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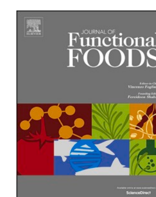

# Resistant potato starch supplementation reduces serum histamine levels in healthy adults with links to attenuated intestinal permeability

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A B S T R A C T

Histamine from our diet or gut microbes can trigger gastrointestinal disturbances, and resistant potato starch (RPS) has previously been shown to alleviate these symptoms while increasing levels of health-associated bacteria such as *Akkermansia* through unknown mechanisms. *Post hoc* exploratory metabolomic analysis of serum amino acid, amine, and carnitine metabolites in participants consuming 3.5 g/day RPS or placebo (n = 48) was performed using liquid chromatography-mass spectrometry to determine whether RPS positively influences histamine metabolism and related parameters. Histamine levels were significantly reduced by RPS treatment, but histamine-degrading enzyme products were unaffected by RPS. RPS also reduced histamine-secreting *Haemophilus* and *Lactobacillus*. Further, metabolites associated with intestinal permeability, including 5-hydroxylysine, acetylspermidine, and short- and medium-chain carnitines ratios, were significantly reduced by RPS treatment, suggesting decreased serum histamine might be related to enhanced gut barrier function. These metabolomic findings expand the value of supplementing the diet with RPS.

## 1. Introduction

Histamine intolerance (HIT) is a gastrointestinal disorder arising from excessive levels of the inflammatory amino acid histamine and is generally thought to be caused by impaired activity of diamine oxidase (DAO), which degrades histamine (Maintz & Novak, 2007; Kettner et al., 2022). Patients with HIT typically report food allergy-like symptoms, as well as gastrointestinal issues like diarrhea and constipation (Schnedl et al., 2019; Kettneret al., 2022). In healthy individuals, DAO is mainly released by mucosal epithelial cells lining the intestinal tract (Biegański et al., 1983), which halts histamine signaling via deamination of histamine to imidazole-4-acetaldehyde (Kettner et al., 2022; Zimatkin & Anichtchik, 1999). Impaired DAO activity can lead to excessive levels of histamine in the gut and/or systemic circulation, presenting as HIT (Kettner et al., 2022).

HIT may also occur independent of DAO impairment. Histamine is prevalent in fermented foods, meat, and seafood (Naila et al., 2010), and can be produced by numerous bacteria in the gut (Pessione & Cirrincione, 2016; Mou et al., 2021). Decarboxylation of histidine by microbial enzymes is responsible for elevated histamine in both food and in the gut (Naila et al., 2010; Pessione & Cirrincione, 2016). Defective intestinal barrier integrity, also referred to as 'leaky gut', may contribute

*Abbreviations:* ANOVA, Analysis of variance; DAO, Diamine oxidase; HIT, Histamine Intolerance; HIV, Human immunodeficiency virus; HNMT, Histamine N-methyltransferase; IBD, Inflammatory bowel disease; IBS, Irritable bowel syndrome; IQR, Interquartile range; IS, Internal standard; LC-MS, Liquid chromatography-mass spectrometry; NSAID, Non-steroidal anti-inflammatory; OTU, Operational taxonomic unit; QQQ, Triple-quadrupole; RPS, Resistant potato starch; RS, Resistant starch; SIV, Simian immunodeficiency virus; SD, Standard deviation; SEM, Standard Error of the Mean; UPLC-MRM/MS, Ultrahigh-performance liquid chromatography: multiple-reaction monitoring mass spectrometry.

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to HIT by allowing elevated levels of histamine from food and gut microbial sources to enter the blood stream (Schink et al., 2018).

Generally, proper food handling and storage can limit bacterial growth on meat and seafood, decreasing the conversion of histidine to histamine and keeping dietary histamine levels low (Naila et al., 2010). Elimination of foods containing high levels of histamine, including seafood, cheese, and other fermented foods, may be therapeutic for people with HIT, which aims to systemically lower dietary histamine levels, but long-term use of low histamine diets is impractical for most people (Reese et al., 2021). Histamine-secreting bacteria are an abundant, heterogeneous group (Mou et al., 2021), making it difficult to treat HIT by lowering endogenously produced histamine levels via microbiome modulators like probiotics. Alternative HIT therapies include dietary supplementation with DAO, which decreases histamine levels in the gut and reduces absorption of this amino acid, but therapeutic efficacy is inadequate (Kettner et al., 2022). Notably, the effect of prebiotics on HIT have not been investigated.

A dysbiosis, or a compositional imbalance in the microbes present in the gut (McBurney et al., 2019), tends to be a hallmark of HIT, although dysbiotic signatures often vary among the cohorts that have been studied (Mou et al., 2021). Elevated Proteobacteria (Schink et al., 2018; Sánchez-Pérez et al., 2022a; 2022b), and relatively lower levels of *Bifidobacteriaceae* and Verrucomicrobia (Schink et al., 2018) have been described in patients with HIT. At the genus level, abundance of *Bifidobacterium* and *Akkermansia* tend to be lower in patients with HIT relative to controls (Schink et al., 2018). Supplementation with low doses of resistant potato starch (RPS) significantly increase the relative abundance of *Bifidobacterium* and *Akkermansia* while reducing constipation and diarrhea related symptoms compared to placebo in an otherwise healthy adult population (Bush et al., 2023). Given that HIT dysbiosis and symptomatology, including gastrointestinal distress (Schnedl et al., 2019), were improved with RPS supplementation in healthy individuals, prebiotic RPS administration might provide a novel therapeutic avenue for HIT.

RPS is a type 2 (native) form of resistant starch (RS), which resists human α-amylase digestion in the upper digestive tract due to its granular structure and reaches the large intestine intact where the insoluble dietary fiber is fermented (Cummings & Englyst, 1991; Englyst et al., 1996). Clinical trials have demonstrated various health benefits associated with RPS consumption, either in isolation or in a potato food matrix (Raben et al., 1994; Sanders et al., 2021; Cummings et al., 1996; Wutzke et al., 2010; Wutzke & Scholübbers, 2013; Cao et al., 2022). Isolated RPS has been demonstrated to have a prebiotic effect in humans including increases in *Bifidobacterium* and *Akkermansia* along with improvements in glucose and insulin metabolism. Reductions in diarrhea- and constipation-associated bowel movement scores have also been reported (Alfa et al., 2018a, 2018b; Bush et al., 2023). While benefits of RPS consumption are demonstrated, connections between changes in the gut microbiota and improvements in health status have not been elucidated.

