Role of Resistant Starch in Improving Gut Health, Adiposity, and Insulin Resistance[1–4]

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ABSTRACT

The realization that low–glycemic index diets were formulated using resistant starch led to more than a decade of research on the health effects of resistant starch. Determination of the metabolizable energy of the resistant starch product allowed for the performance of isocaloric studies. Fermentation of resistant starch in rodent studies results in what appears to be a healthier gut, demonstrated by increased amounts of short-chain fatty acids, an apparent positive change in the microbiota, and increased gene expression for gene products involved in normal healthy proliferation and apoptosis of potential cancer cells. Additionally, consumption of resistant starch was associated with reduced abdominal fat and improved insulin sensitivity. Increased serum glucagon-like peptide 1 (GLP-1) likely plays a role in promoting these health benefits. One rodent study that did not use isocaloric diets demonstrated that the use of resistant starch at 8% of the weight of the diet reduced body fat. This appears to be approximately equivalent to the human fiber requirement. In human subjects, insulin sensitivity is increased with the feeding of resistant starch. However, only 1 of several studies reports an increase in serum GLP-1 associated with resistant starch added to the diet. This means that other mechanisms, such as increased intestinal gluconeogenesis or increased adiponectin, may be involved in the promotion of improved insulin sensitivity. Future research may confirm that there will be improved health if human individuals consume the requirement for dietary fiber and a large amount of the fiber is fermentable. *Adv Nutr* 2015;6:198–205.

Keywords: functional foods, intestinal health, nurtigenomics, obesity, resistant starch

Beginning of Resistant Starch Research at the Louisiana State University Agricultural Center

In the 2002, a member of our research group attended a research presentation by Dr. Jennifer Brand-Miller at the Experimental Biology meeting on how low-glycemic diets reduced body fat fairly dramatically in rodents (1). For several years, we tried to reduce body fat in genetic models of obesity, such as the Zucker obese rats, with very limited success (2). Thus, effects of low-glycemic diets were very impressive, although at that time the research did not include Zucker obese rats. Our research group planned to conduct some similar studies and contacted Dr. Brand-Miller, who informed us that, to lower the glycemic load of her rodent diets, she used resistant starch (RS)[12] (1). At that point, our group decided to focus our laboratory research on RS.

Current State of Knowledge

RSs are broadly characterized into 4 categories: RS1–RS4 (3, 4). RS1 is found in whole grains (WGs) and legumes and is entrapped in a nondigestible matrix. Ungelatinized starch granules are found in foods such as raw potatoes and high-amylose cornstarch and comprise the RS2 category. The RS3 category includes foods that have undergone "retrogradation,"

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[2] A summary of the symposium "Dietary Whole Grain–Microbiota Interactions: Insights into Mechanisms for Human Health" was published in the September 2014 issue of *Advances in Nutrition*.

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[12] Abbreviations used: GLP-1, glucagon-like peptide 1; HAMRS2, high-amylose maize resistant starch type 2; PYY, peptide YY; RS, resistant starch; WG, whole grain; ZDF, Zucker diabetic fatty.

which occurs when foods containing starches are cooked and then cooled. Examples of foods in this category include potatoes cooled after cooking and puddings. Chemically modifying starches with the addition of ester and ether groups and cross-linking amylose strands usually render them resistant to digestion. These starches are found in breads and cakes, and they are categorized as RS4. Much of the research with RS uses high-amylose products. The term "resistant starch" was first used by Englyst et al. (5), as "a small fraction of starch that was resistant to hydrolysis by exhaustive amylase and pullulanase treatment in vitro." His group later confirmed that this same type of starch also resisted hydrolysis in vivo using healthy ileosteomy subjects (6). Thus, RS is defined as the amount of starch that reaches the large intestine. Note that the FDA does not allow the term "resistant starch" on food labels; Hi-maize 260, a purified RS product (Ingredion), is assayed for fiber content, and that amount can be placed on the food label. This product is often referred to as high-amylose maize resistant starch type 2 (HAMRS2).

