UC Davis
UC Davis Previously Published Works

Title

Conserved and variable responses of the gut microbiome to resistant starch type 2

Permalink

Authors

Bendiks, Zachary A
Knudsen, Knud EB
Keenan, Michael J
et al.

Publication Date

DOI

Peer reviewed

Conserved and variable responses of the gut microbiome to resistant starch type 2

Zachary A. Bendiks[1], **Knud E.B. Knudsen**[2], **Michael J. Keenan**[3], **Maria L. Marco**[1]

[1]Department of Food Science & Technology, University of California-Davis, Davis, California

[2]Department of Animal Science, Aarhus University, 8830, Tjele, Denmark

[3]School of Nutrition and Food Sciences, Louisiana State University Agricultural Center, Baton Rouge, Louisiana

Abstract

Resistant starch type 2 (RS2) is a dietary fiber comprised solely of glucose which has been extensively studied in clinical trials and animal models for its capacity to improve metabolic and systemic health. Because the health modulatory effects of RS2 and other dietary fibers are thought to occur through modification of the gut microbiome, those studies frequently include assessments of RS2-mediated changes to intestinal microbial composition and function. In this review, we identify the conserved responses of the gut microbiome among 13 human and 35 animal RS2 intervention studies. Consistent outcomes of RS2 interventions include reductions in bacterial alpha-diversity, increased production of lumenal short-chain fatty acids (SCFA), and enrichment of *Ruminococcus bromii, Bifidobacterium adolescentis,* and other gut taxa. However, different taxa are usually responsive in animal models and many RS2-mediated changes to the gut microbiome vary within and between studies. The root causes for this variation are examined with regard to methodological and analytical differences, host genetics and age, species differences (e.g. human, animal), health status, intervention dose and duration, and baseline microbial composition. The significant variation found for this single dietary compound highlights the challenges in targeting the gut microbiome to improve health with dietary interventions. This knowledge on RS2 also provides opportunities to improve the design of nutrition studies targeting the gut microbiome and ultimately identify the precise mechanisms via which dietary fiber benefits human health.

Keywords

microbiota; starch; amylose; RNA; ribosomal; 16S; fatty acids; volatile; gastrointestinal microbiome

Corresponding author: Maria L. Marco, One Shields Avenue, University of California, Davis, CA 95616, Phone: 530-574-4893. mmarco@ucdavis.edu.

1. Introduction

The human gastrointestinal (GI) tract is colonized by approximately 10^{13} bacterial cells [1]. comprised of nearly 2000 species [2] which perform diverse functions including energy harvest, immunomodulation, and regulation of behavior and neurotransmitter production [3–5]. Gut microbiome composition and function are also relevant to the etiology of complex human disorders including inflammatory bowel diseases, type 1 and type 2 diabetes, and cardiovascular disease [6–9]. Although experiments with germ-free mice and human microbiota transfer models have shown that certain bacterial consortia cause increased adiposity and obesity [10–13], a causative role for the gut microbiome in the development of many chronic diseases remains to be demonstrated.

Diet is a major factor determining gut microbiota composition and function [14–17]. The gut microbiomes of individuals consuming a 'Western-style diet' (WSD) high in fats and refined carbohydrates promote increased energy harvest and have a low abundance of fiber-degrading bacteria [14,18]. Conversely, low-fat diets rich in fruits, vegetables, and cereals, such as the Mediterranean diet, are associated with microbiomes adapted towards dietary fiber degradation and production of health-relevant, bacterial metabolites such as short chain fatty acids (SCFA) [14,16,19]. Because healthy diets are linked to a reduced incidence of acute and chronic diseases, significant efforts have been made to identify how those diets alter the gut microbiome.

Fiber is a dietary category that has been of considerable interest for its potential to increase the abundance of health-promoting bacteria in the intestine [20]. In 2008 the Codex Alimentarius Commission defined dietary fibers as "carbohydrate polymers with ten or more monomeric units which are not hydrolyzed by the endogenous enzymes in the small intestine of humans" [21]. This definition includes arabinoxylans, β-glucans, resistant starches, and other digestion-resistant carbohydrates [22]. Dietary fibers reach the colon where some are metabolized by members of the gut microbiota, and these interactions help shape the bacterial composition of the human GI tract [23,24]. In this review we focus on a particular dietary fiber, resistant starch type 2 (RS2), and what is known about how consuming that dietary fiber alters the mammalian gut microbiome.

2. Type 2 Resistant Starch

Starch is one of the most important carbohydrates in plants, where it functions as the primary storage polysaccharide. Due to its presence in staple crops, including potatoes, rice, wheat, and maize [25], starch is likely the most widely consumed carbohydrate in the human diet. Starch is a glucose heteropolysaccharide comprised of both amylose and amylopectin. Amylose is an α–1,4-linked linear chain whereas amylopectin is a branched polymer composed of approximately 95% α–1,4 and 5% α–1,6 linkages [26]. Starches resist digestion due to being embedded in a food matrix or seed (resistant starch type 1), forming compact granules that resist digestive enzymes (resistant starch type 2), cooking and cooling with subsequent retrogradation (resistant starch type 3), food manufacturing-induced chemical modifications (resistant starch type 4), or forming complexes with lipids (resistant starch type V) [27]. Most resistant starches rely on interactions with nearby dietary

components or additional processing of the native starch structure to achieve their digestion-resistant properties. However, resistant starch type 2 (RS2) is naturally enriched in amylose, causing the starch to form tightly packed granules which are inaccessible to digestive enzymes [26].

In humans, RS2 has been mainly investigated for its effects on host metabolic health. A review of 30 human studies concluded that RS2 from high-amylose cereal grains attenuates acute postprandial glucose and insulin responses when replacing rapidly digestible carbohydrates [28]. However, this review also argued that there is insufficient evidence to claim RS2 improves long-term insulin resistance or sensitivity [28]. A separate systematic review and meta-analysis of 13 case-control human studies concluded that RS2 improves fasting glucose and insulin levels as well as insulin sensitivity in diabetics and overweight subjects [29]. Additionally, a meta-analysis of 14 human studies, encompassing 20 trials, found that resistant starch consumption lowers total serum cholesterol and low-density cholesterol [30]. While RS2 was not the sole focus of this meta-analysis, 18 of the 20 trials used RS2 [30]. RS2 derived from high-amylose maize has been a particular focus of human studies, and the beneficial outcomes led the FDA to approve the qualified health claim that "high-amylose maize resistant starch may reduce the risk of type 2 diabetes" (https://www.regulations.gov/docket?D=FDA-2015-Q-2352).

However, not all human studies have shown health benefits with RS2 consumption. For example, RS2 did not improve fasting or long-term glycemic control, ectopic fat, energy expenditure, or any other cardiovascular risk factors in a randomized controlled trial with pre-diabetic adults [31]. A meta-analysis of 22 randomized control trials showed that while RS2 significantly reduces serum triglycerol concentrations in healthy subjects and body weight in patients with type 2 diabetes mellitus, it otherwise conferred limited cardiometabolic benefits when supplemented for 12 weeks or less [32]. It was also noted that the health benefits described were skewed by the small number of studies which reported positive results [32]. Furthermore, the inclusion of RS2 in diets low in total carbohydrates was found to increase serum levels of trimethylamine-N-oxide (TMAO) [33], a microbiota-derived metabolite associated with cardiovascular disease [34] and chronic kidney disease (CKD) risk [35].

Examination of RS2 effects in animal models have also shown metabolic benefits in some studies but not others. In aged C57Bl/6J mice, RS2 was found to improve glucose sensing and motor coordination [36] and increase colonic peptide YY (PYY) and proglucagon gene expression [37]. Zucker Diabetic Fatty rats fed RS2 for 9 weeks had delayed onset of diabetes symptoms [38], possibly due to RS2-enhanced glucose metabolism and improved pancreatic function [39,40]. RS2 was also shown to potentially alleviate CKD in Sprague Dawley rats [41,42], and those findings might have been due to reducing the expression of host-derived cecal proteins associated with aldehyde metabolism and the humoral immune response [43]. However, RS2 increased urine trimethylamine (TMA) in two C57Bl/6J mouse studies [44,45], indicating a similar diet-dependent response to what was observed in humans [33]. Two other rodent studies also reported that RS2 consumption was associated with anxiety-like behaviors [46,47]. Although many human and animal studies have shown

that RS2 confers health benefits, more work is needed to understand any potential deleterious effects.

3.   The Mammalian Gut Microbiome Following RS2 Consumption

RS2 was first shown to alter the bacterial composition in the distal intestine in 1997 [48]. Since then, there have been at least 13 human studies and 35 animal RS2-focused studies that included assessments of the gut microbiome composition and/or function (Table 1). Although most of those investigations relied on community16S rRNA marker gene sequencing, other techniques such metagenomics, metaproteomics, and metabolomic analyses were increasingly employed (Table 1). The large number of studies on RS2 provides an opportunity for cross-study comparisons assessing how a single dietary ingredient impacts the gut microbiome.

3.1   Changes to bacterial diversity

Some effects of RS2 consumption on intestinal microbial ecology are consistent across studies. At least four human and ten animal studies [40,44,49–61] found reductions in fecal bacterial richness and/or evenness, referred to as alpha-diversity (Table 1). The lower alpha-diversity was presumably due to the enrichment of a subset of gut taxa that efficiently metabolized the RS2 polysaccharides and/or degradation products. Additionally, RS2 was repeatedly found to alter bacterial beta-diversity in the distal intestines of humans and animals [40,41,44,49,53,55,58,61–63], showing that RS2 changes the global structure of the microbiome.

Although RS2 consumption is associated with phylum-level changes in fecal bacterial composition, those changes are not consistent between studies. Human microbiomes are dominated by two phyla - the Firmicutes and Bacteroidetes [64]. While RS2 intake correlated with a lower Firmicutes to Bacteroidetes ratio in elderly human subjects [56] and in several rodent studies [43,44,60,61,65], a higher Firmicutes to Bacteroidetes ratio was found for subjects with insulin resistance and increased body mass index (BMI) [66] as well as middle-aged individuals [56]. Two studies also reported that Actinobacteria was the most responsive phylum to RS2 supplementation [57,58].

3.2   Enrichment of specific bacterial taxa in the human gut

RS2 consumption typically alters the abundance of at least some intestinal bacterial genera and species (Table 2). Certain taxonomic groups were commonly enriched (*Ruminococcus bromii, Bifidobacterium adolescentis, Faecalibacterium prausnitzii, Eubacterium*), others varied between studies (*Ruminococcus* and *Ruminococcaceae, Lactobacillus, Bacteroides*), and the proportions of members of the Clostridiales class (*Oscillospira, Lachnospiracea, Blautia*), were frequently reduced as a consequence of RS2 intake (Table 2). Importantly, the relative abundance of no single taxonomic group was significantly altered in all investigations. For the purpose of discussion here, taxa that were significantly enriched with RS2 intake in at least two human studies are discussed in more detail below.

The proportions of the *Ruminococcaceae* family and the *Ruminococcus* genus were elevated with dietary RS2 supplementation in multiple human and animal studies (Table 2). At the

species level, *Ruminococcus bromii* abundance was increased in five human studies [58,62,63,67,68], four rat studies [43,55,69,70] and one pig study [59]. Experiments in an *in vitro* model of the human colon showed that *R. bromii* degrades [U-13C]-labelled potato starch [71]. A follow-up study concluded that *R. bromii* is a keystone species for resistant starch metabolism [72]. More recently, metagenome analysis showed that *R. bromii* is one of the most RS2-responsive taxa in human subjects and this species contributes large fractions of the amylases and a glucan-branching enzyme associated with RS2 degradation [73]. Human and rumen-associated *R. bromii* isolates appear to be well-adapted for growth on starch and act as primary RS2 degraders [74,75], which could explain why this species is enriched in the intestine during many RS2 intervention studies. Other gut-associated *Ruminococcus* that do not degrade RS2, such as *Ruminococcus gnavus* might also benefit from *R. bromii* RS2 metabolism by consuming glucose and malto-oligosaccharides released from the starch granule [76]. Overall there is strong *in vitro* and *in vivo* evidence supporting the role of *R. bromii* as a primary RS2 degrader in the human gut.

