Also In This Issue

■ Effect of Fermented Soyfoods on the Microbiota

STUDIES SHOW SOY CONSUMPTION MAY PROMOTE A HEALTHY GUT MICROBIOTA

By Elaine S. Krul, PhD

A Quick Taste

Elie Metchnikoff, working at the Pasteur Institute in Paris in the early 1900s, was one of the first scientists to recognize that the types of bacteria (microbiota) residing in our gut have a significant impact on our overall health.[1] He noted that increasing the content of lactic acid producing bacteria, through the consumption of soured milk products, contributed to increased well-being.[1] Today it is common for people to consume live lactic-acid bacteria as supplements or in foods for their potential health benefits (probiotics).[2] Another way to promote the growth of beneficial microbes is to consume foods (prebiotics) that contain "selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health."[3] This article summarizes the emerging data suggesting that soy products may promote a healthy gut microbiota.

Gut microbiota play a critical role in human metabolism and health by processing nutrients and drugs, synthesizing vitamins and inhibiting growth of potential pathogens. The gut microbiota co-evolved with humans in a symbiotic manner, so that those microbes that thrive on ingredients in the human diet serve to provide additional metabolic activity to the host (gut microbiota have 100 times more genes than human), thereby enhancing nutrient availability while also affording protection against opportunistic pathogens. The large intestine harbors most of the gut microbiota and is the major site for fermentation of dietary ingredients which are not efficiently digested in the small intestine. The efficient extraction of energy from the diet made possible by the metabolic activity of the colonic microbiota, in some cases, has undesirable consequences. For instance, gut microbiota have a causal role in the development of obesity in mice. Ridaura et al.[4] demonstrated that when gut microbiota from human twins who were discordant for obesity were transferred to germ-free mice, the mice receiving the microbiota from the obese twin had significantly more body mass and fat tissue than mice receiving the microbiota from the lean twin. The obese individuals had a higher ratio of certain phyla of bacteria (*Firmicutes* to *Bacteroidetes)* and overall reduced bacterial diversity in the gut compared with lean individuals.[5]

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Currently, there is no scientific consensus as to what constitutes a "healthy gut microbiome," but bacteria can be categorized as being either beneficial or potentially deleterious based on their metabolic activities and fermentation products. Bacteria having almost exclusively saccharolytic metabolism (breakdown of carbohydrate for energy) with little peptolytic metabolism (breakdown and metabolism of peptides) such as lactobacilli and bifidobacteria are considered potentially beneficial.[3] Researchers are actively trying to discern what gut microbial metabolites or patterns of metabolites may be predictive of an optimal host-microbe-metabolism through advances in metabolomics and metagenomics.[6] A recent study identified specific gut microbial metabolites, measured in plasma and urine, that were present in higher concentrations in patients with coronary heart disease (CHD) compared with healthy subjects, suggesting that a deranged gut microbiota composition (dysbiosis) is present in the CHD patients.[7] There is a large and growing body of evidence indicating that gut dysbiosis may be central in contributing to metabolic, immune and cognitive dysfunction as well as cardiovascular disease and cancer.[8-14]

The composition of each individual's gut microbiota is influenced by the environment, genetics and most importantly by diet, and remains relatively stable over time.[15] People possess a "core" microbiome which appears to reflect the metabolic pathways and systems used by the resident microbial population to generate energy from the host diet.[5] Some researchers have demonstrated that the "core" microbiomes can be classified based on the characteristics of the predominant microbial genera.[16,17] Differences in habitual diets among groups within a given social group (e.g. urban America) can show large variations in plasma metabolomes (circulating metabolites largely produced by the gut microbiota) while differences in respective gut bacterial communities are relatively modest, indicating that the core gut microbiome can accommodate and efficiently metabolize substrates from a range of varied diets.[18] Core microbial profiles show major differences between populations with radically different diets and environments.[19] That being said, the microbiome demonstrates a significant ability to adapt to sudden or significant changes in diet composition, such as switching from animal to a plant-based diet or vice versa by quickly altering the bacterial composition to meet the functional demands to metabolize alternative substrates.[20-24]

Plant-based diets tend to promote the growth of saccharolytic bacteria and plant fiber is the main source of carbohydrate for the colonic bacteria.[3] A major end product of saccharolytic bacteria are short chain fatty acids (SCFAs) which are absorbed into the blood stream. Societies that consume a plant-rich diet exhibit a gut microbiome that is characterized by a higher *Bacteroidetes* to *Firmicutes* ratio,[19] which is opposite to that seen in obese subjects in the U.S.[5] Up to 90 percent of ingested plant polyphenols, such as soybean isoflavones, make their way to the colon where they can interact directly with the microbiota or can be used as bacterial substrates to produce SCFA and other metabolites.[25]

Soy and the Microbiota

Soy contains four major components which can impact the composition of the microbiota in a potentially prebiotic manner: fiber, oligosaccharides, isoflavones and protein. A brief summary of the evidence for plausible beneficial effects of these components is presented below.