RS, by definition, is starch that reaches the large intestine in which it is fermented by bacteria. Therefore, RS is a type of fermentable fiber and could be considered 1 type of prebiotic, i.e., provides "food" for bacteria living in the large intestine. Fermentation of RS results in production of SCFAs and a reduction in pH in the proximal large intestine. In the rodent, the cecum is more distinct than in humans, and generally cecal contents are analyzed for fermentation products and pH. Additionally, the weight of the cecal contents and empty cecum increase in response to fermentation (7, 8). HAMRS2 feeding also dramatically changes the composition of the microbiota that inhabits the cecum and the gene expression of the cecal cells.

Overview of Research Design and Defining RS

In our research, we used proof-of-concept, mechanistic studies generally using RS at ~23–28% of the weight of the diet. However, we did perform 2 dose–response studies using 0%, 18%, and 36%, and 0%, 15%, and 28% of the diet. Our group used an AIN-recommended semipurified diet formulated in 1993 (9) as a base and substituted 1 carbohydrate starch source for another as the source of dietary carbohydrate starch. In our control diets, we used a 100% amylopectin corn starch (Amioca starch; Ingredion) that is essentially 100% digestible. To investigate the effects of dietary RS, our group replaced the 100% amylopectin starch with a corn starch that is ~60% amylose and ~40% amylopectin (Hi-maize 260 resistant starch; Ingredion). Generally, corn starch that can be bought in the supermarket is ~80% amylopectin and 20% amylose. Thus, the Hi-maize product is considered a high-amylose maize starch. This Hi-maize product also assays as ~50% RS using the modified Englyst assay (6). The other ~50% of the starch consists of rapidly digestible starch and slowly digestible starch. Rapidly digestible starch can be digested to glucose within 20 min after initiation of treatment of the high-amylose maize starch with amylase and other enzymes in an in vitro assay. Slowly digestible starch is digested to glucose within 20–120 min. RS is any of the starch that is digested after 120 min. It is important to note that there is another commercial assay that is harsher than the Englyst assay and gives lower values of RS for samples (Megazyme International), and there is some debate about which assay is the best for measuring RS.

Fermentation of RS Leading to Improved Gut Health

Our group performed targeted analysis of the microbiota using culture and qPCR-based methodologies to measure bacteria in bacterial genera that initially ferment the starch granule and then produce lactate and acetate, which in turn are converted by bacteria in other bacterial genera to butyrate (10). Additionally, a global view of the microbiota using 454 pyrosequencing of bacterial DNA was performed (11). The targeted bacterial measurements were conducted using the cecal contents from ovariectomized and sham rats. We found increased amounts of *Lactobacillus* species, *Bifidobacterium* species, species in *Clostridial* clusters IV and XIVa + b, and an increase in the bacterial domain (total bacteria using universal primers) in sham and ovariectomized rats fed HAMRS2. Elderly (aged 20 mo) C57BL/6J (black 6) mice were used for the global measurement of the microbiota. In general, with the global analysis, the phylum *Firmicutes* was reduced with increasing doses of HAMRS2, and *Bacteroidetes* was increased. However, bacterial species in *Allobaculum* genera in *Firmicutes* were increased. Thus, the biodiversity of *Firmicutes* declined because of the fermentation of HAMRS2 in the diet. Surprisingly, bacteria in *Clostridial* clusters IV and XIVa + b were reduced with HAMRS2 feeding, but this was in a different model than the female rats described above (10). The genus *Bifidobacteria* increased within the phylum *Actinobacteria*, and *Akkermansia muciniphila* increased in the phylum *Verrucomicrobia*. These bacterial changes in mice fed HAMRS2 were correlated positively with measurements of fermentation.

Initially, our studies were limited to gene expression measurement of 2 well-known hormones produced by the gastrointestinal tract peptide YY (PYY) and glucagon-like peptide 1 (GLP-1). Both the PYY and proglucagon (gene for GLP-1) genes were increased with HAMRS2 feeding (7, 8). Along with the increased amounts of gene expression, the amounts of these hormones in the blood were also increased. GLP-1 and PYY are considered as satiety hormones (12), but rodents fed HAMRS2 tend to consume more food than control rodents. Our group demonstrated that GLP-1 with HAMRS2 feeding is elevated persistently over a 24-h period because of the continual fermentation of HAMRS2 rather than peaking after a meal and dropping between meals without HAMRS2 feeding (13). These chronically higher plasma amounts appear not to produce satiety. Primary culture of colonocytes demonstrated that SCFAs in the media increased expression of PYY and proglucagon genes, thus linking fermentation products with the production of these gastrointestinal tract hormones (13).