Bifidobacterium was also enriched during RS2 interventions in numerous human and animal studies (Table 2). For human trials in which *Bifidobacterium* was identified at the species level, *B. adolescentis* was the most commonly enriched *Bifidobacterium* species [56,58,63,68]. *B. adolescentis* is likely a primary RS2 degrader, as indicated by the growth of strains of that species on RS2 as a sole carbon source [77] and the ability to adhere directly to $\alpha-1,4$-linked glucose units within starch granules [78]. These physical interactions could provide a protective barrier; a finding which may explain why RS2 increases the *in vivo* survival of amylolytic bifidobacteria in the GI tract [79]. Additionally, *B. adolescentis* increases butyrate production by *Eubacterium hallii* and *Anaerostipes caccae* when co-cultured on an RS2 substrate [80], and a similar interaction was reported when *B. adolescentis* was co-cultured with *F. prausnitzii* [81]. These effects are likely due to metabolic cross-feeding resulting in butyrate and energy production by microorganisms able to utilize lactate and acetate produced by *B. adolescentis* during growth on RS2 [80]. However, not all *B. adolescentis* strains have the capacity to grow on starch or starch derivatives [82], and therefore this capacity should not be generalized across all members of the species.

The proportions of *Eubacterium rectale* were increased after maize RS2 consumption in two human studies [66,67] (Table 2). Furthermore, *E. rectale* was shown to degrade starch *in vitro* and shows a preference for maize starch over potato starch [83]. *E. rectale* is a commensal bacterium that has long been associated with butyrate production [84] and was recently proposed to use RS2 degradation products to form butyrate in the human colon [63]. Furthermore, *in vitro* experiments have directly implicated *E. rectale* in the trophic chain of starch degradation [71]. An investigation on RS2-mediated butyrate responses in humans found that *E. rectale* proportions were elevated in the subset of individuals who displayed high butyrate levels throughout the study [68], and the abundance of this species was recently correlated with potato RS2-mediated increases in fecal butyrate production [63]. These results suggest that *E. rectale* contributes to the butyrogenic effect of RS2 and may also contribute to primary degradation of RS2 in the gut.

F. prausnitzii is a numerically dominant human commensal and is considered to be one of the most important butyrate producers in the human gut [85]. Two human studies have reported increases of this species during RS2 consumption [66,86] (Table 2). A multi-omics investigation with human subjects proposed that *F. prausnitzii* is a primary degrader of RS2 in addition to producing butyrate [66]. However, *F. prausnitzii* is unable to grow on purified starch [87] and is less efficient at degrading maize RS2 than *B. adolescentis* or *R. bromii* [88], suggesting it would be outcompeted for RS2 metabolism by neighboring gut microbes. These findings indicate that the enrichment of *F. prausnitzii* with RS2 might be due to cross-feeding [81] rather than metabolizing RS2 polysaccharides directly.

RS2-mediated enrichment of members of the Bacteroidetes phylum (*Bacteroides,* S24–7*, Rikenellaceae, Prevotella*) is commonly observed in rodent studies but has only been reported in two human trials [56,57] (Table 1). In one study, the proportions of *Prevotella* and *Alistipes* were significantly higher in elderly subjects consuming RS2, but neither genus was responsive to RS2 in middle-aged adults [56]. A metaproteomics investigation in humans found increased expression of *Bacteroides vulgatus* enzymes associated with RS2 polysaccharide breakdown during RS2 consumption as well as butyrate kinases [66]. Increases in Bacteroidetes after fiber consumption could be due to the presence of sus loci (starch utilization system) in mammalian gut-associated members of this phylum [89]. The *sus* genes encode proteins required for extracellular breakdown, import, and metabolism of starch polysaccharides [90]. Because there does not appear to be a host-specific association of *sus* loci, it is not clear why Bacteroidetes are more consistently responsive to RS2 in the rodent microbiome than the human microbiome.

3.3 Metabolic and functional changes

Seven human and twenty animal RS2 studies reported significant increases in intestinal or systemic levels of SCFA, namely acetate, propionate, and/or butyrate (Table 1). RS2 consumption also increased the presence of genes for butyrate production in the human gut metaproteome [66]. Those genes were associated with *F. prausnitzii* conversion of acetyl-coA to butyrate via the acetyl-CoA:butyryl-CoA transferase pathway and *B. vulgatus* and *Coprococcus* spp. conversion of butanoyl-phosphate to butyrate with butyrate kinase [66]. However, SCFA levels following RS2 consumption show significant subject-subject variation [68,91] and some animal interventions have reported no change [49,92] or reduced concentrations [44,93] after RS2 feeding. Importantly, fecal SCFA concentrations represent the cumulative effects of absorption, production, and microbial cross-feeding in the colon and may not be representative of SCFA production throughout the GI tract [94].

Besides changes in SCFA production, RS2 has been associated with alterations in carbohydrate metabolism by the gut microbiota. Metaproteomic analysis performed on human fecal samples revealed higher numbers of carbohydrate metabolism and transport systems with RS2 supplementation [66]. There was also an enrichment in uncharacterized polyketides as well as the enzyme phosphoenolpyruvate carboxykinase involved in pyruvate metabolism [66]. In another study, KEGG Orthology and CAZyme annotations of metagenomics data showed that starch-degradative enzymes, such as amylases and polysaccharide binding proteins, were increased after RS2 was introduced into the diet [73].

R. bromii contributed significant fractions of the enzymes associated with starch degradation, although starch degradative enzymes from other bacteria were also increased albeit in lower amounts [73].

4. Sources of variation in microbial composition in RS2 studies

4.1 Methodological variation

It is well understood that study-to-study comparisons of the gut microbiota are hindered by a variety of procedural and technical factors [95,96]. 16S rRNA DNA sequencing is a frequently used method for investigating the bacterial composition of the gut microbiome, including studies investigating RS2 (Table 1). However, no standardized protocols for sample preparation and 16S rRNA gene sequence analysis exist. There is also variation between sample collection, storage, and homogenization methods, DNA extraction protocols, 16S rRNA gene variable regions targeted, PCR conditions, and the DNA sequencing platform used [97–99]. Additionally, numerous analysis software applications are available and employ different filtering cut-offs for low-abundance features or outlier samples, normalization strategies for sample-sample comparisons, levels of depth for taxonomic resolution, and statistical tests for identifying significant differences between experimental groups [100,101]. For example, most 16S rRNA studies of the microbiome clustered similar reads into operational taxonomic units (OTUs) that have relatively low taxonomic resolution. Newer methods resolve reads at the single nucleotide level into amplicon sequence variants (ASVs) with improved taxonomic resolution at the sub-family level [102,103]. However, even with those sample preparation and analysis discrepancies, it is notable that inter-individual variation is frequently much greater than variation introduced by methodological differences [104]. Moreover, certain RS2-enriched taxa including *R. bromii* and *Bifidobacterium* were repeatedly found in different human and animal studies even though different methods were used (Table 1 & 2). These findings indicate that, despite methodological differences, 16S rRNA gene surveys are sufficiently robust to identify the dominant changes to the gut microbiota with RS2 consumption.

4.2 Vertebrate host species and host genetics

RS2-responsive taxa can be grouped based on host species origin. For example, *F. prausnitzii, Ruminococcus* [105], and *Bifidobacterium* [106] are more frequently associated with human GI tract microbiomes compared to animal models (Table 2). On the other hand, *Lactobacillus* and *Turicibacter* are more commonly associated with mouse microbiomes [106]. Compared to animal studies, the relative contributions of RS2 to gut microbiome structure is comparatively smaller in humans and inter-individual variations between subjects often dominate the findings [66,68]. The more limited effect of RS2 supplementation among human subjects is likely due to dietary and genetic differences between people and lower quantities of RS2 consumed (Table 2). However, despite the taxonomic differences between humans and animal models, RS2 consistently enriched bacteria with the enzymatic capacity to metabolize RS2. For example, *Bacteroides* and *Lactobacillus* were indicated to be the primary degraders of dietary polysaccharides in the mouse gut [107], and primary RS2 degradation in human microbiomes was proposed to be governed by *R. bromii* [63,66], *F. prausnitzii* and *B. vulgatus* [73], and *Bifidobacterium* spp.

[63]. Hence, the enzymatic capacity to break down RS2 is shared between different bacterial taxa that are host species specific.

Differences in host-species genotype are also associated with gut microbiome composition. A recent study showed that higher endogenous copy numbers of *AMY1*, a gene encoding a salivary amylase, are associated with increased *Ruminococcaceae* and *Ruminococcus* abundance and SCFA production in the human gut [108]. Metagenomic analysis showed that the gut microbiomes of subjects with high *AMY1* copy numbers were depleted in glycoside hydrolases and polysaccharide lyases relative to subjects with low *AMY1* copy numbers [108]. Interestingly, ex-germ-free mice that received the fecal microbiota from high *AMY1* copy number subjects had significantly higher adiposity than mice that were transplanted with the fecal microbiota from low-copy number subjects [108]. This difference in amylase gene content and the associated variation in *Ruminococcus* abundance and gut function could affect the quantity of RS2 reaching the large intestine and the degree to which RS2 is metabolized by the microbiome.

4.3 Health status

Host health is an important determinant of gut microbiome composition [109,110]. For example, obesity is associated with reductions in gut bacterial diversity [111] and an enrichment of taxa which can increase energy harvest from the diet [10,112]. Therefore, host physiology, and the impact it confers on gut microbiome composition could influence how RS2 changes the gut microbial ecosystem. Among the human studies performed thus far, four out of seven RS2 trials conducted in healthy adult subjects reported significant increases in *R. bromii* [62,63,67,68]; whereas an RS2 intervention performed in stunted Malawi children reported a significant increase in *Lactobacillus* but no change in *R. bromii* abundance [57]. In human studies where *F. prausnitzii* was identified as an RS2 responsive taxon, this species was elevated with RS2 intake among insulin-resistant subjects with high BMI [66] and end-stage renal disease patients [86]. In animal models, RS2 supplementation in CD obesity-prone rats resulted in increased cecal fermentation and alterations to gut microbiome composition compared to obesity-resistant rats [65]. Those differences indicate that host health and physiological status influence which intestinal taxa are enriched by RS2.

4.4. Subject age

Aging is also associated with significant changes to gut microbiome structure and function [113]. For example, the microbiomes of healthy adults have relatively high ratios of Firmicutes to Bacteroidetes, but this ratio can decrease with age [114]. Middle-aged and elderly subjects given a RS2 supplement for three months showed significant age-associated differences in fecal microbiome composition [56]. RS2 supplementation increased *Bifidobacterium* abundance and lowered the Firmicutes/Bacteroidetes ratio in both age groups compared to age-matched placebo groups, but only elderly subjects showed significantly more fecal butyrate at the end of the study. Furthermore, *Bifidobacterium* increases were the only conserved taxonomic response across both age groups. [56]. Hence, age-related physiological changes might also influence RS2-associated niche occupation by members of the gut microbiota but more work is needed to further explore this association.

4.5   RS2 source and quantities consumed

Most studies have used RS2 from either high-amylose maize or potatoes (Table 2). Those sources of RS2 are distinct because maize starch forms granules that are smaller than potato starch granules, maize starch has less amylose overall than potato starch, and the amylose within maize starch has a lower degree of polymerization than potato starch amylose [115]. Although modifications could be introduced to alter RS2 content prior to or after ingredient purchase, differences in granule structure help explain why maize starch can have higher *in vitro* total digestibility (i.e. less RS) than potato starch [116].