The primary objective of this study was to expand the previously reported findings of the low dose RPS study (3.5 g/day for four weeks) (Bush et al., 2023), to determine the effect of RPS induced gut microbiome-related changes on histamine metabolism and related parameters in healthy adults.

## 2. Materials and methods

### 2.1. Investigational product

The resistant potato starch (RPS) used in this study was Solnul™ (MSP Starch Products Inc., Carberry, MB), an unmodified RS Type 2 produced via a unique processing method to preserve resistant starch (RS), with an RS content of at least 60% (AOAC 2002.02). The placebo used was a high amylopectin corn starch (Amioca; Ingredion, Brampton, ON) previously shown to be fully digested with no detectible effects on the gut microbiota (Deehan et al., 2020).

### 2.2. Study design

The clinical trial design, sample size estimations, participant selection, and study procedures have previously been described in detail (Bush et al., 2023). Briefly, the study was a double-blind, randomized, placebo-controlled three arm study that evaluated the effects of daily 3.5 g RPS (containing 2.1 g RS) for 4 weeks on fecal bacteria composition and bowel movement consistency. The study protocol was approved by Canadian Shield Ethics Review Board (REB Tracking Number: 19–10-001; Burlington, ON, Canada). Healthy participants (n = 25/arm) between 18 and 69 years of age with a body mass index between 18.0 and 34.9 kg/m$^2$ were recruited. Participants agreed not to use any dietary supplements, including vitamins or minerals, 14 days prior to treatment randomization until completion of the final visit. Participants were advised to maintain their activity level and habitual diet. Twenty-four participants completed the study in both the 3.5 g/day RPS and placebo arms. In the present study, *post hoc* analysis of serum amino acid, amine, and carnitine metabolites was performed to determine whether the 3.5 g/day RPS dose had beneficial effects on histamine metabolism in comparison to the placebo.

### 2.3. Measurement of serum metabolites in amino acid anabolism and catabolism

An internal standard (IS) cocktail of 50 isotope-labeled analogues of amino acids and amines was dissolved in 30% aqueous acetonitrile. A standard-substance solution containing 110 amino acids, amines and metabolic intermediates of amino acid anabolism and catabolism was prepared in 70% aqueous acetonitrile and at a concentration of 50 μM for each compound, and serially diluted with the same solvent to have 10-point calibration solutions. For analysis of amino and phenolic hydroxyl functional group containing metabolites, the quantitation was carried out using a chemical derivatization – ultrahigh-performance liquid chromatography – multiple-reaction monitoring mass spectrometry (UPLC-MRM/MS) method that we described previously (Han et al., 2018a), with necessary modifications. In brief, 20 μL of participant serum stored at −80 °C was mixed with 180 μL of 80% aqueous acetonitrile, vortexed for 30 s, sonicated in a water bath for 2 min, and then centrifuged at 21,000 g for 10 min. 50 μL of the clear extractant of each sample or each of the calibration solutions was mixed in turn with 50 μL of the IS solution, 100 μL of 10-mM dansyl chloride solution, and 50 μL of borate buffer, pH 9, and allowed to react at 40 °C for 30 min. 5 μL aliquots were then injected to run UPLC-MRM/MS on an Agilent 1290 UPLC system coupled to an Agilent 6495B triple-quadrupole (QQQ) mass spectrometer equipped with an atmospheric pressure electrospray ion source and operated with positive-ion detection (Agilent Technologies, Santa Clara, CA). A C18 column (2.1*150 mm, 1.8 μm) was used for chromatographic separation with a mobile phase composed of 0.1% formic acid in water (A) and in acetonitrile – isopropanol (1:1, v/v) (B) for binary-solvent gradient elution (20% to 85% B over 18 min) at 0.3 mL/min and 55 °C. For analysis of those non-derivatizable metabolic intermediates of amino acid anabolism and catabolism, 20 μL of the clear extractant of each sample or each of the calibration solutions, was mixed with 30 μL of the IS solution. 5 μL aliquots of the resultant solutions were injected to run UPLC-MRM/MS on the same liquid chromatography-mass spectrometry (LC-MS) instrument with positive-ion detection. An amide-type column (2.1*100 mm, 1.7 μm) was used for chromatographic separation and a mobile phase composed of 0.1% formic acid in water (A) and in acetonitrile (B) was used for binary-solvent elution using a gradient of 90% to 20% B over 15 min, at 0.35 mL/min and 40 °C. The UPLC-MRM/MS data were recorded and processed using the Agilent *MassHunter*® software suite (Agilent Technologies). The quantitation was performed with internal standard calibration. Linearly regressed calibration curves of individual metabolites

were constructed with the data acquired from injection of the calibration solutions in each set of UPLC-MRM/MS runs. Molar concentrations of the compounds detected in serum were calculated by interpolating the calibration curves of individual metabolites with the analyte-to-IS peak area ratios measured from injection of the sample solutions.

### 2.4. Measurement of serum carnitines

Quantitation of carnitines was carried out using a chemical derivatization – UPLC-MRM/MS method described previously (Han et al., 2018b), with necessary modifications. A standard-substance solution of 27 free and acyl carnitines was prepared at a concentration of 10 μM for each compound in 80% methanol, then serially diluted in a ratio of 1 to 4 (v/v) to have 10-point calibration solutions. Next, 40 μL of each calibration solution, or a mixture of 20 μL of serum and 20 μL of methanol, was mixed with 40 μL of 150 mM of 3-nitrophenylhydrazine solution, 40 μL of 120 mM of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimideacetonitrile – 4% pyridine solution, vortexed for 30 s, reacted at 40 °C for 30 min, then centrifuged at 21,000 g for 10 min. 50 μL of the supernatant of each solution was mixed with an equal volume of a pre-made IS solution containing $^{13}C_6$-3-nitrophenylhydrazine derivatives of all the targeted carnitines. 10-μL aliquots of the mixed solutions were injected to run UPLC-MRM/MS on the same Agilent 1290 UHPLC-6495B QQQ MS system with positive-ion detection (Agilent Technologies), following the procedures using the LC-MS operating parameters previously described (Han et al., 2018b). Quantitation was performed similarly to measurements of the amino acid catabolism and anabolism metabolites.