Later, our group performed a gene array for cecal cells to determine a more global approach on what gene expression

is increased (14). Overall, we observed increases in >2000 genes and specifically in genes associated with cell growth, proliferation, differentiation, and apoptosis, all likely tied in a complex manner to improved gut health. For example, our results included increased gene expression for galactose-4-epimerase, which catalyzes the formation of UDP-*N*-acetylgalactosamine from UDP-*N*-acetylglucosamine and represents the first committed step in mucin biosynthesis (15). The gut plays a major role in detoxifying agents from food and microbes. After the ingestion of HAMRS2, gut flora induces the chemopreventive enzyme glutathione transferase gene (16) expression in the colon of the rat (14). Our group found that HAMRS2 feeding was associated with a 3-fold elevation of the glutathione *S*-transferase A5 subunit and a 10-fold increase in the glutathione *S*-transferase Yc2 subunit. The most upregulated gene in the gene array was dual-specificity protein phosphatase, which responds to environmental stress and prevents oncogenesis (17). Adrenomedullin was also upregulated (5-fold), and the protein in the blood is reported to counteract the oxidative stress that leads to insulin resistance (18).

Many other genes involved with gut health were upregulated in our gene-array study (14). Muscle and microspikes rat sarcoma (RAS) is involved in the reorganization of the cytoskeleton of cells (19). Bone morphogenetic factors 2 and 3 genes are important for tissue architecture (20). Hypoxia inducible factor 1, α subunit (21) and vascular endothelial growth factor A (22) are genes important for blood flow in tissues. Amphiregulin promotes the growth of normal epithelial cells (23) and inhibits the growth of some cancerous cell lines (24) but was also reported to be increased in association with some tumors. The use of HAMRS2 appears to promote the beneficial effect of amphiregulin. Le Leu et al. (25) reported that HAMRS2 promotes apoptosis of precancerous cells but does not reduce cell proliferation of healthy cells. Cyclin-dependent kinase inhibitor 1A promotes apoptosis of potentially proinflammatory cells (26). Several TNF receptors were increased, and, during binding of TNF proteins to TNF receptors, 1 of 3 pathways can be stimulated based on cell type and conditions (27, 28). TNF can be involved in either promoting or preventing apoptosis and stimulating the immune response. Ras homolog gene family, member B inhibits NF-κB, leading to the promotion of apoptosis of damaged cells (29). NF-κB increases the protein cellular myc, which represses gene expression of growth arrest and DNA damage–inducible α and β. The result is the escape from programmed cell death (30). NF-κB inhibitor β binds to NF-κB, leading to increased apoptosis. Several of the growth arrest and DNA damage–inducible α and β proteins promote apoptosis by activating MAPK kinase, which then increases MAPK kinase 4 and then phosphorylation of c-Jun N-terminal kinase (30). Growth arrest and DNA damage–inducible α and β increases when NF-κB is inhibited. Neurotensin, a hormone that regulates gut motility, is also increased (31).

Several solute carrier 16 family genes were upregulated. These are also known as monocarboxylate transporters. Solute carrier 16a1 transports SCFAs (32, 33). Butyrate is the main energy source for the cells that line the colon (34) and is implicated in colon cancer prevention because it has the "potential to act on secondary chemoprevention by slowing growth and activating apoptosis in colon cancer cells" (35). Thus, increased uptake of SCFAs should improve the health of the gut.