Differences in physicochemical properties between RS2 sources likely influence intestinal microbiota responses. A recent human trial comparing the effects of RS2 from potato starch and high-amylose maize found that only potato RS2 significantly increased the fecal concentrations of butyrate and acetate [63]. Additionally, potato RS2 was associated with increased fecal *Bifidobacterium* levels while maize RS2 conferred increases in *R. bromii* proportions [63]. Those findings are consistent with the observation that increases in *B. adolescentis* levels have thus far only been observed in human studies where potato RS2 was consumed [56,58,63,68] (Table 1). In pigs, potato and high-amylose maize RS2 consumption both resulted in increased SCFA concentrations in the portal blood, however, only potato RS2 conferred increased portal blood butyrate levels [117]. Furthermore, the two RS2 sources differed in their effects on SCFA production in the cecum and colon of Spague Dawley rats [118]. That study also reported that wheat RS2 enriched lactic acid-producing bacteria from clostridial cluster XVI while maize RS2 enriched members of clostridial cluster XIVa, specifically *Eubacterium ventriosum* and *Roseburia cecicola* [118]. Differences in RS source might influence health outcomes as was shown in a Spague Dawley rat study which tested the capacities of potato RS2, maize RS2, and butyrylated maize RS2 to mitigate the deleterious effects of a high-protein meat diet [55]. Each diet resulted in a distinct gut microbiome composition, and maize RS2 and butyrylated maize RS2 shifted fecal fermentation patterns towards carbohydrate metabolism. In that study, maize RS2 was unable to mitigate the expression of an oncogenic miRNA cluster associated with colorectal cancer, while potato RS2 and butyrylated maize RS2 significantly reduced the expression of those miRNA [55].

The quantities of dietary RS2 are also associated with changes to GI tract microbial composition and the production of microbiota-derived metabolites [33,119,120]. A study comparing diets with 0, 18, or 36% w/w RS2 by weight in aged C57BL/6J mice found dose-dependent differences in microbial composition [119]. *Bifidobacterium* proportions were significantly increased with both 18 and 36% w/w RS2; whereas *Allobaculum* and *Akkermansia* were enriched in mice fed 36% w/w RS2. Dose-dependent differences in cecal pH and SCFA levels were found in Sprague-Dawley rats consuming diets with 10, 20, 30, or 40% w/w RS2 [121]. Diets containing at least 10% w/w RS2 resulted in significantly reduced DNA damage in rat colonocytes and this outcome was found to be dose-dependent and correlated with cecal butyrate concentrations [121].

4.6 Study duration

There is considerable variation in the literature with regard to the length of RS2 interventions. Human and animal model studies have ranged from as short as 4 hours to well over 200 days (Table 2). Although the human gut microbiota responds to dietary shifts in as little as one day [16,122], long-term dietary patterns have a greater impact on gut composition and microbiota-derived metabolite production [123,124]. To this regard, a meta-analysis concluded that the beneficial effects of RS2 on serum total cholesterol and LDL cholesterol were most prominent when RS2 was administered for 4 weeks or longer [30]. A study with C57BL/6J mice found that the concentrations of fecal acetate increased between day 3 and day 11 after the initiation of RS2 feeding [125]. However, more work is needed to understand short- and long-term microbiome dynamics during RS2 consumption.

4.7 Host diet

Dietary components consumed alongside RS2 might influence the extent to which RS2 affects the gut microbiome and health. For example, a cross-over trial comparing the effects of RS2 when combined with low carbohydrate (39–40% energy)/high fat or high carbohydrate (51–53% energy)/low fat diets found that subjects consuming high amounts of RS2 with the low carbohydrate/high fat diet had significantly higher levels of serum TMAO [33]. It is presently not clear whether it was the quantities of fat in the diet or inclusion of other carbohydrates that were most important in influencing study outcomes. It is well established that high-fat diets alter the composition of the mammalian gut microbiome [126,127]. For example, a study with healthy young adults reported that consumption of a high-fat diet reduced fecal SCFA concentrations and the abundance of *Faecalibacterium* [128]. Sprague-Dawley rats fed RS2 combined with a high-fat diet, as opposed to a low-fat diet, had lower cecal weights, thereby indicating lower levels of intestinal microbial fermentation [129]. Alternatively, a human study combining RS2 and β-glucan yielded greater improvements in postprandial insulin and glucose responses than either fiber individually in healthy and overweight women [130]. In pigs, the GI tract fermentation characteristics with less digestible, recalcitrant dietary fibers (e.g. non-glucosyl polysaccharides from rapeseed meal) were altered by the presence of other more readily fermented fibers (β-glucans) [131].

Besides the potential effects of combining RS2 with other carbohydrates, there is evidence that dietary proteins also interact with RS2 in ways that can affect the outcomes of dietary interventions. Proteins can escape digestion and reach the colon where they are metabolized by the gut microbiota [132]. The amount of dietary nitrogen in is a major determinant of gut composition [133]. To this regard, ammonia derived from endogenous urea was shown to influence the response of the rat microbiome to RS2 [70]. Interestingly, a study with Sprague-Dawley rats which utilized resistant potato protein and RS2 in a factorial design reported significant interaction effects between potato protein and RS2 on SCFA concentrations throughout the GI tract [134]. The inclusion of dietary red meat fed to Balb/c mice inhibited the responses of specific gut taxa to RS2, including *Ruminococcus* and *F. prausnitzii* [135]. However, it is not presently clear how dietary proteins alters RS2 metabolism by the gut microbiota.

Beyond the influence of individual dietary components, dietary patterns likely also shape the ecological interactions between RS2 and an individual's gut microbiota. For example, *Prevotella* abundance is associated with consumption of plant-rich diets [136], and further stimulation of those bacteria with RS2 might be dependent on the extent to which other plant-based foods are consumed. To this regard, when germ free mice received human donor microbiota and were then supplemented with RS2, mice containing donor microbiota which initially contained low levels of *Ruminococcaceae* and *Faecalibacterium* and high levels of *Prevotellaceae* showed the greatest RS2-mediated change in microbial composition and cecal butyrate [137]. Therefore, it may be useful to define microbial enterotypes prior to the initiation of RS2 interventions, particularly the identification of *Prevotella*-dominant enterotypes [138]. This could help predict which individuals will show the greatest benefits from RS2 consumption.

4.8 Baseline Gut Microbiome Composition and Microbial Responsiveness

The gut microbiome composition prior to the start of an intervention (baseline) is a predominant factor driving inter-individual responses to RS2 consumption. To this regard, differences in baseline fecal bacterial composition were directly associated with fiber (barley kernel)-mediated improvements in glucose metabolism [139]. In another human study, strong associations were found between the baseline abundances of *Streptococcus*, *Ruminococcus torques*, *Eubacterium halii*, and *Eubacterium eligens* and RS2-mediated hormonal and physiological changes in humans, including GLP-1 and insulin secretion [140]. Interestingly, a study in which obese subjects were fed resistant starch type 3 reported a 2.5-fold variation in alpha diversity indices, and this variation was attributed to baseline differences between the individuals [141]. While not an RS2 study, this finding highlights that baseline differences can influence the microbiological outcomes of resistant starch interventions.

Another source of variation is the responsiveness of the gut microbiome to diet-induced alterations. This was indicated for RS2 by Venkataraman et al. who classified the butyrogenic response of human subjects to RS2 consumption as low, high, or enhanced following a seven-day intervention [68]. They found that subjects with 'enhanced' butyrogenic responses, whose fecal butyrate levels increased the greatest amount during RS2 intervention, also showed significant increases in the proportions of *B. adolescentis* and *R. bromii* during the intervention period. Subjects in the 'high' group, whose baseline and post-intervention butyrate concentrations were high, were enriched in *B. adolescentis, R. bromii,* and the butyrate-producer *Eubacterium rectale*. Interestingly, the abovementioned species were present in similar proportions among all three response groups prior to the RS2 intervention, indicating that the observed variation in butyrate production was driven by the responsiveness of RS2-associated taxa to the diet intervention. However, the factors that drive the responsiveness of individual taxa to diet interventions are not well understood.

5. Conclusions and future directions

Considerable attention has been given to the physiological and ecological effects of RS2 in the GI tract. It is clear that RS2, along with other dietary fibers, are important constituents of

the human diet that modify the gut microbiota. It is also increasingly understood that people have highly individualized responses to RS2 [66] and that stratifying individuals based on their responses to RS2, such as fecal SCFA and undigested RS2 levels, shows associations between microbiome composition and health-relevant outcomes [68]. However, this inter-individual variation poses a significant research challenge because it hinders statistical power and obscures biological signals with noise. Furthermore, caution is required to avoid over-stating results obtained in one study or for groups of individuals within a study, as they cannot be generalized to RS2 or other fermentable fiber as a whole. Adding to the challenges are the differences between the gut microbiota responses to RS2 in humans and animal models, thereby hindering mechanistic studies of RS2-mediated health outcomes. Lastly, without an understanding of how environmental, host (e.g. health status, age, genetics), study design, and methodological factors affect assessments of RS2-induced microbiome changes, our ability to predict whether RS2 is an appropriate microbiome-modulatory fiber for personalized nutrition interventions is limited. Future studies on RS2 and other dietary fibers should take each of those variables into account.

More work is needed to fully understand the other factors that impact microbe-RS2 interactions. For example, sex is an important determinant of the gut microbiome [24] but only a single human study to our knowledge has described RS2 responses between men and women [58]. Furthermore, to better predict how individuals will respond to RS2 interventions it would be beneficial to identify microbial composition and classifications of enterotypes at baseline in addition to collecting host metadata, such as long-term dietary habits, that can be related to downstream responses. Studies should not only examine the composition of the gut microbiome, but also explore functional and metabolomic changes to provide a more comprehensive understanding of the conserved intestinal responses to RS2. Finally, the development of quantitative microbiome profiling techniques allows investigators to enumerate cell numbers rather than relative abundances [142]. It is possible for bacterial proportions to be identical across two habitats while the overall cell counts vary drastically, and this could impact SCFA concentrations, acidification of the gut environment, the degree of RS2 degradation, and the potential health effects. Ultimately, the extensive number of studies on RS2 have provided unique insight into how a single dietary ingredient alters the gut microbiota. Because of this knowledge, new studies on RS2 should now use and build upon those findings to investigate how diet-based approaches can benefit human health by targeting the gut microbiome.

Acknowledgments

Abbreviations:

RS2 resistant starch type 2

SCFA	short-chain fatty acids
GI	gastrointestinal
CKD	chronic kidney disease
WSD	Western-style diet
PYY	Peptide YY
BMI	Body mass index
TMAO	Trimethylamine-N-oxide
PD	phylogenetic diversity

References

[1]. Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. Cell 2016;164:337–40. 10.1016/j.cell.2016.01.013. [PubMed: 26824647]

[2]. Almeida A, Mitchell AL, Boland M, Forster SC, Gloor GB, Tarkowska A, et al. A new genomic blueprint of the human gut microbiota. Nature 2019;568:499–504. 10.1038/s41586-019-0965-1. [PubMed: 30745586]

[3]. Cornejo-Pareja I, Muñoz-Garach A, Clemente-Postigo M, Tinahones FJ. Importance of gut microbiota in obesity. Eur J Clin Nutr 2018 10.1038/s41430-018-0306-8.

[4]. Arleevskaya MI, Aminov R, Brooks WH, Manukyan G, Renaudineau Y. Editorial: Shaping of human immune system and metabolic processes by viruses and microorganisms. Front Microbiol 2019;10 10.3389/fmicb.2019.00816.