### 2.5. Microbial analysis

Microbial analysis was previously described (Bush et al., 2023). Briefly, 16S amplicons of the v4 region were obtained from fecal samples collected in OMNIgene-Gut kits (DNA Genotek) and sequenced on the MiSeq platform (Illumina, San Diego, CA, USA) at Microbiome Insights (Vancouver, BC, Canada). MiSeq-generated Fastq files were quality-filtered and clustered into 97% similarity operational taxonomic units (OTUs) using the Greengenes v13.8 database and the mothur software package (Schloss et al., 2009). Bacterial genera with histamine-secreting capabilities were identified by literature review (Mou et al., 2021) and the effect of RPS versus placebo was evaluated. Further analysis is underway and sequence data will be deposited once this work is completed.

### 2.6. Statistical analysis

Changes in metabolite levels and bacterial relative abundance were calculated by subtracting the baseline level from Week 1 or Week 4 level. Short- and medium-chain carnitine to free carnitine and long-chain carnitine to free carnitine ratios were calculated. Shifts in these ratios were then calculated by subtracting the baseline ratio from the Week 1 or Week 4 ratio. Changes in metabolites, metabolite ratios, and bacterial relative abundance were subjected to outlier analysis using Tukey's fences method (Tukey, 1977), where outliers were considered those that were three times the interquartile range greater or less than the median (Table 1). After correcting for outliers, mean changes in metabolite levels and bacterial levels were compared between placebo and RPS treatment arms using a two-way ANOVA to assess the overall effect of treatment (Microsoft Excel, Redmond, WA). Spearman's rho and false discover rate (FDR) adjusted $q$ values were generated to compare changes in metabolites and genera using GraphPad Prism (v9.5.1; GraphPad Software, Boston, MA), with changes from baseline to Week 1 and Week 4 analyzed together (i.e., 48 data points per arm). Statistical significance was considered at $P < 0.05$ and $q < 0.10$.

## 3. Results

Histamine levels were significantly decreased in the RPS treatment compared to the placebo (Fig. 1A; treatment effect P = 0.0397). Histamine can be produced from histidine via histamine decarboxylase, either by human immune cells or microbes in the gut (Smolinska et al., 2022). Histidine levels were not significantly affected by RPS (Fig. 1B; P = 0.07245), suggesting that RPS-dependent effects on histamine are not due to upstream changes in histidine. Imidazole propionate can also be synthesized from histidine by gut microbes (Koh et al., 2018), but this metabolite was not captured in the targeted analysis. Decreases in histamine levels could be due to increased activity of histamine N-methyltransferase (HNMT) and accumulation of 1-methylhistamine and/or diamine oxidase (DAO) and accumulation of imidazole-4-acetaldehyde (Maintz & Novak, 2007). Levels of 1-methylhistamine increased in the placebo arm but were not affected by RPS (Fig. 1C; P = 0.0415), suggesting that RPS does not decrease histamine levels by enhancing HNMT activity.

Serum imidazole-4-acetaldehyde levels were undetected by the targeted metabolomic assays, so it was not possible to determine if RPS decreased histamine by increasing DAO activity. However, DAO acts on other substrates, including polyamines putrescine and spermidine (Schwelberger & Bodner, 1997). If histamine levels decrease in response to RPS via enhanced DAO activity, putrescine and spermidine levels should similarly decrease. Putrescine levels tended to increase in the

**Table 1**
Changes in metabolite levels identified as outliers.

| Metabolite | Treatment | Time Point | Median Value | 3*IQR Fence | Outlier Value |
|---|---|---|---|---|---|
| Dicarboxyl Acylcarnitines | Placebo | Week 1 | 0.0730075 | −1.56786 | −1.59323 |
| Dicarboxyl Acylcarnitines | Placebo | Week 4 | 0.0161145 | 1.10633 | 5.576043 |
| Glutamine | Placebo | Week 4 | −1.6925 | −42.2255 | −142.497 |
| Histamine | Placebo | Week 1 | −0.00007445 | −0.00748 | −0.00934 |
| Histamine | Placebo | Week 4 | −0.0002445 | −0.00761 | −0.01036 |
| Histamine | Placebo | Week 4 | −0.0002445 | −0.00761 | −0.00882 |
| Hydroxylysine | RPS | Week 1 | −0.01668 | −0.25242 | −0.26285 |
| Hydroxylysine | Placebo | Week 1 | 0.001425 | 0.222248 | 0.25252 |
| Hydroxylysine | RPS | Week 4 | −0.01268 | −0.21593 | −0.26984 |
| Hydroxylysine | RPS | Week 4 | −0.01268 | −0.21593 | −0.24526 |
| Hydroxylysine | RPS | Week 4 | −0.01268 | 0.167675 | 0.18064 |
| Spermine | Placebo | Week 1 | −0.0004735 | −0.00427 | −0.01016 |
| Spermine | Placebo | Week 1 | −0.0004735 | 0.003574 | 0.004854 |
| Spermine | RPS | Week 4 | 0.000028 | 0.008548 | 0.00901 |
| Spermine | Placebo | Week 4 | 0.0003995 | −0.00886 | −0.00919 |

Outliers were detected by comparing changes in metabolites levels to the upper and lower three times interquartile range (3*IQR) fences (Tukey, 1977). Those greater than the upper fence or lower than the lower fence were considered outliers.

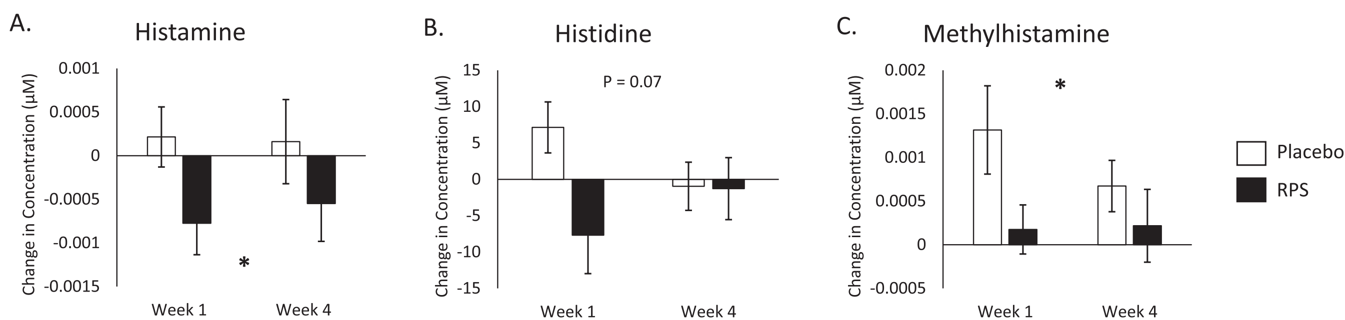


**Fig. 1.** Changes in histamine (A), histamine precursor histidine (B), and methylhistamine (C) were compared between RPS and placebo treatment arms. *, P < 0.05.

placebo arm but were not significantly affected by RPS (Fig. 2A; P = 0.0633). Spermidine levels also tended to increase in the placebo arm but were not affected by RPS (Fig. 2B; P = 0.0524), suggesting that RPS does not decrease histamine levels by enhancing DAO activity. Levels of the related polyamine spermine were also unaffected (Fig. 2C; P = 0.4635).