Effects of HAMRS2 on Phenotype

Body fat

Initially, our research group did not know the metabolizable energy of the Hi-maize product, and we simply replaced Amioca control cornstarch with Hi-maize. The more HAMRS2 we used and the longer the study, the greater the reduction in abdominal fat (36). Later, we performed a study using collection cages and bomb calorimetry to determine the metabolizable energy of the Hi-maize product (37, 38). Our group teamed with a laboratory in Holland that used another technique: gain of dry body weight gain on the basal diet with 0–5 g of glucose added compared with dry body weight gain in rats fed different types of starches (37). The value measured with both techniques was ~2.8 kcal/g. Once we knew the metabolizable energy for the Hi-maize product, we were able to design studies with control and test groups fed isocaloric diets. To produce isocaloric diets, cellulose was added to the control diet because the product containing HAMRS2 has a lower metabolizable energy than the control amylopectin cornstarch, and purified cellulose provides 0 kcal/g because it is not fermented in rodents (39). Our initial studies without isocaloric diets were actually testing 2 effects of adding HAMRS2 to the diet: *1*) dilution of dietary metabolizable energy; and *2*) fermentation and production of fermentation products. Using a fermentable fiber/prebiotic in the diet not only lowers the energy of the diet, as with a nonfermentable fiber, but also affects the gut microbiota as a result of fermentation products produced by the bacteria. Both the microbiota and the fermentation products affect the health of the host, such as the behavior of the host through the endocrine, immune, and nervous systems (40). Using isocaloric diets, which allowed our group to test the effects of fermentation on abdominal fat amounts, also resulted in reduced abdominal fat. Our research group previously published these results. The results included reduced abdominal fat in male C57BL/6J mice (41), male Sprague-Dawley rats (7, 42, 43), female Goto-Kakizaki rats (44), and female ovariectomized and sham-surgery rats (10). The fat pads excised for measurement of their weights were epididymal (male) or ovarian (female) and perirenal (associated with the kidneys) and retroperitoneal (the remaining fat on both the left and right sides of the abdominal cavity).

The mechanism for this reduction in abdominal fat appears to be an increase in energy expenditure and increased oxidation of fat (41). C57BL/6J mice, placed in indirect calorimetry cages, demonstrated a significant ($P < 0.05$) reduction in the respiratory quotient (also called respiratory exchange ratio), and their heat production increased during the dark cycle (approached significance, $P = 0.07$). This

means that rodents fed HAMRS2 had increased fat oxidation and also may have increased energy expenditure. No effect was observed on physical activity, indicating that energy metabolism, and particularly oxidation of fat, was increased. Similarly, Shimotoyodome et al. (45) used the chemically modified version of RS, RS4, in C57BL/6J mice to prevent high-fat diet–induced obesity by increasing FA oxidation in the liver. So et al. (46) also demonstrated results similar to those of our research group using HAMRS2. Mice fed HAMRS2 had similar body weights but lower percentages of body obesity (subcutaneous and visceral), intrahepatocellular lipids, plasma leptin, plasma adiponectin, and plasma insulin/glucose than mice fed readily digestible starch. Additionally, adipocytes from epidiymal fat pads were smaller in mice fed HAMRS2 but had greater insulin-stimulated glucose uptake. The latter indicates greater insulin sensitivity. One major difference between the study by our research group and the study by So et al. (46) is that our studies demonstrated reduced body fat with HAMRS2 feeding compared with a control diet that had an equivalent energy content as the HAMRS2 diet. However, the diet with HAMRS2 in their study had a lower energy density (10 kJ/g) than the diet with readily digestible starch (15 kJ/g). With isocaloric diets, rodents fed HAMRS2 usually have numerically greater amounts of food and energy intake ($P \geq 0.05$), but it is not a statistically significant difference. In the study by So et al. (46), the mice fed HAMRS2 consumed significantly greater amounts of food but had lower energy intake. They argued that this lower energy intake was the result of greater neuronal activity in regions of the hypothalamus involved in appetite regulation.

Belobrajdic et al. (47) reported a dose–response study in obese-prone Sprague-Dawley rats. The results showed that addition of HAMRS2 to the diet reduced body fat when HAMRS2 was added at 8% of the weight of the diet but not at 4%. The researchers did not feed isocaloric diets, and, thus, the effect on the obese-prone rats is because of both reduction of the dietary energy of the diet containing HAMRS2 and fermentation of HAMRS2. This is essentially what would occur with humans if they added a source of HAMRS2 to their diets. These results are encouraging because of the possibility of reducing body fat in humans who consume adequate amounts of fermentable fiber. The estimated value for rodents was 10% of the weight of the diet (G Fahey, University of Illinois-Urbana, personal communication). Therefore, the use of products containing RS would appear to reduce body fat in humans who meet their dietary fiber requirement that includes a substantial amount of fermentable fiber.