[5]. Zheng P, Zeng B, Liu M, Chen J, Pan J, Han Y, et al. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. Sci Adv 2019;5 10.1126/sciadv.aau8317.

[6]. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat Microbiol 2019;4:293–305. 10.1038/s41564-018-0306-4. [PubMed: 30531976]

[7]. Kostic AD, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hämäläinen A-MM, et al. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. Cell Host Microbe 2015;17:260–73. 10.1016/j.chom.2015.01.001. [PubMed: 25662751]

[8]. Hartstra A V, Bouter KEC, Bäckhed F, Nieuwdorp M Insights into the role of the microbiome in obesity and type 2 diabetes. Diabetes Care 2015;38:159–65. 10.2337/dc14-0769. [PubMed: 25538312]

[9]. Jie Z, Xia H, Zhong SL, Feng Q, Li S, Liang S, et al. The gut microbiome in atherosclerotic cardiovascular disease. Nat Commun 2017;8 10.1038/s41467-017-00900-1.

[10]. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–31. 10.1038/nature05414. [PubMed: 17183312]

[11]. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 2009;1:1–12. 10.1126/scitranslmed.3000322.

[12]. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013;341 10.1126/science.1241214.

[13]. Ussar S, Griffin NW, Bezy O, Bry L, Gordon JI, Kahn CR, et al. Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and metabolic syndrome. Cell Metab 2015;22:1–15. 10.1016/j.cmet.2015.07.007. [PubMed: 26154048]

[14]. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 2010;107:14691–6. 10.1073/pnas.1005963107. [PubMed: 20679230]

[15]. Claesson MJ, Jeffery IB, Conde S, Power SE, O'connor EM, Cusack S, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012;488:178–84. 10.1038/nature11319. [PubMed: 22797518]

[16]. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505:559–63. 10.1038/nature12820. [PubMed: 24336217]

[17]. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, et al. Environment dominates over host genetics in shaping human gut microbiota. Nature 2018;555:210–5. 10.1038/nature25973. [PubMed: 29489753]

[18]. Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 2008;3:213–23. 10.1016/j.chom.2008.02.015. [PubMed: 18407065]

[19]. De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, et al. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. Gut 2016;65:1812–21. 10.1136/gutjnl-2015-309957. [PubMed: 26416813]

[20]. Zhao L, Zhang F, Ding X, Wu G, Lam YY, Shi Y, et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. Science 2018;359:1151–6. 10.1126/science.aao5774. [PubMed: 29590046]

[21]. Codex Alimentarius Commission. Report of the 30th session of the Codex Committee on nutrition and foods for special dietary uses. 2009.

[22]. Stephen AM, Champ MMJ, Cloran SJ, Fleith M, Van Lieshout L, Mejborn H, et al. Dietary fibre in Europe: Current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. vol. 30 2017 10.1017/S095442241700004X.

[23]. Tasse L, Bercovici J, Pizzut-Serin S, Robe P, Tap J, Klopp C, et al. Functional metagenomics to mine the human gut microbiome for dietary fiber catabolic enzymes. Genome Res 2010;20:1605–12. 10.1101/gr.108332.110. [PubMed: 20841432]

[24]. Dominianni C, Sinha R, Goedert JJ, Pei Z, Yang L, Hayes RB, et al. Sex, body mass index, and dietary fiber intake influence the human gut microbiome. PLoS One 2015;10:1–14. 10.1371/journal.pone.0124599.

[25]. Tester RF, Karkalas J, Qi X. Starch - Composition, fine structure and architecture. J Cereal Sci 2004;39:151–65. 10.1016/j.jcs.2003.12.001.

[26]. Gallant DJ, Bouchet B, Buleon A, Perez S. Physical characteristics of starch granules and susceptibility to enzymatic degradation Eur. J. Clin. Nutr, MacMillan Press Ltd.; 1992, p. S3–16.

[27]. Fuentes-Zaragoza E, Sánchez-Zapata E, Sendra E, Sayas E, Navarro C, Fernández-Lõpez J, et al. Resistant starch as prebiotic: A review. Starch/Staerke 2011;63:406–15. 10.1002/star.201000099.

[28]. Harris KF. An introductory review of resistant starch type 2 from high-amylose cereal grains and its effect on glucose and insulin homeostasis. Nutr Rev 2019;0:1–17. 10.1093/nutrit/nuz059.

[29]. Wang Y, Chen J, Song YH, Zhao R, Xia L, Chen Y, et al. Effects of the resistant starch on glucose, insulin, insulin resistance, and lipid parameters in overweight or obese adults: a systematic review and meta-analysis. Nutr Diabetes 2019;9 10.1038/s41387-019-0086-9.

[30]. Yuan HC, Meng Y, Bai H, Shen DQ, Wan BC, Chen LY. Meta-analysis indicates that resistant starch lowers serum total cholesterol and low-density cholesterol. Nutr Res 2018;54:1–11. 10.1016/j.nutres.2018.02.008. [PubMed: 29914662]

[31]. Peterson CM, Beyl RA, Marlatt KL, Martin CK, Aryana KJ, Marco ML, et al. Effect of 12 wk of resistant starch supplementation on cardiometabolic risk factors in adults with prediabetes: A randomized controlled trial. Am J Clin Nutr 2018;108:492–501. 10.1093/ajcn/nqy121. [PubMed: 30010698]

[32]. Snelson M, Jong J, Manolas D, Kok S, Louise A, Stern R, et al. Metabolic effects of resistant starch type 2: A systematic literature review and meta-analysis of randomized controlled trials. Nutrients 2019;11:1833 10.3390/nu11081833.

[33]. Bergeron N, Williams PT, Lamendella R, Faghihnia N, Grube A, Li X, et al. Diets high in resistant starch increase plasma levels of trimethylamine-N-oxide, a gut microbiome metabolite associated with CVD risk. Br J Nutr 2016;116:2020–9. 10.1017/S0007114516004165. [PubMed: 27993177]

[34]. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57–65. 10.1038/nature09922. [PubMed: 21475195]

[35]. Tang WHW, Wang Z, Kennedy DJ, Wu Y, Buffa JA, Agatisa-Boyle B, et al. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. Circ Res 2014;116:448–55. 10.1161/CIRCRESAHA.116.305360. [PubMed: 25599331]

[36]. Zhou J, Keenan MJ, Fernandez-Kim SO, Pistell PJ, Ingram DK, Li B, et al. Dietary resistant starch improves selected brain and behavioral functions in adult and aged rodents. Mol Nutr Food Res 2013;57:2071–4. 10.1002/mnfr.201300135. [PubMed: 23818307]

[37]. Zhou J, Keenan MJ, Keller J, Fernandez-Kim SO, Pistell PJ, Tulley RT, et al. Tolerance, fermentation, and cytokine expression in healthy aged male C57BL/6J mice fed resistant starch. Mol Nutr Food Res 2012;56:515–8. 10.1002/mnfr.201100521. [PubMed: 22174009]

[38]. Hedemann MS, Hermansen K, Pedersen S, Bach Knudsen KE. Resistant starch but not enzymatically modified waxy maize delays development of diabetes in Zucker Diabetic Fatty rats. J Nutr 2017 10.3945/jn.116.243899.

[39]. Sun H, Ma X, Zhang S, Zhao D, Liu X. Resistant starch produces antidiabetic effects by enhancing glucose metabolism and ameliorating pancreatic dysfunction in type 2 diabetic rats. Int J Biol Macromol 2018 10.1016/j.ijbiomac.2017.11.162.

[40]. Goldsmith F, Guice J, Page R, Welsh DA, Taylor CM, Blanchard EE, et al. Obese ZDF rats fermented resistant starch with effects on gut microbiota but no reduction in abdominal fat. Mol Nutr Food Res 2017;61 10.1002/mnfr.201501025.

[41]. Kieffer DA, Piccolo BD, Vaziri ND, Liu S, Lau WL, Khazaeli M, et al. Resistant starch alters gut microbiome and metabolomic profiles concurrent with amelioration of chronic kidney disease in rats. Am J Physiol - Ren Physiol 2016;310:F857–71. 10.1152/ajprenal.00513.2015.

[42]. Vaziri ND, Liu SM, Lau WL, Khazaeli M, Nazertehrani S, Farzaneh SH, et al. High amylose resistant starch diet ameliorates oxidative stress, inflammation, and progression of chronic kidney disease. PLoS One 2014;9:1–15. 10.1371/journal.pone.0114881.

[43]. Zybailov BL, Glazko GV., Rahmatallah Y, Andreyev DS, McElroy T, Karaduta O, et al. Metaproteomics reveals potential mechanisms by which dietary resistant starch supplementation attenuates chronic kidney disease progression in rats. PLoS One 2019;14:e0199274. 10.1371/journal.pone.0199274.

[44]. Barouei J, Bendiks Z, Martinic A, Mishchuk D, Heeney D, Hsieh YH, et al. Microbiota, metabolome, and immune alterations in obese mice fed a high-fat diet containing type 2 resistant starch. Mol Nutr Food Res 2017;61:1–14. 10.1002/mnfr.201700184.

[45]. Koay YC, Wali JA, Luk AWS, Macia L, Cogger VC, Pulpitel TJ, et al. Ingestion of resistant starch by mice markedly increases microbiome-derived metabolites. FASEB J 2019;33:8033–42. 10.1096/fj.201900177r. [PubMed: 30925066]

[46]. Hanstock TL, Clayton EH, Li KM, Mallet PE. Anxiety and aggression associated with the fermentation of carbohydrates in the hindgut of rats. Physiol Behav 2004;82:357–68. 10.1016/j.physbeh.2004.04.002. [PubMed: 15276799]

[47]. Lyte M, Chapel A, Lyte JM, Ai Y, Proctor A, Jane J-L, et al. Resistant starch alters the microbiota-gut brain axis: Implications for dietary modulation of behavior. PLoS One 2016;11:e0146406. 10.1371/journal.pone.0146406.

[48]. Kleessen B, Stoof G, Proll J, Schmiedl D, Noack J, Blaut M. Feeding resistant starch affects fecal and cecal microflora and short-chain fatty acids in rats. J Anim Sci 1997;75:2453–62. 10.2527/1997.7592453x. [PubMed: 9303464]

[49]. Kieffer DA, Piccolo BD, Marco ML, Kim EB, Goodson ML, Keenan MJ, et al. Mice fed a high-fat diet supplemented with resistant starch display marked shifts in the liver metabolome

concurrent with altered gut bacteria. J Nutr 2016;146:2476–90. 10.3945/jn.116.238931. [PubMed: 27807042]

[50]. Hald S, Schioldan AG, Moore ME, Dige A, Lærke HN, Agnholt J, et al. Effects of arabinoxylan and resistant starch on intestinal microbiota and short-chain fatty acids in subjects with metabolic syndrome: A randomised crossover study. PLoS One 2016;11:1–18. 10.1371/journal.pone.0159223.

[51]. Sun Y, Su Y, Zhu W. Microbiome-metabolome responses in the cecum and colon of pig to a high resistant starch diet. Front Microbiol 2016;7 10.3389/fmicb.2016.00779.

[52]. Herrmann E, Young W, Rosendale D, Conrad R, Riedel CU, Egert M. Determination of resistant starch assimilating bacteria in fecal samples of mice by in vitro RNA-based stable isotope probing. Front Microbiol 2017;8:1–13. 10.3389/fmicb.2017.01331. [PubMed: 28197127]

[53]. Shang W, Si X, Zhou Z, Li Y, Strappe P, Blanchard C. Characterization of fecal fat composition and gut derived fecal microbiota in high-fat diet fed rats following intervention with chito-oligosaccharide and resistant starch complexes. Food Funct 2017;8:4374–83. 10.1039/C7FO01244F. [PubMed: 29068034]

[54]. Herrmann E, Young W, Reichert-Grimm V, Weis S, Riedel CU, Rosendale D, et al. In vivo assessment of resistant starch degradation by the caecal microbiota of mice using rna-based stable isotope probing—a proof-of-principle study. Nutrients 2018;10 10.3390/nu10020179.