While DAO degrades putrescine and spermidine via oxidative deamination, these polyamines are also subject to acetylation, which can decrease the intracellular pools by promoting their export from the cell (Casero Jr & Pegg, 2009). Serum levels of acetylputrescine tended to decrease in response to RPS (Fig. 2D; P = 0.0687), while acetylspermidine levels significantly decreased in response to RPS treatment (Fig. 2E; P = 0.006).

In addition to dietary sources of histamine, several gut bacteria are known to secrete histamine, which contributes to endogenous histamine levels in the gut (Mou et al., 2021). We detected ten histamine-secreting bacteria genera previously described by Mou et al., including *Bacteroides*, *Clostridium*, *Eggerthella*, *Escherichia*, *Haemophilus*, *Lactobacillus*, *Lactococcus*, *Pseudomonas*, *Shigella*, and *Streptococcus* (Fig. 3A-J) (Mou et al., 2021). RPS supplementation led to significant reductions in the relative abundance of *Haemophilus* (Fig. 3E; P = 0.0304) and *Lactobacillus* (Fig. 3F; P = 0.0249) compared to the placebo. RPS-dependent reductions in *Haemophilus* and *Lactobacillus* were modest but it was possible that reductions in these bacteria could at least partially explain the serum histamine decreases in participants consuming RPS. However, changes in histamine levels were not correlated with changes in any of the histamine-secreting bacteria ($q > 0.10$; Table 2).

Given that RPS appears to have no meaningful effect on the activity of histamine degrading enzymes and minimal effects on histamine-secreting bacteria, we reasoned that RPS-dependent reductions in serum histamine could result from enhanced gut barrier function (Nofrarías et al. 2007; Cao et al., 2022; Qin et al., 2023). Using a targeted amino acid and amine metabolomics approach, several metabolites reported to be elevated in cases of intestinal permeability were detected (Yamamoto et al. 2019; Shah et al., 2022; Qin et al., 2023), including arginine, asparagine, glutamine, hydroxylysine, lysine, ornithine, proline, serine, tryptophan, and tyrosine (Fig. 4A-J). Levels of asparagine (Fig. 4B; P = 0.0015), hydroxylysine (Fig. 4D; P = 0.0216), ornithine (Fig. 4F; P = 0.0169), serine (Fig. 4H; P = 0.001), and tyrosine (Fig. 4J; P = 0.0014) were all significantly reduced in response to RPS. Arginine (Fig. 4A; P = 0.0869), glutamine (Fig. 4C; P = 0.2183), lysine (Fig. 4E; P = 0.1309), and tryptophan (Fig. 4I; P = 0.416812) levels were unaffected by RPS treatment. Proline levels significantly increased in response to the placebo (Fig. 4G; P = 0.0486).

Short- and medium-chain carnitines have been shown to negatively correlated with intestinal *Zo-1* expression and associated with

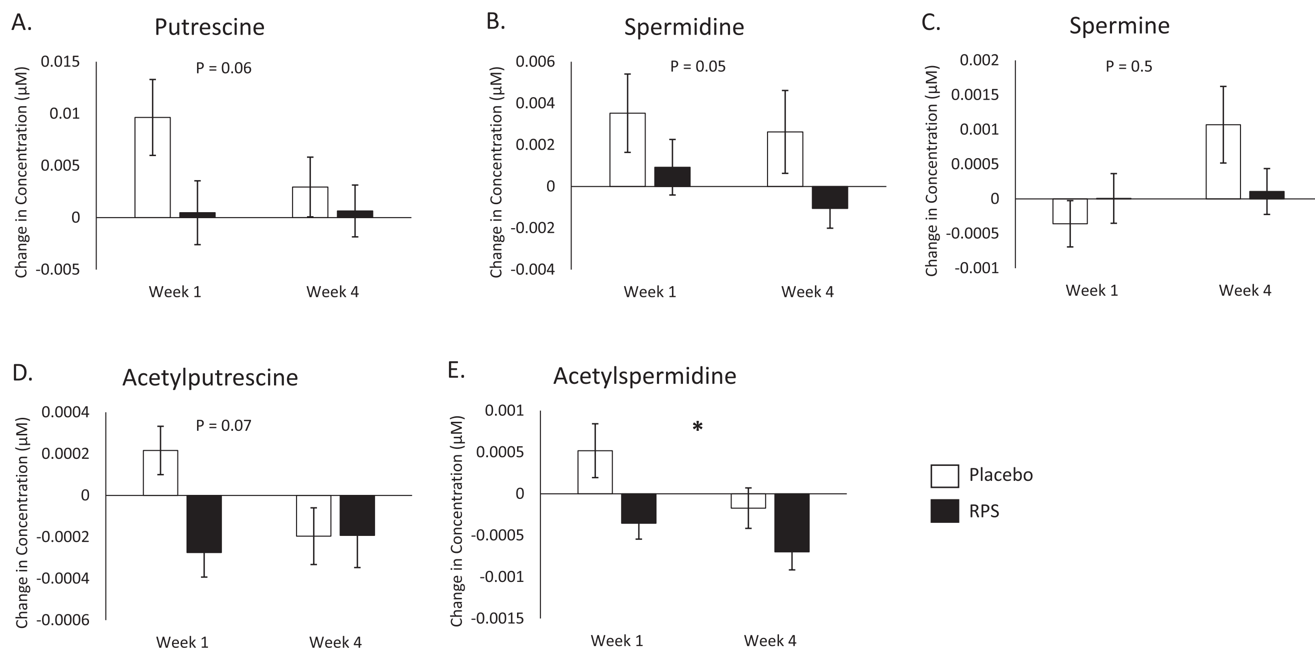


**Fig. 2.** Changes in putrescine (A), spermidine (B), spermine (C) acetylputrescine (D), and acetylspermidine (E) were compared between RPS and placebo treatment arms. *, P < 0.05.