Insulin resistance/sensitivity

In rodent studies, diets containing HAMRS2 improved insulin sensitivity measured with a glucose tolerance test in mice made partially diabetic with a streptozotocin injection but had no effect on normal mice (13). Our group then investigated the effects of HAMRS2 in a lean model of type 2 diabetes, the Goto-Kakizaki rat (44). Inclusion of HAMRS2 in the diet of Goto-Kakizaki rats improved insulin sensitivity and increased pancreatic mass compared with control rats.

In human subjects, the effects of HAMRS2 on insulin sensitivity were well documented by the Robertson laboratory in the United Kingdom (48–50) and by Maki et al. (51). The Robertson laboratory group did not observe an increase in the incretin hormone GLP-1 in humans in their studies despite its increase in many animal species with HAMRS2 treatment (7, 8, 13, 43, 44, 52, 53). However, in their most recent study, they observed increased serum GLP-1 (54). Two questions from their research include the following: *1*) why is there no GLP-1 response in many humans in several of their studies?; and *2*) what is the mechanism for improved insulin sensitivity in humans with HAMRS2 feeding who did not produce increased GLP-1? The answer to the first question appears to be that some individuals produce a defective transcription factor that normally interacts with the promoter region of the proglucagon gene in the intestinal L endocrine cells, and the result is lower production of the proglucagon gene transcript and lower amounts of the protein products that include GLP-1 (55). These individuals have a much greater risk of type 2 diabetes and have a polymorphism in the T cell factor 7 lymphoid enhancer-binding factor 2. The answer to the second question is unknown, but 1 possible reason for improved insulin sensitivity may be increased intestinal gluconeogenesis (56). Recently, De Vadder et al. (56) reported increased insulin sensitivity in mice fed fructooligosaccharide, butyrate, or propionate. The glucose produced by intestinal gluconeogenesis binds to receptors in the portal vein, and this signals the brain to inform the liver to reduce hepatic gluconeogenesis. Butyrate binds to its receptor in the intestinal cells and signals to the brain through a cAMP mechanism. Propionate is a substrate for gluconeogenesis, and its binding to its receptor also signals to the brain. Using knockout mice for glucose-6-phosphatase prevented the effects on insulin sensitivity. Intraperitoneal treatment with capsaicin, presumably damaging nerves from the portal vein, also knocked out the insulin sensitivity associated with fructooligosaccharide, butyrate, or propionate. However, when our group used intraperitoneal capsaicin to destroy nonmylenated neurons in the gut, we still observed beneficial health effects of HAMRS2 (43). Thus, there may be other unknown mechanisms that cause the increased insulin sensitivity in individuals given dietary HAMRS2 that do not produce an increase in serum GLP-1. Our group also showed that mice fed HAMRS2 had increased serum amounts of adiponectin (57), which may also be another possible mechanism for increased insulin sensitivity in human subjects.

WG RS

Other forms of RS besides HAMRS2 were also reported to have beneficial health effects if consumed. For example, RS4 was reported to prevent high-fat diet–induced obesity (45). Currently, there is much interest in WGs for their potential health benefits (58). Epidemiologic studies demonstrated beneficial health effects, such as improved satiety, blood cholesterol concentrations, and bowel functioning