[55]. Nielsen TS, Bendiks Z, Thomsen B, Wright ME, Theil PK, Scherer BL, et al. High-amylose maize, potato, and butyrylated starch modulate large intestinal fermentation, microbial composition, and oncogenic miRNA expression in rats fed a high-protein meat diet. Int J Mol Sci 2019;20:2137 10.3390/ijms20092137.

[56]. Alfa MJ, Strang D, Tappia PS, Graham M, Van Domselaar G, Forbes JD, et al. A randomized trial to determine the impact of a digestion resistant starch composition on the gut microbiome in older and mid-age adults. Clin Nutr 2018;37:797–807. 10.1016/j.clnu.2017.03.025. [PubMed: 28410921]

[57]. Ordiz MI, May TD, Mihindukulasuriya K, Martin J, Crowley J, Tarr PI, et al. The effect of dietary resistant starch type 2 on the microbiota and markers of gut inflammation in rural Malawi children. Microbiome 2015;3:37 10.1186/s40168-015-0102-9. [PubMed: 26334878]

[58]. Flowers SA, Baxter NT, Ward KM, Kraal AZ, McInnis MG, Schmidt TM, et al. Effects of atypical antipsychotic treatment and resistant starch supplementation on gut microbiome composition in a cohort of patients with bipolar disorder or schizophrenia. Pharmacotherapy 2019;39:161–70. 10.1002/phar.2214. [PubMed: 30620405]

[59]. Sun Y, Zhou L, Fang L, Su Y, Zhu W. Responses in colonic microbial community and gene expression of pigs to a long-term high resistant starch diet. Front Microbiol 2015;6:1–10. 10.3389/fmicb.2015.00877. [PubMed: 25653648]

[60]. Wang X, Pan Q, Ma F, Li P, Xu B, Chen C. Amelioration of growth performance, lipid accumulation, and intestinal health in mice by a cooked mixture of lean meat and resistant starch. Mol Nutr Food Res 2019;1801364:1–11. 10.1002/mnfr.201801364.

[61]. Hu Y, Le Leu RK, Christophersen CT, Somashekar R, Conlon MA, Meng XQ, et al. Manipulation of the gut microbiota using resistant starch is associated with protection against colitis-associated colorectal cancer in rats. Carcinogenesis 2015;37:366–75. 10.1093/carcin/bgw019.

[62]. Abell GCJ, Cooke CM, Bennett CN, Conlon MA, McOrist AL. Phylotypes related to Ruminococcus bromii are abundant in the large bowel of humans and increase in response to a diet high in resistant starch. FEMS Microbiol Ecol 2008;66:505–15. 10.1111/j.1574-6941.2008.00527.x. [PubMed: 18616586]

[63]. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. MBio 2019;10:e02566–18. 10.1128/mbio.02566-18.

[64]. Mahowald MA, Rey FE, Seedorf H, Turnbaugh PJ, Fulton RS, Wollam A, et al. Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. Proc Natl Acad Sci U S A 2009;106:5859–64. 10.1073/pnas.0901529106. [PubMed: 19321416]

[65]. Obanda D, Page R, Guice J, Raggio AM, Husseneder C, Marx B, et al. CD obesity-prone rats, but not obesity-resistant rats, robustly ferment resistant starch without increased weight or fat accretion. Obesity 2018;26:570–7. 10.1002/oby.22120. [PubMed: 29464911]

[66]. Maier T V, Lucio M, Lee H, Verberkmoes NC, Brislawn CJ, Bernhardt J, et al. Impact of dietary resistant starch on the human gut microbiome, metaproteome, and metabolome. MBio 2017;8:1–16. 10.1128/mBio.01343-17.

[67]. Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. PLoS One 2010;5 10.1371/journal.pone.0015046.

[68]. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. Microbiome 2016;4:33 10.1186/s40168-016-0178-x. [PubMed: 27357127]

[69]. Abell GC J, Christophersen CT, McOrist AL, Clarke JM Dietary resistant and butyrylated starches have different effects on the faecal bacterial flora of azoxymethane-treated rats. Br J Nutr 2011;105:1480–5. 10.1017/s0007114510005349. [PubMed: 21255474]

[70]. Kalmokoff M, Zwicker B, O'Hara M, Matias F, Green J, Shastri P, et al. Temporal change in the gut community of rats fed high amylose cornstarch is driven by endogenous urea rather than strictly on carbohydrate availability. J Appl Microbiol 2013;114:1516–28. 10.1111/jam.12157. [PubMed: 23383759]

[71]. Kovatcheva-Datchary P, Egert M, Maathuis A, Rajili -Stojanovi M, De Graaf AA, Smidt H, et al. Linking phylogenetic identities of bacteria to starch fermentation in an in vitro model of the large intestine by RNA-based stable isotope probing. Environ Microbiol 2009;11:914–26. 10.1111/j.1462-2920.2008.01815.x. [PubMed: 19128319]

[72]. Ze X, Duncan SH, Louis P, Flint HJ. Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. ISME J 2012;6:1535–43. 10.1038/ismej.2012.4. [PubMed: 22343308]

[73]. Vital M, Howe A, Bergeron N, Krauss RM, Jansson JK, Tiedje JM. Metagenomic insights into the degradation of resistant starch by human gut microbiota. Appl Environ Microbiol 2018;84:1–13. 10.1128/AEM.01562-18.

[74]. Ze X, David Y Ben, Laverde-Gomez JA, Dassa B, Sheridan PO, Duncan SH, et al. Unique organization of extracellular amylases into amylosomes in the resistant starch-utilizing human colonic Firmicutes bacterium Ruminococcus bromii. MBio 2015;6.

[75]. Mukhopadhya I, Moraïs S, Laverde-Gomez J, Sheridan PO, Walker AW, Kelly W, et al. Sporulation capability and amylosome conservation among diverse human colonic and rumen isolates of the keystone starch-degrader Ruminococcus bromii. Environ Microbiol 2018;20:324–36. 10.1111/1462-2920.14000. [PubMed: 29159997]

[76]. Crost EH, Le Gall G, Laverde-Gomez JA, Mukhopadhya I, Flint HJ, Juge N. Mechanistic insights into the cross-feeding of Ruminococcus gnavus and Ruminococcus bromii on host and dietary carbohydrates. Front Microbiol 2018;9:1–13. 10.3389/fmicb.2018.02558. [PubMed: 29403456]

[77]. Crittenden RG, Morris LF, Harvey ML, Tran LT, Mitchell HL, Playne MJ. Selection of a Bifidobacterium strain to complement resistant starch in a synbiotic yoghurt. J Appl Microbiol 2001;90:268–78. 10.1046/j.1365-2672.2001.01240.x. [PubMed: 11168730]

[78]. Crittenden R, Laitila A, Forssell P, Mättö J, Saarela M, Mattila-Sandholm T, et al. Adhesion of Bifidobacteria to granular starch and its implications in probiotic technologies. Appl Environ Microbiol 2001;67:3469–75. 10.1128/AEM.67.8.3469-3475.2001. [PubMed: 11472921]

[79]. Wang X, Brown IL, Evans AJ, Conway PL. The protective effects of high amylose maize (amylomaize) starch granules on the survival of Bifidobacterium spp. in the mouse intestinal tract. J Appl Microbiol 1999;87:631–9. 10.1046/j.1365-2672.1999.00836.x. [PubMed: 10594702]

[80]. Belenguer A, Duncan SH, Calder a G, Holtrop G, Louis P, Lobley GE, et al. Two routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. Society 2006;72:3593–9. 10.1128/AEM.72.5.3593.

[81]. Rios-Covian D, Gueimonde M, Duncan SH, Flint HJ, De Los Reyes-Gavilan CG. Enhanced butyrate formation by cross-feeding between Faecalibacterium prausnitzii and Bifidobacterium adolescentis. FEMS Microbiol Lett 2015;362 10.1093/femsle/fnv176.

[82]. Duranti S, Turroni F, Lugli GA, Milani C, Viappiani A, Mangifesta M, et al. Genomic characterization and transcriptional studies of the starch-utilizing strain Bifidobacterium adolescentis 22L. Appl Environ Microbiol 2014;80:6080–90. 10.1128/aem.01993-14. [PubMed: 25063659]

[83]. Cockburn DW, Suh C, Medina KP, Duvall RM, Wawrzak Z, Henrissat B, et al. Novel carbohydrate binding modules in the surface anchored α-amylase of Eubacterium rectale provide a molecular rationale for the range of starches used by this organism in the human gut. Mol Microbiol 2018;107:249–64. 10.1111/mmi.13881. [PubMed: 29139580]

[84]. Barcenilla A, Pryde SE, Martin JC, Duncan SH, Stewart CS, Henderson C, et al. Phylogenetic relationships of butyrate-producing bacteria from the human gut. Appl Environ Microbiol 2000;66:1654–61. 10.1128/AEM.66.4.1654-1661.2000. [PubMed: 10742256]

[85]. Miquel S, Martín R, Rossi O, Bermúdez-Humarán LG, Chatel JM, Sokol H, et al. Faecalibacterium prausnitzii and human intestinal health. Curr Opin Microbiol 2013;16:255–61. 10.1016/j.mib.2013.06.003. [PubMed: 23831042]

[86]. Laffin MR, Tayebi Khosroshahi H, Park H, Laffin LJ, Madsen K, Kafil HS, et al. Amylose resistant starch (HAM-RS2) supplementation increases the proportion of Faecalibacterium bacteria in end-stage renal disease patients: Microbial analysis from a randomized placebo-controlled trial. Hemodial Int 2019:1–5. 10.1111/hdi.12753.

[87]. Scott KP, Martin JC, Duncan SH, Flint HJ. Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. FEMS Microbiol Ecol 2014;87:30–40. 10.1111/1574-6941.12186. [PubMed: 23909466]

[88]. Lim YM, Barnes MB, Gras SL, McSweeney C, Lockett T, Augustin MA, et al. Esterification of high amylose starch with short chain fatty acids modulates degradation by Bifidobacterium spp. J Funct Foods 2014;6:137–46. 10.1016/j.jff.2013.09.027.

[89]. Martens EC, Koropatkin NM, Smith TJ, Gordon JI. Complex glycan catabolism by the human gut microbiota: The bacteroidetes sus-like paradigm. J Biol Chem 2009;284:24673–7. 10.1074/jbc.R109.022848. [PubMed: 19553672]

[90]. Foley MH, Cockburn DW, Koropatkin NM. The Sus operon: a model system for starch uptake by the human gut Bacteroidetes. Cell Mol Life Sci 2016;73:2603–17. 10.1007/s00018-016-2242-x. [PubMed: 27137179]

[91]. Bird AR, McOrist AL, Noakes M, Conlon MA, Keogh JB, Miller RB, et al. Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. J Nutr 2011;141:883–9. 10.3945/jn.110.128504. [PubMed: 21430242]

[92]. Leblois J, Massart S, Soyeurt H, Grelet C, Dehareng F, Schroyen M, et al. Feeding sows resistant starch during gestation and lactation impacts their faecal microbiota and milk composition but shows limited effects on their progeny. PLoS One 2018;13:1–22. 10.1371/journal.pone.0199568.

[93]. Si X, Shang W, Zhou Z, Strappe P, Wang B, Bird A, et al. Gut microbiome-induced shift of acetate to butyrate positively manages dysbiosis in high fat diet. Mol Nutr Food Res 2018;1700670. 10.1002/mnfr.201700670.

[94]. Müller M, Hermes GDA, Canfora EE, Smidt H, Masclee AAM, Zoetendal EG, et al. Distal colonic transit is linked to gut microbiota diversity and microbial fermentation in humans with slow colonic transit. Am J Physiol Liver Physiol 2019;53:ajpgi.00283.2019. 10.1152/ajpgi.00283.2019.