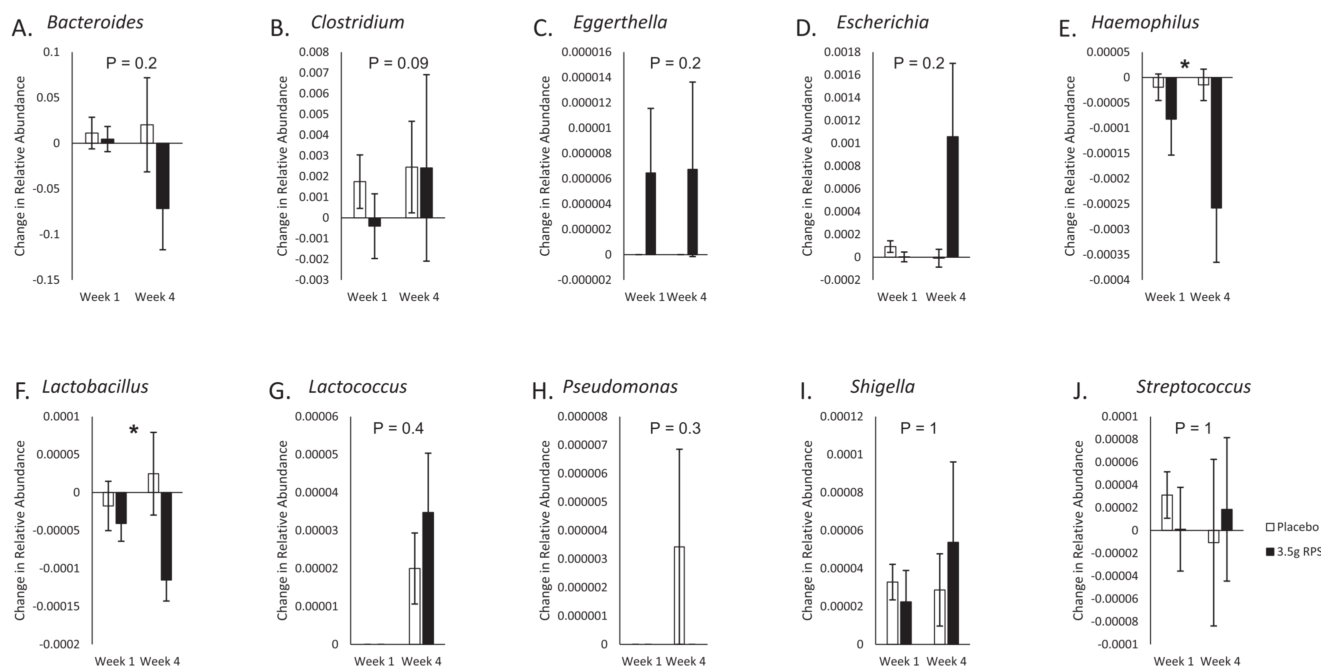


**Fig. 3.** Changes in histamine-secreting gut bacteria *Bacteroides* (A), *Clostridium* (B), *Eggerthella* (C), *Escherichia* (D), *Haemophilus* (E), *Lactobacillus* (F), *Lactococcus* (G), *Pseudomonas* (H), *Shigella* (I), and *Streptococcus* (J) were compared between RPS and placebo treatment arms. *, P < 0.05.

**Table 2**
Correlation analysis comparing changes in gut bacteria and changes in serum histamine levels.

| Genus | RPS Spearman's rho | *q* value | Placebo Spearman's rho | *q* value |
|---|---|---|---|---|
| *Bacteroides* | −0.23524 | 0.326 | −0.35215 | 0.256 |
| *Clostridium* | 0.2057 | 0.430 | 0.0432 | 0.781 |
| *Eggerthella* | −0.1254 | 0.562 | N/A | N/A |
| *Escherichia* | 0.1420 | 0.551 | 0.1199 | 0.562 |
| *Haemophilus* | −0.1816 | 0.465 | 0.1209 | 0.562 |
| *Lactobacillus* | −0.2779 | 0.326 | 0.1866 | 0.455 |
| *Lactococcus* | 0.1061 | 0.591 | 0.2721 | 0.326 |
| *Pseudomonas* | N/A | N/A | 0.2739 | 0.326 |
| *Shigella* | 0.1905 | 0.455 | 0.1589 | 0.482 |
| *Streptococcus* | −0.2905 | 0.326 | 0.0449 | 0.781 |

mitochondrial fatty acid oxidation dysfunction and permeability in a primate virus model of inflammation-associated leaky gut (Crakes et al., 2019), which prompted us to examine serum levels of carnitines. RPS treatment shifted the ratio between short- and medium-chain carnitines (C2 to C10) to free carnitine when compared to the placebo arm (Fig. 5A; P = 0.0444). Changes in long chain carnitine (C12 to C22) to free carnitine ratios were not different between intervention arms (Fig. 5B; P = 0.8517), nor were changes in dicarboxyl acylcarnitines (Fig. 5C; P = 0.2384). These results suggest that RPS influences mitochondrial β-oxidation but has no effect on peroxisomal β-oxidation or ω-oxidation in the endoplasmic reticulum.

Finally, we assessed correlations between changes in *Akkermansia* and *Bifidobacterium*, with changes in histamine, methylhistamine, acetylputrescine, acetylspermidine, and short- and medium-chain carnitine rations, as these metabolites were shown to be influenced by RPS treatment. Increases in *Akkermansia* were inversely correlated with decreases in short- and medium-chain carnitine ratios (Fig. 6; $r_s = -0.34$, $q = 0.083$), linking the enrichment of *Akkermansia* to host changes previously associated with improved intestinal barrier function. Changes in histamine were not correlated with responses in either genus but were significantly correlated with both changes in acetylspermidine ($r_s = 0.383$, $q = 0.052$) and short- and medium-chain carnitine ratios ($r_s = -0.386$, $q = 0.052$) (Fig. 6). No statistically significant correlations were detected in the placebo arm ($q > 0.10$).