No human studies have directly evaluated the effects of soy fiber (derived from the soybean cotyledon) on the gut microbiome, but benefits of it on some aspects of bowel function and metabolic health have been known for some time.[26,27] Kapadia et al.[28] evaluated the fermentation characteristics of soy fiber *in vitro* using human fecal bacteria. Compared with the control and oat fiber substrates, soy fiber produced significantly more SCFAs.[28] A similar experiment using dog fecal bacteria showed that soy fiber was moderately fermentable and produced equivalent SCFAs to that of sugar beet fiber or pulp, citrus pectin and pea fiber.[29]

Kapadia et al.[28] also evaluated soy oligosaccharides (carbohydrates made up of 3 to 9 monosaccharides) in human fecal bacterial cultures and found a 4.6-times increase in SCFAs compared with soy fiber, indicating that soy oligosaccharides have a much higher "prebiotic" potential on a weight basis. The major soybean oligosaccharides are

raffinose and stachyose, which are not digested in the small intestine and enter the colon as substrates for the resident bacteria. Commercial preparations of soy oligosaccharides are available and presumably marketed for their prebiotic potential.[30] Soy oligosaccharides appear to promote the growth of bifidobacteria, and not bacteria such as *Clostridium difficile* or *E coli,* since only the former specifically utilizes these oligosaccharides as substrates.[3,31-33] Fermentation studies using human fecal bacteria mixtures have confirmed that soy oligosaccharides tend to promote the growth of bifidobacteria.[30,34-36] Studies on people consuming either pure raffinose[37] or soy oligosaccharide mixtures[36,38-40] also show a bifidogenic property of soy oligosaccharides. Nevertheless, additional studies are needed to better understand the potential beneficial effects of soy oligosaccharides on the overall microbiota profile.

Studies to evaluate the effect of soy protein on gut microbial changes are challenged by the fact that the protein is associated with isoflavones, and possibly fiber and oligosaccharides, depending on the source of soy protein (isolate, concentrate or whole soy). Human studies are limited to analyses of fecal microbiota, which does not permit direct evaluation of the microbial populations in the gut. However, short-term[41,42] and long-term[43] studies of soy protein with isoflavone consumption in postmenopausal women have demonstrated consistent increases in fecal *Bifidobacterium* and other microbial differences that were unique to each study. All three studies noted that specific changes in microbial profiles showed significant correlations to the equol producing status of the subjects, which may be expected since the conversion of the isoflavone daidzein to equol is mediated by specific gut bacterial species.[44] A two-week study found the consumption of fermented, in comparison with non-fermented, soymilk (100 g/day) caused significant increases in fecal bifidobacteria and lactobacilli with reductions in clostridia.[36] Unfermented soymilk produced similar findings but the results were not statistically significant. In addition, the presence of live bacteria in the fermented soymilk confounds the interpretation of this study.

Finally, Fernandez-Raudales et al.[45] reported that obese adult men consuming a low glycinin (a fraction of soy protein) or conventional soymilk for three months had significantly lower fecal *Firmicutes* to *Bacteroidetes* ratios and lower fecal *Bifidobacterium* compared with bovine milk. It is not clear why this study reported a reduction in bifidobacteria with soy protein consumption and why all test products reduced bacterial diversity over three months.

A study of infants switched from cow's milk formula to soy-based formula for one month showed that the diversity of bacteria and presence of beneficial bifidobacteria and ruminococci were similar to that seen with cow-milk formula,[46] indicating that the microbiota of formula-fed infants may be similar regardless of protein source. More research

applying sequence analyses of gut microbiota are needed in this area. Malawian infants aged 6 to 18 months, also did not show significant differences in their fecal microbial profiles (using sequencing methods) after receiving one of four interventions: Control, lipid-based nutrient supplements (cow milk or soy-based) or corn-soya blend.[47] The lack of difference may be due to the high inherent gut bifidobacteria presence in this population, as well as dominance by other species such as *Prevotella* and *Faecalibacterum.[47]*