[95]. Klurfeld DM, Davis CD, Karp RW, Allen-Vercoe E, Chang EB, Chassaing B, et al. Considerations for best practices in studies of fiber or other dietary components and the intestinal microbiome. Am J Physiol - Endocrinol Metab 2018 10.1152/ajpendo.00058.2018.

[96]. Knight R, Navas J, Quinn RA, Sanders JG, Zhu Q. Best practices for analysing microbiomes. Nat Rev Microbiol 2018 10.1038/s41579-018-0029-9.

[97]. Fouhy F, Clooney AG, Stanton C, Claesson MJ, Cotter PD. 16S rRNA gene sequencing of mock microbial populations-impact of DNA extraction method, primer choice and sequencing platform. BMC Microbiol 2016;16 10.1186/s12866-016-0738-z.

[98]. Xue Z, Kable ME, Marco ML. Impact of DNA sequencing and analysis methods on 16S rRNA gene bacterial community analysis of dairy products. mSphere 2018;3 10.1128/msphere.00410-18.

[99]. Gorzelak MA, Gill SK, Tasnim N, Ahmadi-Vand Z, Jay M, Gibson DL. Methods for improving human gut microbiome data by reducing variability through sample processing and storage of stool. PLoS One 2015;10 10.1371/journal.pone.0134802.

[100]. Pollock J, Glendinning L, Wisedchanwet T, Watson M. The madness of microbiome: Attempting to find consensus "best practice" for 16S microbiome studies. Appl Environ Microbiol 2018;84 10.1128/AEM.02627-17.

[101]. Nilakanta H, Drews KL, Firrell S, Foulkes MA, Jablonski KA. A review of software for analyzing molecular Sequences. BMC Res Notes 2014;7 10.1186/1756-0500-7-830.

[102]. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581–3. 10.1038/nmeth.3869. [PubMed: 27214047]

[103]. Callahan BJ, McMurdie PJ, Holmes SP. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J 2017;11:2639–43. 10.1038/ismej.2017.119. [PubMed: 28731476]

[104]. Costea PI, Zeller G, Sunagawa S, Pelletier E, Alberti A, Levenez F, et al. Towards standards for human fecal sample processing in metagenomic studies. Nat Biotechnol 2017;35:1069–76. 10.1038/nbt.3960. [PubMed: 28967887]

[105]. Loan T, Nguyen A, Vieira-Silva S, Liston A, Raes J, Nguyen TLA, et al. How informative is the mouse for human gut microbiota research? Dis Model Mech 2015;8:1–16. 10.1242/dmm.017400. [PubMed: 25561744]

[106]. Lee JE, Lee S, Sung J, Ko G. Analysis of human and animal fecal microbiota for microbial source tracking. ISME J 2011;5:362–5. 10.1038/ismej.2010.120. [PubMed: 20686512]

[107]. Panebianco C, Adamberg K, Adamberg S, Saracino C, Jaagura M, Kolk K, et al. Engineered resistant-starch (ERS) diet shapes colon microbiota profile in parallel with the retardation of tumor growth in in vitro and in vivo pancreatic cancer models. Nutrients 2017;9 10.3390/nu9040331.

[108]. Poole AC, Goodrich JK, Youngblut ND, Luque GG, Ruaud A, Sutter JL, et al. Human salivary amylase gene copy number impacts oral and gut microbiomes. Cell Host Microbe 2019;25:553–564.e7. 10.1016/j.chom.2019.03.001. [PubMed: 30974084]

[109]. Salonen A, Salojärvi J, Lahti L, de Vos WM. The adult intestinal core microbiota is determined by analysis depth and health status. Clin Microbiol Infect 2012;18:16–20. 10.1111/j.1469-0691.2012.03855.x. [PubMed: 22647042]

[110]. Lynch S V, Pedersen O The human intestinal microbiome in health and disease. N Engl J Med 2016;375:2369–79. 10.1056/NEJMra1600266. [PubMed: 27974040]

[111]. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature 2009;457:480–4. 10.1038/nature07540. [PubMed: 19043404]

[112]. Stanislawski MA, Dabelea D, Lange LA, Wagner BD, Lozupone CA. Gut microbiota phenotypes of obesity. Npj Biofilms Microbiomes 2019;5 10.1038/s41522-019-0091-8.

[113]. Claesson MJ, Cusack S, O'Sullivan O, Greene-Diniz R, de Weerd H, Flannery E, et al. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. Proc Natl Acad Sci 2011;108:4586–91. 10.1073/pnas.1000097107. [PubMed: 20571116]

[114]. Mariat D, Firmesse O, Levenez F, Guimarães VD, Sokol H, Doré J, et al. The irmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol 2009;9:1–6. 10.1186/1471-2180-9-123. [PubMed: 19121223]

[115]. Waterschoot J, Gomand S V., Fierens E, Delcour JA. Production, structure, physicochemical and functional properties of maize, cassava, wheat, potato and rice starches. Starch/Staerke 2015 10.1002/star.201300238.

[116]. Corgneau M, Gaiani C, Petit J, Nikolova Y, Banon S, Ritié-Pertusa L, et al. Digestibility of common native starches with reference to starch granule size, shape and surface features towards

guidelines for starch-containing food products. Int J Food Sci Technol 2019;54:2132–40. 10.1111/ijfs.14120.

[117]. Martin LJM, Dumon HJW, Lecannu G, Champ MMJ. Potato and high-amylose maize starches are not equivalent producers of butyrate for the colonic mucosa. Br J Nutr 2000 10.1017/s0007114500002038.

[118]. Conlon MA, Kerr CA, McSweeney CS, Dunne RA, Shaw JM, Kang S, et al. Resistant starches protect against colonic DNA damage and alter microbiota and gene expression in rats fed a Western diet. J Nutr 2012;142:832–40. 10.3945/jn.111.147660. [PubMed: 22457395]

[119]. Tachon S, Zhou J, Keenan M, Martin R, Marco ML. The intestinal microbiota in aged mice is modulated by dietary resistant starch and correlated with improvements in host responses. FEMS Microbiol Ecol 2013;83:299–309. 10.1111/j.1574-6941.2012.01475.x. [PubMed: 22909308]

[120]. Young W, Roy NC, Lee J, Lawley B, Otter D, Henderson G, et al. Changes in bowel microbiota induced by feeding weanlings resistant starch stimulate transcriptomic and physiological responses. Appl Environ Microbiol 2012;78:6656–64. 10.1128/aem.01536-12. [PubMed: 22798356]

[121]. Toden S, Bird AR, Topping DL, Conlon MA. Dose-dependent reduction of dietary protein-induced colonocyte DNA damage by resistant starch in rats correlates more highly with caecal butyrate than with other short chain fatty acids. Cancer Biol Ther 2007;6:253–8. 10.4161/cbt.6.2.3627. [PubMed: 17218781]

[122]. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. Cell 2016;167:1339–1353.e21. 10.1016/j.cell.2016.10.043. [PubMed: 27863247]

[123]. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105–9. 10.1126/science.1208344. [PubMed: 21885731]

[124]. Wu GD, Compher C, Chen EZ, Smith SA, Shah RD, Bittinger K, et al. Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. Gut 2016;65:63–72. 10.1136/gutjnl-2014-308209. [PubMed: 25431456]

[125]. Kaur A, Chen T, Green SJ, Mutlu E, Martin BR, Rumpagaporn P, et al. Physical inaccessibility of a resistant starch shifts mouse gut microbiota to butyrogenic Firmicutes. Mol Nutr Food Res 2019;63:1–8. 10.1002/mnfr.201801012.

[126]. Mullin GE. High-fat diet determines the composition of the murine gut microbiome independently of obesity. Nutr Clin Pract 2010;25:310–1. 10.1177/0884533610368714. [PubMed: 29130781]

[127]. de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE, et al. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. Am J Physiol - Gastrointest Liver Physiol 2010;299:G440–8. 10.1152/ajpgi.00098.2010. [PubMed: 20508158]

[128]. Wan Y, Wang F, Yuan J, Li J, Jiang D, Zhang J, et al. Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: A 6-month randomised controlled-feeding trial. Gut 2019:1417–29. 10.1136/gutjnl-2018-317609.

[129]. Charrier JA, Martin RJ, McCutcheon KL, Raggio AM, Goldsmith F, Goita M, et al. High fat diet partially attenuates fermentation responses in rats fed resistant starch from high-amylose maize. Obesity 2013;21:2350–5. 10.1002/oby.20362. [PubMed: 23512798]

[130]. Behall KM, Scholfield DJ, Hallfrisch JG, Liljeberg-elmståhl HGM. Consumption of both resistant starch and β-glucan improves postprandial plasma glucose and insulin in women. Diabetes Care 2006;29:976–81. 10.2337/dc05-2012. [PubMed: 16644623]

[131]. De Vries S, Gerrits WJJ, Kabel MA, Vasanthan T, Zijlstra RT. β-Glucans and resistant starch alter the fermentation of recalcitrant fibers in growing pigs. PLoS One 2016;11:1–18. 10.1371/journal.pone.0167624.

[132]. Scott KP, Gratz SW, Sheridan PO, Flint HJ, Duncan SH. The influence of diet on the gut microbiota. Pharmacol Res 2013;69:52–60. 10.1016/j.phrs.2012.10.020. [PubMed: 23147033]

[133]. Holmes AJ, Chew YV, Colakoglu F, Cliff JB, Klaassens E, Read MN, et al. Dietmicrobiome interactions in health are controlled by intestinal nitrogen source constraints. Cell Metab 2017;25:140–51. 10.1016/j.cmet.2016.10.021. [PubMed: 27889387]

[134]. Le Leu RK, Brown IL, Hu Y, Morita T, Esterman A, Young GP. Effect of dietary resistant starch and protein on colonic fermentation and intestinal tumourigenesis in rats. Carcinogenesis 2007;28:240–5. 10.1093/carcin/bgl245. [PubMed: 17166881]

[135]. Le Leu RK, Young GP, Hu Y, Winter J, Conlon MA. Dietary red meat aggravates dextran sulfate sodium-induced colitis in mice whereas resistant starch attenuates inflammation. Dig Dis Sci 2013;58:3475–82. 10.1007/s10620-013-2844-1. [PubMed: 23990000]

[136]. Ley RE. Gut microbiota in 2015: Prevotella in the gut: Choose carefully. Nat Rev Gastroenterol Hepatol 2016;13:69 10.1038/nrgastro.2016.4. [PubMed: 26828918]

[137]. Cherbuy C, Bellet D, Robert V, Mayeur C, Schwiertz A, Langella P. Modulation of the caecal gut microbiota of mice by dietary supplement containing resistant starch: Impact is donor-dependent. Front Microbiol 2019;10:1–11. 10.3389/fmicb.2019.01234. [PubMed: 30728808]

[138]. Costea PI, Hildebrand F, Manimozhiyan A, Bäckhed F, Blaser MJ, Bushman FD, et al. Enterotypes in the landscape of gut microbial community composition. Nat Microbiol 2017;3:8–16. 10.1038/s41564-017-0072-8. [PubMed: 29255284]

[139]. Kovatcheva-Datchary P, Nilsson A, Akrami R, Lee YS, De Vadder F, Arora T, et al. Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of Prevotella. Cell Metab 2014:971–82. 10.1016/j.cmet.2015.10.001.

[140]. Zhang L, Ouyang Y, Li H, Shen L, Ni Y, Fang Q, et al. Metabolic phenotypes and the gut microbiota in response to dietary resistant starch type 2 in normal-weight subjects: a randomized crossover trial. Sci Rep 2019;9 10.1038/s41598-018-38216-9.