with higher intake of WGs (58, 59). WG products contain a variety of compounds, including, but not limited to, RS1 (60) and other fermentable (e.g., arabinoxylans and oligosaccharides) and nonfermentable (e.g., cellulose and hemicellulose) fibers (61). The effect of RS1 may be difficult to investigate without the confounding by the many other components in WG products. Additionally, purification of RS1 may destroy the WG structure, such as the cell-wall structure that is needed to make the starch in the WG kernel resistant to digestion (62). Our research group recently completed a study using Zucker diabetic fatty (ZDF) rats (F Goldsmith, J Guice, R Page, AM Raggio, RW Stout, A Gaither, R Elzer, C Pelkman, J Ye, J Finley, RJ Martin, J Geaghan, H Durham, D Coulon, MJ Keenan, unpublished data, 2012). Four dietary groups were used. The first group was an isolated starch control using the 100% amylopectin Amioca product for the starch in an AIN-recommended diet formulated in 1993 for mature rodents. For the second group, the isolated Hi-maize starch product was used to provide HAMRS2. However, a WG version of the Hi-maize product was also used in the third group, and WG dent corn was used for a WG control group. Dent corn is typical corn with ~80% amylopectin and ~20% amylose and would have RS1 in the WG component and a small amount of HAMRS2 in the amylose fraction. The WG version of Hi-maize would have RS1 and a high amount of HAMRS2. Amylopectin starch was replaced with varying amounts of 1 of the 3 other products to result in 4 diets with 0%, 25%, 25%, and 6.9% RS by weight of the diets. Although there were no phenotypic changes observed, the ZDF rats robustly fermented the RS in the 3 diets that contained it. This was demonstrated by lower pH of the cecal contents, increased cecum weights, and increased amounts of SCFAs in the cecal contents. One interesting finding was that, during the study, the ZDF rats did not have soft or loose stools, and it was thought that this type of rat may be a nonresponder to HAMRS2. Most of the studies by our group that used HAMRS2 also used Sprague-Dawley rats. In these studies, our group usually observed soft feces during the study with fermentation of HAMRS2. Additionally, effects of fermentation were significantly greater in rats fed the WG products. This is likely because of components beyond HAMRS2 found in the 2 WG flour–containing diets. As stated above, the WG diets would also have RS1 (3, 4), arabinoxylans (61), cellulose and hemicellulose (59), and polyphenolic (59) compounds. The latter 4 types of compounds would be found in the bran component of the WG (59).

High-fat diets impair the fermentation response

High-fat diets modify negatively the intestinal microbiota and impair gut health compared with low-fat diets (63, 64). Our laboratory demonstrated healthy fermentation in rodents using low-fat (18% of energy) diets (7, 10, 41–44). Because moderate-fat diets (~30% of energy) are recommended for good health and a palatable diet (65), characterization of the effects of moderate-fat diets is important to determine whether changes to the intestinal microbiota [such as an increase in the genus *Allobaculum* of the phylum *Firmicutes* and the genus *Akkermansia* of the phylum *Verrucomicrobiota* (64)] also occur. Very little is known about a moderate-fat diet. Our group demonstrated that fermentation and other beneficial health effects of HAMRS2 feeding are attenuated with the consumption of a high-fat (42% of energy) diet (42). Another study reported similar results in rats with the use of high fats with fermentable fiber in the form of pectin, guar gum, or a mixture of both (66). The researchers found that the high-fat diet reduced the formation of butyrate and increased succinate, inflammation, liver fat, and cholesterol. The dietary fiber only partially counteracted these negative effects.