Animal studies may provide some advantages in helping to learn about the role of dietary protein on the gut microbiota. Responses of the microbiota in animals to changes in dietary protein appear to be similar to what is seen in humans. Lee et al.[48] demonstrated that when 20 percent of the casein in a cholesterol-enriched laboratory diet was substituted by soy protein from freeze-dried soymilk for six weeks, rats had an increased fecal *Firmicutes to Bacteroidetes* ratio compared to the cholesterol-enriched diet alone. An et al.[49] found microbial diversity was significantly higher for rats fed soy protein for 16 days compared with casein, but not compared with a diet containing fish meal. In another recent study, Zhu et al.[50] found distinctions in microbiota of Sprague-Dawley rats fed diets differing only in protein source for 90 days. Analyses of the microbial sequences revealed that the meat-fed groups had more similar gut microbiota compared with the non-meat (casein and soy-fed) groups, with the meat protein groups having a higher *Firmicutes* to *Bacteroidetes* ratio compared with the non-meat group.[50] Analyses of the feces revealed that the soy protein fed group had the highest content of SCFAs compared to all other groups.[51]

Another study found that in comparison with milk protein, soy protein increased the microbial diversity throughout the gut of hamsters.[52] Increased microbial diversity in humans is associated with a "lean" phenotype[5] and has been shown to be associated with metabolic health, while individuals with low richness have a relatively higher incidence of dyslipiemia, higher fat mass, insulin resistance, inflammation and frailty (elderly).[53-55] In this hamster study, three differently processed soy proteins were evaluated and all soy proteins showed significant differences in microbial profiles compared to the milk protein and were most similar to each other.[52] Importantly, microbial families present at significantly higher concentrations in the gut of soy protein-fed groups were correlated with lower blood lipid concentrations and the expression of hepatic genes that could account for the observed lipid concentrations. Conversely, those microbial families more abundant in the milk protein-fed groups correlated with higher plasma lipid concentrations and expression of hepatic genes that contribute to the higher lipid concentrations.[52] This study provides evidence that soy protein may exert its cholesterol lowering activity in large part through its ability to modulate the gut microbiome.

Summary and Conclusions

The emerging data on the potential prebiotic properties of soy products indicate that consuming these products can help promote a healthy gut microbiota. The role of the diet in maintaining health is currently receiving more attention given that the diet is one of the most significant factors impacting the gut microbial profile. Future research on the gut microbiota and soy promises to provide more insights into how soy consumption contributes to maintaining overall health.

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ABOUT THE AUTHOR

Elaine S. Krul, PhD, is a senior technical fellow in the Global Nutrition Innovation group at DuPont Nutrition & Health and adjunct associate professor at Washington University in St. Louis. Her research focuses on identifying the biochemical mechanisms of how food ingredients provide nutrition and confer health benefits. She obtained her PhD in Biochemistry from McGill University and has over three decades of experience conducting academic and industry research.

Effect of Fermented Soyfoods on the Microbiota

By Mark Messina, PhD

Fermented soyfoods play an important role in the cuisines of many Asian countries. Fermentation produces changes in the composition of the food itself but the health implications of these changes aren't precisely understood. Limited evidence suggests fermented soyfoods exert favorable changes on the composition of the intestinal microbiota.

There are numerous ways in which fermentation can potentially enhance the healthfulness of soy, such as by decreasing phytate,[1] protease inhibitor[2] and oxalate[3] content, thereby potentially improving protein digestion[4] and mineral absorption.[5-7] Fermentation may also create antioxidants[8-9] and in the case of natto, fermentation causes this food to be an excellent source of vitamin K[10,11] and nattokinase, an enzyme which exhibits fibrinolytic activity.[12-13] However, the nutritional and physiological relevance of these fermentation-induced changes remains to be established. This lack of clarity is because there is adaptation to the inhibitory effects of phytate on mineral absorption,[14] the digestion of protein from unfermented soy is excellent[15] and despite containing oxalate (and phytate) the absorption of calcium from fortified soymilk is similar to the absorption of calcium from cow's milk.[16]

In addition to the above-cited changes, fermentation causes more of the isoflavones naturally present in the soybean to be in the aglycone rather than glycoside form.[17-20] Since isoflavones are absorbed as aglycones, there has been speculation that by bypassing the first step in the absorption of isoflavone glycosides, which is the hydrolysis of the glucose molecule from the isoflavone backbone, the absorption of isoflavones from fermented foods would be greater than from unfermented ones. However, despite years of investigation, no consensus on this issue has emerged,[21-26] although it is accepted that aglycones are absorbed more quickly.[21-23]