[141]. Salonen A, Lahti L, Salojärvi J, Holtrop G, Korpela K, Duncan SH, et al. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. ISME J 2014;8:2218–30. 10.1038/ismej.2014.63. [PubMed: 24763370]

[142]. Vandeputte D, Kathagen G, D'Hoe K, Vieira-Silva S, Valles-Colomer M, Sabino J, et al. Quantitative microbiome profiling links gut community variation to microbial load. Nature 2017;551:507–11. 10.1038/nature24460. [PubMed: 29143816]

[143]. Fang L, Jiang X, Su Y, Zhu W. Long-term intake of raw potato starch decreases back fat thickness and dressing percentage but has no effect on the longissimus muscle quality of growing-finishing pigs. Livest Sci 2014;170:116–23. 10.1016/j.livsci.2014.10.004.

[144]. Nielsen TS, Lærke HN, Theil PK, Sørensen JF, Saarinen M, Forssten S, et al. Diets high in resistant starch and arabinoxylan modulate digestion processes and SCFA pool size in the large intestine and faecal microbial composition in pigs. Br J Nutr 2014;112:1837–49. 10.1017/s000711451400302x. [PubMed: 25327182]

[145]. Trachsel J, Briggs C, Gabler NK, Allen HK, Loving CL. Dietary resistant potato starch alters intestinal microbial communities and their metabolites, and markers of immune regulation and barrier function in swine. Front Immunol 2019;10:1–13. 10.3389/fimmu.2019.01381. [PubMed: 30723466]

[146]. Bindels LB, Segura Munoz RR, Gomes-Neto JC, Mutemberezi V, Martínez I, Salazar N, et al. Resistant starch can improve insulin sensitivity independently of the gut microbiota. Microbiome 2017;5:1–16. 10.1186/s40168-017-0230-5. [PubMed: 28086968]

[147]. Paturi G, Nyanhanda T, Butts CA, Herath TD, Monro JA, Ansell J. Effects of potato fiber and potato-resistant starch on biomarkers of colonic health in rats fed diets containing red meat. J Food Sci 2012;77 10.1111/j.1750-3841.2012.02911.x.

[148]. Zhou Z, Ren X, Jiang Y, Zhang Q, Zhang M, Strappe P, et al. Responses of fecal bacterial communities to resistant starch intervention in diabetic rats. Starch/Staerke 2016;68:1008–15. 10.1002/star.201500139.

[149]. Keenan MJ, Janes M, Robert J, Martin RJ, Raggio AM, McCutcheon KL, et al. Resistant starch from high amylose maize (HAM-RS2) reduces body fat and increases gut bacteria in ovariectomized (OVX) rats. Obesity 2013;21:981–4. 10.1002/oby.20109. [PubMed: 23784900]

[150]. Le Leu RK, Brown IL, Hu Y, Bird AR, Jackson M, Esterman A, et al. A synbiotic combination of resistant starch and Bifidobacterium lactis facilitates apoptotic deletion of carcinogen-

damaged cells in rat colon. J Nutr 2005;135:996–1001. 10.1093/jn/135.5.996. [PubMed: 15867271]

[151]. Jiminez JA, Uwiera TC, Abbott DW, Uwiera RRE, Inglis GD. Impacts of resistant starch and wheat bran consumption on enteric inflammation in relation to colonic bacterial community structures and short-chain fatty acid concentrations in mice. Gut Pathog 2016;8:1–20. 10.1186/ s13099-016-0149-6. [PubMed: 26759607]

[152]. Haenen D, Zhang J, da Souza CS, Bosch G, van der Meer IM, van Arkel J, et al. A diet high in resistant starch modulates microbiota composition, SCFA concentrations, and gene expression in pig intestine. J Nutr 2013;143:274–83. 10.3945/jn.112.169672. [PubMed: 23325922]

[153]. Tachon S, Lee B, Marco ML. Diet alters probiotic Lactobacillus persistence and function in the intestine. Environ Microbiol 2013 10.1111/1462-2920.12297.

Highlights

- Resistant starch type 2 (RS2) consumption alters the human gut microbiome

- The effects of RS2 on the gut microbiome vary within and between studies

- No single factor explains the variations in gut microbiota responses to RS2
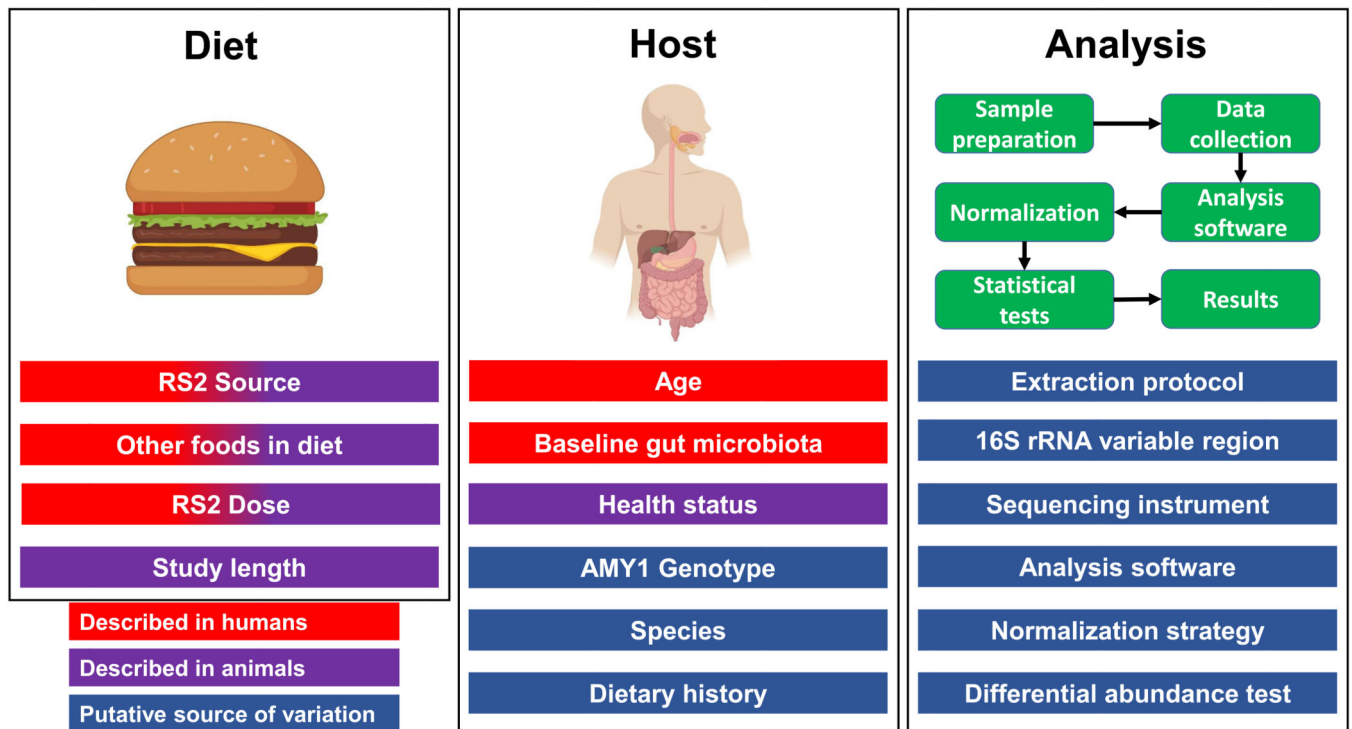
Figure 1. Sources of variation in RS2-based investigations of the gut microbiome.
Displayed are known and putative sources of variation in RS2-based studies on gut ecology. Boxes in red show factors that have been tested directly in human RS2 interventions, while purple boxes show factors that have been tested in animals. Factors in blue are known to contribute to variation in gut ecology studies but have not been tested directly for their impact on RS2 intervention studies. Dietary sources of variation that have been shown to affect RS2 outcomes include the source of RS2 [55,63,117,118], the presence and amount of other foods in the diet [33,129,130,134], the amount of RS2 consumed [33,119–121], and the feeding duration [125]. Host sources of variation include the age of the host [56], their gut microbiome composition prior RS2 intervention [140], and the health status of the host [65].

Table 1.

RS2 human and animal model studies in which the gut microbiome was examined

Host	Host characteristics	Analysis method[a]	RS2 source, dose, and other dietary information	Intervention duration	Bacterial α-diversity	SCFA[c]	Ref.
Human	Healthy	Metagenome+ 16S rRNA V3-V4 + LC/ESI/MS/MS	Maize; 66 g/2500 kcal [10460 kJ] (high CHO arm) and 48 g/2500 kcal [10460 kJ] (low CHO arm)	2 weeks	ND	ND	[33,73]
	Healthy	DGGE + qPCR	Barley, bean, wheat, and maize; 22 g RS2 with 25 g total fiber	4 weeks	No significant differences	↑ fecal butyrate, acetate, total SCFA conc.	[62]
	Healthy; ages 17 to 29	16S rRNA V4 + HPLC	Maize (50% RS2); 20–24 g/day Potato (70% RS2); 28–34 g/day	2 weeks	No significant differences	↑ fecal butyrate, acetate, total SCFA conc. (Potato RS2)	[63]
	Healthy; BMI < 24 kg/m²; ages 18 to 35	16S rRNA, V3 + UPLC-MS + GC	Maize; 40 g/day	4 weeks	No significant differences	↑ serum acetate conc.	[140]
	Healthy; ages 19 to 20	16S rRNA, V4 + HPLC	Potato (50% RS2); 48 g/day	1 week	No significant differences	↑ fecal butyrate and acetate conc., but variable	[68]
	Healthy; age; 23 to 38	16S rRNA, V1-V3 + PCR-DGGE + qRT-PCR	Maize; 33 g/day	3 weeks	No significant differences	ND	[67]
	Healthy; mid-aged; ages 30 to 50 and elderly; > 70 years	16S rRNA V4 + GC	Potato (70% RS2), MSPrebiotic®; 21 g/day	12 weeks	↓ Shannon (mid-aged) + inverse Simpson	↑ fecal butyrate:SCFA ratio (elderly subjects)	[56]
	Insulin resistant (HOMA IR > 50th percentile) + BMI between 20 and 35 kg/m²	16S rRNA V4-V6 + LC-MS/MS + FT-ICR-MS + UHPLC-MS	Maize; 48 g/2500 kcal [10460 kJ]	2 weeks	ND	Trend towards ↑ butyrate and propionate conc., but variable	[66]
	Metabolic syndrome; ages 39–75	16S rRNA V4 + G-LC	Maize, potato mix; 20.7 g/day; wheat arabinoxylans included	4 weeks	↓ observed OTUs + Chao1 + Faith's PD	↑ fecal acetate, butyrate, total SCFA conc. compared to WSD	[50]
	Stunted rural Malawi children; ages 3 to 5	16S rRNA V1-V3 and V4-V5 + qPCR + GC/MS	Maize (70% amylose); 8.5 g/day	4 weeks	↓ Shannon	↑ fecal propionate, ↓ acetate conc.	[57]
	End-stage renal disease patients undergoing hemodialysis	16S rRNA V3-V4	Maize; 20 g/day for one month, then 25 g/day for one month	8 weeks	ND	ND	[86]
	Bipolar or schizophrenic; undergoing atypical antipsychotic (AAP) treatment	16S rRNA V4	Potato (50% RS2); 24 g/day	3 weeks	↓ inverse Simpson (women only)	ND	[58]
Pig	Duroc × Landrace × Large White; age 70 days	GC	Potato; 230 g/kg (growing) then 280 g/kg (weaning)	100 days	ND	↑ colonic butyrate, acetate, propionate, total SCFA conc.	[143]
	Duroc × Landrace × Large White; age 70 days	16S rRNA V1-V3 + GC-MS	Potato; 230 g/kg (growing) then 280 g/kg (finishing)	100 days	↓ Chao1 (colon, not cecum)	ND	[51]
	Duroc × Landrace × Large White; age 70 days	16S rRNA V4-V5 + qPCR + RNA microarray	Potato; 230 g/kg growing) then 280 g/kg (finishing)	100 days	↓ Chao1 + ACE	ND	[59]