## 4. Discussion

This study aimed to determine the effect of RPS-induced gut microbiome-related changes on histamine metabolism and related parameters in healthy adults. We report a significant decrease in histamine levels and certain histamine-secreting gut microbiome in the RPS treatment group compared to the placebo. However, there were no significant changes in histamine-degrading enzyme products. Rather, decreases in acetylated polyamines, select amino acids, and the ration of short- and medium-chain carnitine to free carnitine support a model whereby RPS enhances intestinal permeability to promote the retention of histamine within the gut.

Clinical trials have previously demonstrated health benefits associated with RPS consumption, either in isolation or in a potato food matrix, including improved postprandial metabolism (Raben et al., 1994; Sanders et al., 2021), laxation (Cummings et al., 1996), ammonia metabolism (Wutzke et al., 2010; Wutzke & Scholübbers, 2013), and intestinal permeability (Cao et al., 2022). Building on previous work demonstrating improvements in beneficial microbiota and reductions in both constipation- and diarrhea-associated bowel movements in response to RPS supplementation, we explored whether decreased histamine levels connected microbiota changes to improvements in host wellness. Metabolomic analysis confirmed that RPS consumption led to decreased serum histamine and demonstrated that this decrease was not likely due to enhanced DAO or HNMT enzyme activity. Furthermore, decreases in histamine-secreting bacteria were insufficient to explain RPS-induced reductions in serum histamine. Rather, acetylated polyamine, amino acid, and carnitine metabolite changes provide suggestive evidence that RPS decreases histamine levels by enhancing intestinal barrier integrity.

Intolerance to histamine likely stems from multiple issues, such as inadequate DAO metabolism, consumption of improperly stored food, and/or increased histamine production by microbiota within the gut

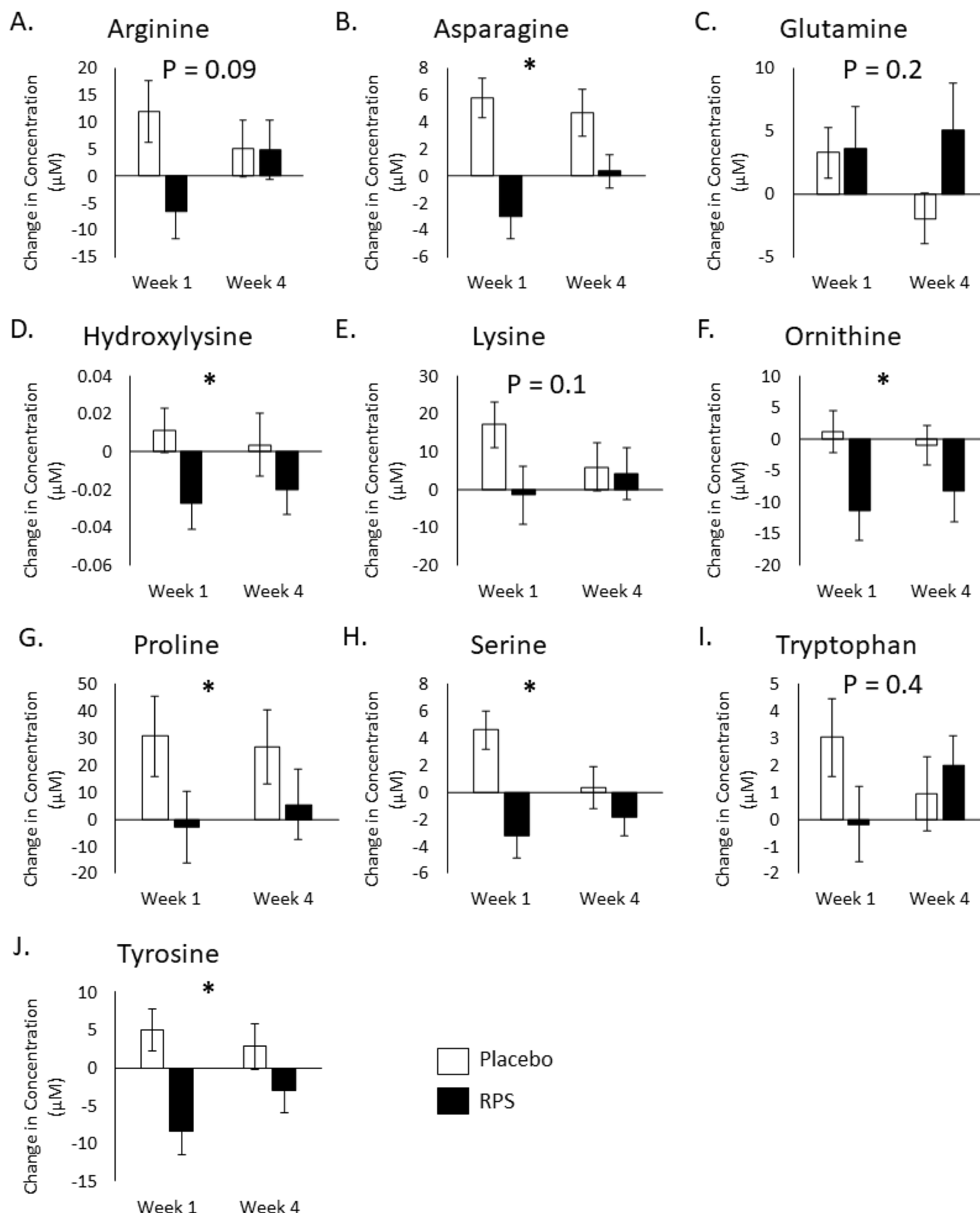


**Fig. 4.** Changes in amino acids elevated in cases of intestinal permeability arginine (A), asparagine (B), glutamine (C), hydroxylysine (D), lysine (E), ornithine (F), proline (G), serine (H), tryptophan (I), and tyrosine (J) were compared between RPS and placebo treatment arms. *, $P < 0.05$.

