders similarly exhibited improvements in plasma triglyceride, total cholesterol, and CRP levels. These observations suggest that variation in CAZyme-related functional potential, specifically CBM49 and CBM8, may be associated with metabolic responsiveness, in a manner consistent with previously described roles of these enzymes in fiber degradation. However, direct measures of enzymatic activity or downstream metabolic outputs were not assessed in the present study. Associations involving GH5 and several of its subfamilies suggest that these CAZymes may warrant further investigation in relation to immune-metabolic responses. GH5 enzymes catalyze the breakdown of a wide range of fibers, including arabinoxylan, xyloglucans, mannans and β-glucans[51]. Arabinoxylan and xyloglucans both showed some potential health benefits, including anti-diabetic effects in rats for the former[52], and β-glucans effects have been highlighted above. Similar to GH9, these GH5 subfamilies may be associated with the differential metabolic responses observed in the present study. PL42, identified from differential analysis as positively expressed during the intervention for responders compared to non-responders, and previously known as GH145, encompasses enzymes that exhibit both PL and GH activities, depending on specific structural features[53]. This dual function may suggest a versatile capacity for degrading complex polysaccharides, which could be relevant to gut microbiome composition and function. However, no studies have yet explored the potential health benefits of PL42, and this interpretation remains speculative in the context of this study. GH30_8, which was linked to improvement in CRP in responders, is known to degrade xylans into xylo-oligosaccharides (XOS)[54], which has been linked to multiple beneficial effects such as reduced inflammation, risk of some cancers[55], and they have a prebiotic effect for bacteria in the gut microbiome[56]. Raspberries contain xylans in their hemicelluloses which highlights the importance of CAZymes capable of

catalyzing it into XOS[57]. PL10 on the other hand, whose subfamily PL10_2 was found to be significantly higher in pre-intervention responder samples in this study, degrades pectin[58]. This soluble fiber can affect cholesterol levels and potentially help prevent certain cancers[59]. These examples illustrate plausible links between CAZymes, dietary fibers and health-related outcomes, and support further investigation into whether such interactions may contribute to the variability in metabolic responses observed following red raspberry consumption.

In a previous study using the same biological samples and responder groups, analyses of microbiome composition and diversity revealed that the Firmicutes-to-Bacteroides ratio was significantly lower in responders compared to non-responders at baseline, and that it further decreased in non-responders following the intervention. Alpha-diversity indexes, Shannon and Simpson, were higher, though not significantly, in non-responders at baseline but decreased after the intervention, whereas the opposite trend was observed in responders[10]. Further results involving the microbiome composition had been reported in this previous study[10]. It has been widely shown that the gut microbiome composition can be very quickly modulated from many different factors such as diet, age, exercise and environment, within days or even hours[19,60]. However, whether these factors, particularly long-term fiber consumption, can meaningfully shape individual CAZyme profiles remain unclear. Sustained modulation of the gut microbiome can be achieved through consistent adherence to a diet rich in fruits, vegetables, and fiber, as well as through the regular use of prebiotics and probiotics[61]. Personalized strategies for modulating the gut microbiome are increasingly emerging, driven by advances in bioinformatics, machine learning and multi-omics approaches that account for the microbiome's heterogeneity and the interindividual variability observed[41].

The primary objective of this study was to build upon previous analyses of an 8-week raspberry consumption intervention, specifically a transcriptomic-based clustering analysis, and to investigate whether baseline gut microbiome functional features may be associated with the interindividual variability observed in metabolic responses. The main limitations of this study were: (1) the small sample size of this secondary analysis, which limits statistical power; (2) the lack of multiple testing correction for the regressions, restricting the interpretability of the results and increasing the risk of false-positives; (3) the need for further investigations into the key CAZymes to clarify their functional roles in immunometabolic health and determine whether microbiome-targeted interventions could enhance their abundance and therapeutic potential; and (4) the limited panel of inflammatory markers analyzed, as including additional biomarkers beyond CRP could have provided a more comprehensive understanding of the heterogeneity in inflammatory responses across participants. Future studies should also explore participants' long-term dietary habits to assess how they shape individual gut microbiomes and whether they influence immunometabolic responses to nutritional interventions. It is also worth noting that the 280 g daily raspberry dose used in this intervention was designed as a standardized scientific protocol and should not be interpreted as a dietary recommendation. Dietary fiber intake is best achieved through a varied and balanced diet encompassing a wide range of whole food sources including fruits, vegetables, whole grains, legumes, and nuts, consistent with the World Health Organization's recommendation of at least 25 g of fiber per day for individuals aged 10 and above[62]. Investigating CAZyme profiles in responders and non-responders could also give valuable insights into the variability of their responses. Finally, CAZymes alone are not sufficient to fully account for the degradation and utilization of complex carbohydrates. A holistic approach that integrates adjacent systems, including transporters and accessory pathways, may help clarifying the broader biological processes associated with distinct metabolic responses.

In conclusion, we have identified CAZymes as candidate contributors to the interindividual variability observed in responses to this 8-week raspberry consumption intervention. By complementing previous multi-omics analyses on these data, we provided new preliminary insights into the role of the gut microbiome in shaping immunometabolic responses. These findings highlight potential functional microbiome features associated with interindividual variability in dietary responses, though they require confirmation in larger, independent cohorts, and lay the groundwork for future mechanistic and experimental investigations to advance precision nutrition strategies.

Data availability

The data that support the findings of this study are available from the corresponding author, MCV, upon reasonable request. However, due to the sensitive nature of the research and the fact that participants did not provide written consent for public data sharing, access to the data may be restricted.

Received: 11 December 2025; Accepted: 23 March 2026
Published online: 29 March 2026

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Acknowledgements
This article would not have been possible without the valuable guidance of Prof. M.C.V., and the technical counsel of Juan de Toro-Martín. Many thanks to both. The authors also thank the study participants for their exceptional collaboration and acknowledge the contributions of the clinical coordinators and the staff of the Clinical Investigation Unit. M.C.V. is Tier 1 Canada Research Chair in Genomics Applied to Nutrition and Metabolic Health.

Author contributions
V.B. did the analyses, wrote the main manuscript, and prepared all figures and tables. J.dT.M. provided technical counsel on the shotgun metagenomics pipeline and omics analyses. V.G. coordinated the randomized control trial from which data were used in this study, and P.C. provided medical supervision during the trial. M.C.V. is the principal investigator of the project, supervised the study, and provided guidance and directions for the analyses, with the assistance of co-investigators D.R., C.C., and A.M. All authors reviewed the manuscript.

Funding
Dr Vohl's work was supported by the Washington Red Raspberries Council (WRRC).

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-45955-7.

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