Host	Host characteristics	Analysis method[a]	RS2 source, dose, and other dietary information	Intervention duration	Bacterial α-diversity	SCFA[c]	Ref.
	Landrace x Pietrain	16S rRNA V3-V4 + HPLC + RT-qPCR	Pea; 33% of diet	106 days	ND	No significant differences	[92]
	Females	qPCR + GC	Maize; 2.7% of body weight	3 weeks	ND	↑ large intestinal acetate, butyrate, propionate, total SCFA pool	[144]
	Large White; 21 d old	16S rRNA V4 + *but* gene survey + GC	Potato; 3.5% diet by weight	3 weeks	ND	↑ cecal butyrate, propionate conc. ↑ fecal butyrate and total SCFA conc.	[145]
Mouse	C57BL/6J; 8 weeks old	16S rRNA V3-V4 + RT-qPCR + RNA stable isotope probing + GC-MS + GC-FID	[U^{13}C]-labeled potato; 0.4 g total	2–4 hours	↓Faith's PD	Tended to ↓ cecal butyrate, acetate, propionate conc., SCFA responses were variable	[54]
	C57BL/6J; 6 weeks old; males	16S rRNA + GC	Potato; 10% of diet	2 weeks	ND	↑ fecal acetate, butyrate, total SCFA conc.	[125]
	C57BL/6J; 4 weeks old; males	16S rRNA V4 + LC-MS/MS	Wheat; 42% diet energy	18 weeks	ND	ND	[45]
	C57BL/6J; 18–20 months old; males	16S rRNA V1-V3 + qPCR	Maize; 18% or 36% of diet	8 weeks	ND	ND	[119]
	C57BL/6J; five weeks old; males	16S rRNA V4 + qPCR + G-LC	Maize; 20% of diet weight; high-fat diet	10 weeks	ND	No significant differences	[49]
	C57BL/6J; diet-induced obesity; males	16S rRNA V4 + RT-qPCR + NMR + metabolite panel	Maize; 20% of diet weight; high-fat diet	6 weeks	↓ observed OTUs	↓ cecal butyrate conc.	[44]
	Conventionalized C57BL/6J; males and C3H; germ-free	16S rRNA V1-V3 + UPLC-MRM-MS	Maize; 16% of diet weight	8 weeks	ND	ND	[146]
	C57BL/6; 4 weeks old; males	16S rRNA V3-V4 + GC	Maize; 100 g/kg diet; included with cooked lean meat	4 weeks	↓Chao1	↑ fecal propionate and butyrate conc., but not when starch and meat were cooked together	[60]
	BALB/c; DSS-induced colitis; males	qPCR	Maize; 10 g/100 g of diet weight	12 days	ND	ND	[135]
	Swiss Webster; 4 weeks old; males	16S rRNA V4	Maize; 55% by weight	6 weeks	No significant differences	ND	[47]
Rat	Sprague Dawley; 4 weeks old; males	qPCR + GC	Potato; 50 g/kg of diet was RS2; 2 wks feeding + 6 wks feeding with 25% cooked beef	8 weeks	ND	↑ cecal, colonic acetate, butyrate, propionate conc.	[147]
	Sprague Dawley; 6 wk old; males	16S rRNA V4 + GC	Maize; 10% diet; high meat protein diet Potato; 10% diet; high meat protein diet	4 weeks	↓ Faith PD (maize)	↑ cecal butyrate, acetate total SCFA (maize + potato) ↑ fecal acetate conc. (maize)	[55]

Host	Host characteristics	Analysis method[a]	RS2 source, dose, and other dietary information	Intervention duration	Bacterial α-diversity	SCFA[c]	Ref.
	Sprague Dawley; males	16S rRNA + GC	Maize (73% amylose Hi Maize); 1.2 g/day; high fat diet	6 weeks	↓ observed OTUs, ACE, Chao1, Shannon	↑ fecal butyrate conc.	[53]
	Sprague Dawley; males	Phylogenetic microarray + G-LC + whole genome expression microarray	Maize; 280 g/kg (high amylose starch); WSD Wheat; 500 g/kg (high amylose whole wheat flour); WSD	11 weeks	ND	↑ colon propionate pool (Wheat) ↑ portal vein acetate concentration, total SCFA conc. (Maize) ↑ cecal acetate, butyrate, propionate, and total SCFA pool (both)	[118]
	Sprague Dawley; adenine-induced CKD; 10 wk old; males	Metaproteomics+ 16S rRNA V4 + GC-TOF-MS	Maize; 59% by weight	3 weeks	↑ metaproteomics ↓ observed OTUs	ND	[41,43]
	Sprague Dawley; streptozotocin-induced diabetes mellitus; males	16S rRNA V1-V3	Maize; 8% of total diet	4 weeks	ND	ND	[148]
	Sprague Dawley; ovariectomized (OVX), females;	qPCR	Maize; 29.7% by weight	6 weeks	ND	ND	[149]
	Sprague Dawley; azoxymethane treated; males	DGGE	Maize; 10%	31 weeks (max)	↓ Shannon	↑ distal colon propionate conc.	[69]
	Sprague Dawley; colitis-associated colorectal cancer; 4 weeks old; males	16S rRNA V4-V5 + GC	Maize; 20 g/100 g of diet	20 weeks	↓ Shannon	↑ cecal and fecal acetate, butyrate, and propionate conc.	[61]
	Sprague Dawley; adult males	GC	Maize; 0, 10, 20, 30, or 40% high-amylose starch by weight; included 25% casein by weight	4 weeks	ND	↑ cecal pooled acetate, propionate, butyrate, total SCFA (20% RS2) ↑ fecal pooled acetate, total SCFA (10% RS2)	[121]
	Sprague Dawley; 6 weeks old; males	G-LC	Maize; 27% by weight; included 15% or 42% fat by energy and 0 or 10% tuna oil by energy	12 weeks	ND	↑ cecal acetate, propionate, butyrate, total SCFA conc.	[129]
	Sprague Dawley; azoxymethane-treated; 5 weeks old; males	GC	Maize; 10%; included 0 or 15% potato protein	30 weeks	ND	↑ cecal acetate, total SCFA conc. ↑ proximal colon acetate, butyrate total SCFA conc. ↑ distal colon acetate, butyrate total SCFA conc. ↑ fecal butyrate total SCFA conc.	[134]
	Sprague Dawley; 5 weeks old; males	Selective culture media + GC	Maize; 10 g RS2/100 g; with or without 1% by weight Lacobacillus acidophilus or Bifidobacterium lactis	4 weeks	ND	↑ cecal acetate, propionate, butyrate, total SCFA conc.	[150]

Host	Host characteristics	Analysis method[a]	RS2 source, dose, and other dietary information	Intervention duration	Bacterial α-diversity	SCFA[c]	Ref.
	Zucker Diabetic Fatty; males	16S rRNA V3-V4	Maize; 25% by weight	11 weeks	↓Chao1	↑ cecal acetate, butyrate, and propionate conc.	[40]
	BioBreeding; 28–42 days old; males	16S rRNA + qPCR	Maize; 5% diet by weight	4 weeks	↓Chao1	↑ fecal acetate, butyrate, propionate, total SCFA conc.	[70]
	CD obesity prone and obesity-resistant rats; 5–6 wk old; males	16S rRNA V3 + qPCR + GC	Maize; 20% diet by weight	4 weeks	ND	↑ serum acetate, butyrate, and propionate pool	[65]
	Wistar; diet-induced obese; 4 wk old; males	16S rRNA V4 + metagenome + GC	Maize; 10% diet by weight	6 weeks	ND	↓ serum acetate conc.	[93]
	Wistar; 8 wk old; male	Selective culture media + GC	Potato; 10% by weight	5 months	ND	↑ fecal and cecal acetate, butyrate, propionate conc.	[48]

LC/ESI/MS/MS, stable-isotope dilution HPLC with online electrospray ionization tandem mass spectrometry; Metagenome, high throughput sequencing of environmental DNA; DGGE, denaturing gradient gel electrophoresis; 16S rRNA, 16S rRNA marker gene survey; HPLC, high-performance liquid chromatography; UPLC-MS, ultra performance liquid chromatography with tandem mass spectrometry; GC, gas chromatography; PCR, polymerase chain reaction; qRT-PCR, reverse transcription quantitative polymerase chain reaction; LC-MS/MS, liquid chromatography with tandem mass spectrometry; FT-ICR-MS, Fourier-transform ion cyclotron resonance mass spectrometry; G-LC, gas-liquid chromatography; qPCR, quantitative polymerase chain reaction; GC-MS, gas chromatography with tandem mass spectrometry; GC-FID, gas chromatography-flame ionization detection; NMR, nuclear magnetic resonance; UPLC-MRM-MS, ultra-performance liquid chromatography electrospray ionization-tandem mass spectrometry in multiple reactions monitoring mode; GC-TOF, gas chromatography with time-of-flight mass spectrometry; ND, not determined; Conc., concentration.

Table 2.

Responsive intestinal bacterial taxa in RS2 studies

Taxonomy Level[a]						Human		Animal	
Phylum	Class	Order	Family	Genus	Species	Increased	Reduced	Increased	Reduced
Firmicutes						[66,73]	[50,56,57]	[92,125]	[40,41,43,44,49,54,60,65,93,119]
	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus		[63]		[40,49,92]	[41,44,49]
				Ruminococcus	R. bromii	[66]	[50]	[40,41,43,59–61,151]	[44,47,49,148]
				Faecalibacterium	F. prausnitzii	[58,62,63,67,68]		[43,55,59,69,70]	
				Faecalibacterium		[66,86]		[93,145,152]	
				Oscillospira					[49,59]
			Lachnospiraceae				[57]	[65,107]	[40,41,49]
				Coprococcus			[57,140]	[51,59,92,93]	[41,60]
				Roseburia		[66]	[57]	[118,151]	[60]
				Blautia			[57,140]	[59]	[41,54]
			Eubacteriaceae	Eubacterium		[66,67]	[50]	[41,43,93,118]	
			Clostridiaceae	Clostridium		[63]		[145,147]	[51,59]
	Bacilli	Lactobacillales	Lactobacillaceae					[49,65,125]	
				Lactobacillus		[57]	[56]	[48,53,147–150]	[40,54,93]
	Erysipelotrichi	Erysipelotrichales	Erysipelotrichidae	Allobaculum				[41,44,45,119]	[60,61]
			Erysipelotrichaceae	Turicibacter				[51,59,92]	[41,60]
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae			[57,58]		[47]	
				Bifidobacterium		[50,56]		[41,47,48,53,61,92,93,119,120,147,149,150]	
				Bifidobacterium	B. adolescentis	[56,58,63,68]			
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae			[56,57]	[50,66,73]	[40,41,43,44,49,65,70,93,119]	[92,125]
				Bacteroides			[50,140]	[40,45,48,70]	[47,59,147]
			S24–7					[40,41,44,55,65,148]	[59]
			Rikenellaceae				[50,57]	[49,119]	[41,47]
			Prevotellaceae	Prevotella		[56]			[54]

Taxonomy Level[a]						Human		Animal	
Phylum	Class	Order	Family	Genus	Species	Increased	Reduced	Increased	Reduced
Verrumicrobia	Verrumicrobia	Verrumicrobiales	Verrumicrobiaceae	Akkermansia				[49,119,153]	
					A. muciniphila	[66]		[151]	

[a]Taxonomy based on the deepest classification level provided that reached significance ($p < 0.05$).