(Biegański et al., 1983; Naila et al., 2010; Mou et al., 2021; Sánchez-Pérez et al., 2022). Our data suggest that impaired histamine containment within the gut might be at least one factor contributing to HIT and the associated gastrointestinal disturbances. This is consistent with gastrointestinal complaints affecting both healthy and diseased individuals, and contributes to the challenges associated with making HIT diagnoses (Maintz & Novak, 2007; Schnedl et al., 2019). Normal basal plasma histamine levels range from 2.7 to 9.0 nM and symptoms of HIT, including elevated gastric acid secretion and increased heart rate, are reported in people with levels above 9 nM (Maintz & Novak, 2007). RPS consumption led to serum histamine reductions of 8 nM at one week and 5 nM at four weeks, corresponding to 18% and 13% reductions from baseline levels, respectively. To our knowledge, RPS is the first prebiotic shown to lower histamine levels in healthy adults. Notably, RPS-dependent decreases in histamine levels are physiologically relevant to individuals with HIT and related conditions.

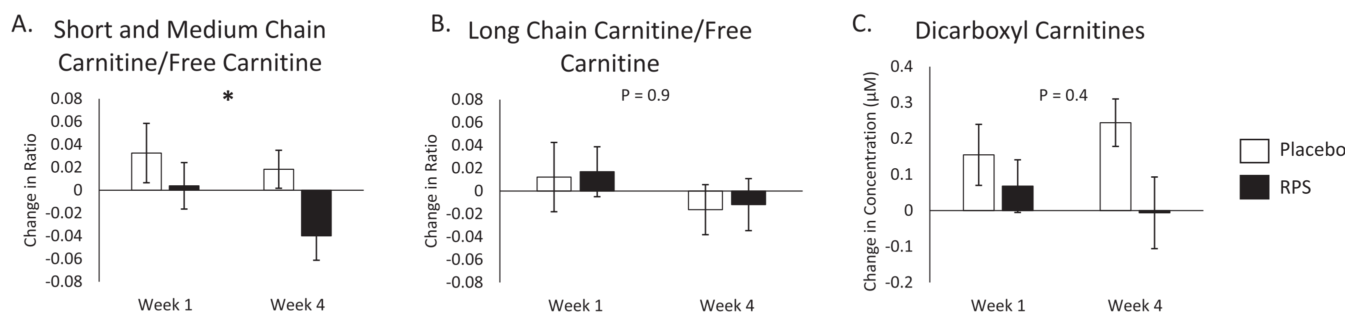


**Fig. 5.** Changes in the ratio of short- and medium-chain carnitines to free carnitine (A), the ratio of long-chain carnitines to free carnitine (B), and dicarboxyl carnitine levels (C) were compared between RPS and placebo treatment arms. *, P < 0.05.

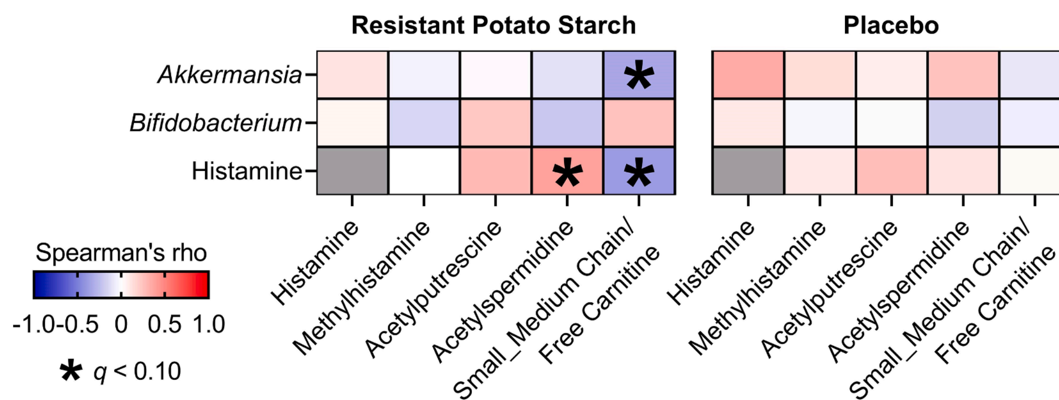


**Fig. 6.** Correlations between changes in *Akkermansia*, *Bifidobacterium*, and metabolomic markers of intestinal permeability histamine, methylhistamine, acetylputrescine, acetylspermidine, and small- and medium-chain carnitine ratios were measured in the resistant potato starch and placebo treatment arms. *, $q < 0.10$.

Consumption of potatoes containing resistant starch decreased serum endotoxin and lactulose/mannitol levels in adults (Cao et al., 2022) and raw potato starch enhanced Claudin-1 while reducing endotoxin levels in ducks (Qin et al., 2023), suggesting that RPS may reduce serum histamine via enhanced intestinal barrier function. RPS significantly increased *Bifidobacterium* and *Akkermansia* in healthy adults (Bush et al., 2023), and *Akkermansia* has been shown to enhance intestinal epithelial barrier integrity *in vitro* (Reunanen et al., 2015; Ottman et al., 2017; Cruz-Lebrón et al., 2021) and counteract high fat diet- and alcohol-induced mucosal barrier dysfunction in mouse models (Everard et al., 2013; Grander et al., 2018). Furthermore, prebiotic, probiotic, and synbiotic ingredients that enhance both *Bifidobacterium* and *Akkermansia* promote enhanced barrier function in various rodent models (Wang et al., 2015; Chen et al., 2020; Oh et al., 2020; Wang et al., 2022; Li et al., 2022).

Neither endotoxins nor dual sugar permeability were measured in this study, but changes in relevant serum metabolites provide an indication that RPS consumption promotes normal barrier function in healthy adults. First, acetylated polyamines have been associated with elevated intestinal permeability (Weiss et al., 2004; Karl et al., 2017) and levels of acetylspermidine decreased in response to RPS. While RPS-dependent changes in acetylputrescine were not statistically significant, decreases in acetylputrescine levels were correlated with reductions in histamine levels only in the RPS treatment arm, directly connecting the changes in relevant metabolites. Stool acetylputrescine levels increase during military training and were correlated with sucralose excretion as a marker of intestinal permeability (Karl et al., 2017). Acetylspermidine levels are elevated in endoscopic biopsies from patients with inflammatory bowel disease and higher inflammatory index scores (Weiss et al., 2004), a condition associated with increased intestinal permeability (Edelblum & Turner, 2009). Acetylated polyamines, including acetylspermidine, are exported from colonocytes via SLC3A2 (Uemura et al., 2008), suggesting that decreases in circulating acetylated polyamines in the RPS arm are due in part to enhanced polyamine metabolism in the colonic epithelium.