Over the past decade or so there has been a surge of interest in understanding the effect of diet on the microbiota and the impact of the microbiota on overall health.[27] Some soyfoods may influence the microbiota because the soybean contains large amounts of oligosaccharides (predominately stachyose).[28-31] Because these sugars are poorly digested by intestinal enzymes, they travel to the colon where they are able to stimulate the growth of bacteria such as *Bifidobacteria* which are considered to be advantageous to the host. For this reason soybean oligosaccharides are classified as prebiotics.[32-34]

Fermented soyfoods may affect the intestinal microflora independent of oligosaccharide content. For example, tempeh made from soybeans was shown to stimulate the growth of bacteria of the genus *Bifidobacterium*, whereas tempeh made from common beans stimulated the growth of *Escherichia coli*.[35] These changes clearly represent potential beneficial effects of soy tempeh. For this in vitro study, tempeh was fermented for 24 hours and then subjected to digestion in conditions simulating the human digestive tract. Human fecal microflora were added at the stage corresponding to the small intestine and the change in the number of microorganisms belonging to the genera *Bifidobacterium, Lactobacillus, E. coli* and *Enterococcus* was determined at the end of the digestion process.

More than a decade ago, the effect of natto on the microbiota was evaluated by Japanese researchers.[36] Natto is an especially popular food in the Eastern regions of Japan. For this study, seven healthy volunteers, 22 to 49 years of age, consumed 50 g/day of natto for 14 days. When compared to the baseline values, during natto consumption, the counts of *Bacillus subtilis* and *Bifidobacterium* were significantly increased, whereas the counts and the frequency of the occurrence of lecithinase-positive clostridia, including *Clostridium perfringens*, were significantly decreased. Natto had previously been shown to enhance the growth of *Bacillus*, *Streptococcus* and *Lactobacillus*, and to reduce *Escherichiacoli,* in rat caeca.[37]

A second human study that utilized a similar experimental design as the previous study, examined the effects of miso soup containing natto on the microbiota.[38] The results of the two studies are similar. Over the 14-day experimental period, the numbers of *Bacillus* and *Bifidobacterium* were increased whereas numbers of *Enterobacteriaceae* were decreased and the numbers of *Clostridium perfringens* tended to decrease. The eight participants in this study consumed 200 ml of miso soup daily containing 50 g of commercially available natto that was boiled for one minute.

Finally, a cross-over study found that fermented soymilk led to desirable changes in the intestinal microflora. Soymilk is typically consumed in the unfer-

mented form but for experimental purposes, Chinese researchers compared the effects of fermented and unfermented soymilk.[39] Twenty-eight participants consumed 250 ml twice a day between meals, of either fermented soymilk or regular soymilk for two weeks and then switched to the other drink for two weeks after a two-week washout period. During the consumption of fermented soymilk, the populations of *Bifidobacterium spp.* and *Lactobacillus spp.* increased as well as the ratios of *Bifidobacterium spp.* and *Lactobacillus spp.* to *Clostridium perfringens*. The population of coliform organisms also decreased in response to fermented soymilk consumption. A more recent study that also compared fermented with unfermented soymilk also found more favorable changes in the intestinal microflora in response to the former.[32] For this study, ten participants, from 21 to 25 years of age consumed 100 ml/day of either fermented or unfermented soymilk for two weeks.

Future research is needed to determine how changes in the microflora in response to fermented soyfoods affect overall health.

History of Fermented Soyfoods

Soyfoods were first consumed in fermented form beginning in China around 2,200 years ago and in Japan approximately 700 years later. Historical records indicate that in China, unfermented soybeans and tofu were consumed beginning approximately 2,000 and 1,000 years ago, respectively.

Today, in Japan, about half of soy consumed is derived from unfermented foods, primarily tofu, and about half comes from the fermented products, miso and natto. In contrast, in China, Hong Kong, and Singapore, nearly all soy consumed is in unfermented form, mostly as soymilk and various forms of tofu. In Indonesia (the birthplace of tempeh), about 60 percent of the soy is consumed in fermented form as tempeh, and about 40 percent as tofu. Finally, in Korea, about 70 percent of the soy is consumed in unfermented form.

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ABOUT THE AUTHOR

Mark Messina, PhD, MS, is the executive director of the Soy Nutrition Institute, and co-owner of Nutrition Matters, Inc., a nutrition consulting company. He is an adjunct professor at Loma Linda University and chairman of *The Soy Connection* Editorial Board. His research focus is on the health effects of soyfoods and soybean components.

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