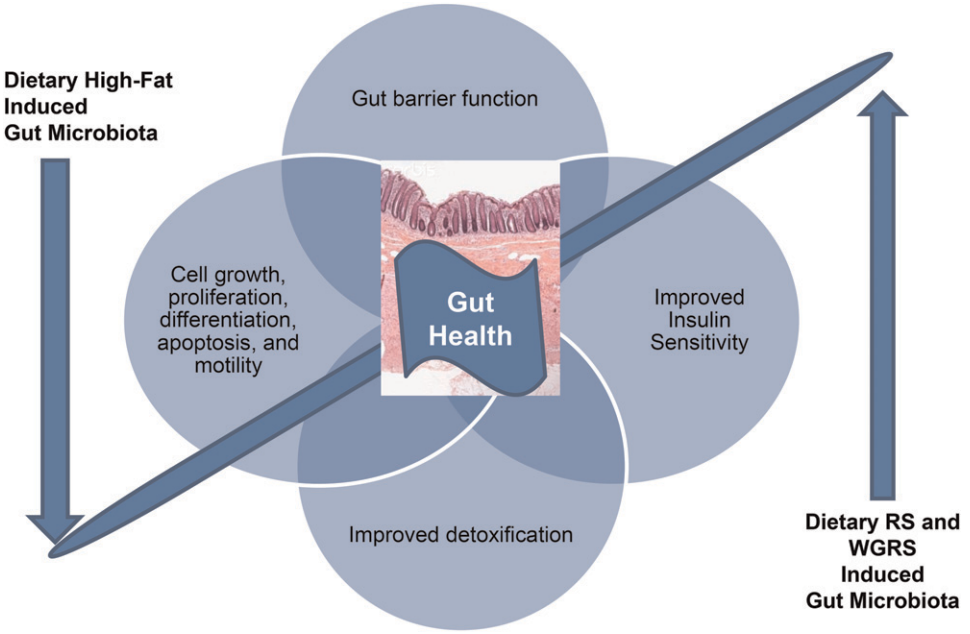

FIGURE 1 A schematic summary of the beneficial gut health effects of a microbiota in response to the feeding of a high-fat diet vs. a gut microbiota in response to the feeding of RS or a whole-grain RS product. The former reduces and the latter increases beneficial health effects. RS, resistant starch; WGRS, whole-grain resistant starch.

Reduction of metabolic endotoxemia

Metabolic endotoxemia is a relatively new concept based on recent evidence that certain gram-negative bacteria enhance the exposure to the LPSs (67–69). LPSs are large glycolipids derived from the outer membrane of gram-negative bacteria. High-fat diets promote a dysbiosis in the microbiota and a "leaky gut," allowing LPSs to enter the blood (67, 70). LPSs cause a condition of "metabolic endotoxemia" characterized by low-grade inflammation and insulin resistance. The LPSs are powerful stimulators of the innate immune system response. After binding to the toll-like receptor 4 and its coreceptors, LPSs trigger a cascade of responses, ultimately resulting in the release of proinflammatory molecules that interfere with the utilization of glucose metabolism and insulin sensitivity. Furthermore, a selective increase of *Bifidobacteria* in the gut improves high-fat diet–induced diabetes in mice through a mechanism associated with decreased endotoxemia (67). We showed that a diet with HAMRS2 increases gut *Bifidobacteria* and improves insulin sensitivity in the Goto-Kakizaki rat, an animal model of diabetes (44). In this study, LPSs were not measured, but other studies demonstrated that the use of the nonsystemic antibiotics neomycin and ampicillin cured a dysbiosis in obese mice and mice fed a high-fat diet (70). This resulted in lower plasma LPS concentration. Possibly, dietary manipulation of gut microbes could be a potent strategy for the control of metabolic diseases, as well.

Bifidobacteria may be too simple to be the only answer for a healthy gut. The genus *Akkermansia* was only discovered 9 y ago, and bacteria in this genus, particularly *Akkermansia muciniphila*, degrade mucin (71). Mucin serves as the carbon and nitrogen source for bacteria in this genus. They degrade N-glycans, and the gene-array study by our group (14) demonstrated that galactose-4-epimerase, which catalyzes the formation of UDP-*N*-acetylgalactosamine from UDP-*N*-acetylglucosamine and represents the first committed step in mucin biosynthesis (72), was upregulated. This enzyme transfers an N-glycan to the oxygen of serines or threonines to produce O-glycans. These O-glycans promote the growth of favorable bacteria in the large intestine. *Akkermansia* is also 1 of the genera increased in mice fed a low-fat diet vs. a high-fat diet (64). With the increase of the study of the microbiome in recent years, other newly discovered genera of bacteria may be increased when investigating the interaction among 3 bioactive dietary components: *1*) moderate fat; *2*) RS; and *3*) WG (**Figure 1**).

Summary

Many studies were performed with RS fed to animal models and demonstrated many health benefits, including increased fermentation leading to improved gut health, reduced adiposity, and improvement in insulin sensitivity. The major beneficial result observed in studies with human subjects is an increase in insulin sensitivity. Most animal studies demonstrate an increase in the gastrointestinal tract incretin hormone GLP-1, but only 1 of the studies demonstrating improved insulin sensitivity reported an increase in serum GLP-1. This may be because many humans who develop insulin resistance and type 2 diabetes have an ineffective allele for a transcription factor that is known to interact with the promoter region of the proglucagon gene and to increase its transcription. The mechanism for improved insulin sensitivity in human subjects in those who do not produce increased amounts of GLP-1 with HAMRS2 feeding is unknown at this time but likely would involve positive changes in the microbiota. Also, very encouraging is the study in obese-prone rats that observed reduced body fat with 8% of the weight of the diet as HAMRS2. This result may mean that humans could reduce body fat if the recommended amounts of dietary fiber are consumed, including a variety of fermentable fibers.

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