Second, RPS administration led to significant decreases in six amino acids previously shown to be elevated in cases of intestinal permeability, including asparagine, hydroxylysine, ornithine, proline, serine, and tyrosine (Yamamoto et al., 2019; Shah et al., 2022; Qin et al., 2023). Levels of asparagine were increased in individuals with intestinal permeability diagnosed via elevated lactulose/rhamnose ratios and pathway enrichment analysis identified significant enrichment in spermidine and spermine metabolism (Shah et al., 2022). Perhaps most informative is the RPS-dependent reduction in hydroxylysine, a modified form of lysine found in various collagen-containing tissues, including the lamina propria (Schofield et al., 1971; Quaroni & Trelstad, 1980; Hendel et al., 1986), and is known as a marker of collagen degradation (Krane et al., 1977; Claus-Walker et al., 1977; Geesin et al., 1986). Reductions in this collagen degradation product support a role for RPS in promoting collagen integrity, in both the lamina propria and other collagen-containing tissues like skin.

Finally, participants in the RPS arm experienced a reduction in the ratio of short- and medium-chain to free carnitines, which is consistent with improvements in mitochondrial β-oxidation (Crakes et al., 2019), and this reduction was significantly correlated with an increase in *Akkermansia*. This ratio has been correlated negatively with *Zo-1* expression in simian immunodeficient virus affected intestinal tissue (Crakes et al., 2019), with increases in short- and medium-chain carnitine levels also positively correlated with intestinal permeability in children with environmental enteric dysfunction (Semba et al., 2017). Medium chain acyl carnitines are also elevated in males with intestinal permeability compared to healthy individuals (Shah et al., 2022), and mitochondrial dysfunction, characterized by excessive short- and medium-chain carnitines, is a primary cause of NSAID-induced

intestinal permeability (Bjarnason et al., 1993).

RPS consumption led to significant reductions in histamine-secreting bacteria, though the mean changes in relative abundance were small and unlikely to explain the histamine reductions in the RPS arm. Collectively, our findings suggest that diverse mechanisms underpin histamine metabolism and intestinal barrier function in healthy people, and that RPS administration is likely working via direct prebiotic effects, indirect effects via the gut microbiota (e.g., shifting dysbiotic communities, enhancing SCFA production via cross-feeding), and via effects independent of the gut microbiome (e.g., RPS granule interaction with the intestinal epithelium or excretion of toxic compounds).

There are several limitations to this study. First, the participants in the clinical trial were healthy individuals for which major diseases and disorders would have constituted exclusion criteria (Bush et al., 2023), so the conclusions here do not represent improvements in a diseased condition. Clinical studies examining the effects of RPS in patients with HIT and/or impaired barrier function are warranted. Second, fecal metabolite analysis was not conducted on samples collected from the participants of this trial, making it difficult to determine the microbial metabolite(s) responsible for these effects. Third, metabolomic changes in the placebo arm suggest that daily consumption of 7 g of digestible starch influences host physiology. While future studies might benefit from using an indigestible carbohydrate as a placebo, many of these substances influence the gut microbiota, making them unsuitable for microbiome studies. Fourthly, we compared effects at week 1 and week 4 collectively, but differences in RPS supplementation duration may influence the metabolites described here. Investigating correlations between changes in metabolites and changes in bacteria levels will help elucidate the role that supplementation duration plays on these effects. Finally, measurements of barrier function, such as quantification of tight junction proteins or administration of a dual sugar intestinal permeability test were not performed. However, the use of dual sugar administration, such as lactulose to rhamnose ratios, to evaluate intestinal permeability are confounded by the fact that lactulose is a rapidly fermenting substance that influences the composition of the gut microbiota (Bouhnik et al., 2004; Riskin et al., 2010; Tayebi-Khosroshahi et al., 2016; Sakai et al., 2019). Further studies of RPS using direct measures of intestinal permeability are warranted.

In conclusion, we performed serum metabolomics analysis to explore the hypothesis that RPS-dependent improvements in constipation and diarrhea, along with increases in *Bifidobacterium* and *Akkermansia*, were functionally related to lower circulating histamine levels. RPS reduces histamine by physiologically relevant levels but had no effect on histamine-degrading enzymes and only modest effects on a subset of histamine-secreting bacterial taxa. Rather, RPS consumption enhanced intestinal barrier integrity, as evidenced by decreases in leaky gut-associated amino acids and improvements in mitochondrial function, which is consistent with other studies using resistant starch from potatoes (Cao et al., 2022; Qin et al., 2023). Taken together, our findings demonstrate the value of metabolomic studies on dietary supplement ingredients and support the importance of supplementing the diet with RPS.

## Clinical Trial Registration

This trial was retrospectively registered at Clinicaltrials.gov as “Effects of Resistant Potato Starch on the Gut Microbiota” with the number NCT05242913 on February 16, 2022.

## Availability of data and materials

The data that support the findings of this study are available from MSP Starch Products Inc., but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of MSP Starch Products Inc. Individual patient data is unavailable as per the informed consent form and the interventional study protocol registration.

## Ethical approval

The study protocol and other related documents were approved by Canadian Shield Ethics Review Board (Burlington, ON) on 29 Oct 2019. This study was conducted in accordance with the protocol and with the consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, applicable ICH Good Clinical Practice guidelines, and applicable local and federal laws and regulations.

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## CRediT authorship contribution statement

**Jun Han:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Edward C. Deehan:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Scott V. Harding:** Writing – review & editing, Validation. **Madhura Maiya:** Writing – review & editing, Validation. **Joshua Baisley:** Writing – review & editing, Validation, Investigation, Conceptualization. **David Schibli:** Writing – review & editing, Validation, Project administration. **David R. Goodlett:** Writing – review & editing, Validation, Project administration.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jason R. Bush is employed by, and Scott V. Harding previously provided consulting services for MSP Starch Products Inc., Carberry, MB, Canada. Joshua Baisley is employed by Nutrasource Pharmaceutical and Nutraceutical Services, Guelph, ON, the company that MSP Starch Products contracted to conduct the clinical trial. Jun Han, David Schibli, and David Goodlett are employed by Genome British Columbia Proteomics Centre, University of Victoria, Victoria, BC, the company that MSP Starch Products contracted to perform metabolomic analyses. MSP Starch Products Inc. sister company McPharma Biotech Inc. holds relevant patents US11058711B2, CA3024201A1, AU2017294806A1, and provisional patent application 63/358,194. The remaining authors declare no conflict of interest.

## Data availability

Data will be made available on request.

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