Urolithins and intestinal health

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SUMMARY There are trillions of microorganisms in the human intestine. They can react to the intestinal micro-environment by metabolizing food or producing small molecular compounds to affect the host’s digestive ability and resist the risk of infection and autoimmune diseases. Many studies have revealed that intestinal flora and its metabolites play an important role in human physiology and the development of diseases. Urolithins are kinds of intestinal microbiota metabolites of ellagitannins (ETs) and ellagic acid (EA) with potent biological activity in vivo. However, different individuals have different intestinal flora. According to the different metabolites from ETs and EA, it is divided into three metabo-types including UM-A, UM-B and UM-O. This paper reviews the origin of urolithins, the urolithin producing microorganisms and the effects of urolithins on regulating intestinal diseases. This review will provide a theoretical basis for the regulation of urolithins in the homeostasis of intestinal flora and a reference for the scientific utilization of urolithins and foods rich in ETs and EA.

Keywords Urolithin, intestinal microbiota, intestinal health

1. Introduction

It is estimated that the number of bacteria in human intestine is about 10 times that of human cells. The micro-ecological balance of intestinal flora affects energy absorption and immune system, thus affecting human health. Studies have reported that small molecular compounds produced by intestinal microbiota have direct correlation with human health. For example, short chain fatty acids (SCFAs), produced by Bacteroides and Firmicutes through anaerobic fermentation of dietary fiber, can enhance host immunity (1,2). Indole produced by intestinal flora can regulate the host’s immune system and inflammatory diseases (3). Flavonoid metabolites of intestinal flora can reduce the prevalence of obesity (4). Meanwhile, specific intestinal bacteria and their products can lead to human diseases. For example, the small molecule TMAO produced by intestinal microbiota from nutrients rich in choline is related to many metabolic diseases (5,6) Colibactin produced by a polyketide synthase positive (PKS+) Escherichia coli can promote the occurrence of colorectal cancer (CRC) (7). Therefore, it is of great significance to study the mechanism of the intestinal microbiota metabolites and their occurrence together with their effects on development of diseases.

Ellagitannins (ETs) and ellagic acid (EA) are natural polyphenols found in fruits and nuts, as well as some traditional Chinese medicines. The physical and chemical properties of ETs with high molecular weight and strong polarity determine their low bioavailability. The micro-ecological balance of intestinal microbiota affects energy absorption from food and immune system, thus affecting human health. As metabolites of intestinal flora, urolithins have many biological activities, such as anti-oxidation (8), anti-inflammation (9), inducing fat browning (10), and regulating lipid metabolism (11). In recent years, the regulatory effects of urolithins on intestinal microbiota and intestinal inflammatory diseases have also received widely attention (12). Here, we review the origin of urolithins, urolithin-producing microorganisms and the regulatory effects on intestinal related diseases.

2. The origin of urolithins

Urolithins (uros) are polyhydroxyl derivatives of diphenylpyran-6-one, which can be considered as a combination of coumarin and isocoumarin in chemical structure. Although uro-M5 has been reported to be isolated from plants Terminalia (13), Rosa chinensis (14), Lagerstroemia speciosa (15), Punica granatum (16), Mallotus furetianus (17), and uro-A from pomegranate (18), uros are not common in nature.
After eating food rich in ETs, most of them are first metabolized to EA in stomach and small intestine of mammals. Then, EA loses a lactone ring to obtain uro-M5 under the action of esterase and decarboxylase in intestinal microbiota, and then gradually loses hydroxyl groups under the action of dehydroxylase to form a class of internal metabolites with different hydroxyl substitutions (19,20). Uro-A and uro-B were first isolated from sheep kidney stones as EA metabolites (21), and then were found in urine, feces, bile, prostate, colon and milk of human, rat, mouse, cow, pig, beaver and other animals. Compared with ETs and EA, urolithins are more easily absorbed in colon and can be detected in blood a few hours later under the action of intestinal microbiota. After that, it is widely distributed in the cells of the body or enters the liver with the blood circulation to participate in phase II metabolism, which is gluconic acidified, sulfated or methylated to further exert biological effects (22,23). The concentrations of phase II metabolites in human plasma were uro-A glucuronide with 0.024-35 μM, isouro-A glucuronide with 0.0045-0.745 μM and uro-B glucuronide with 0.012-7.3 μM (24), respectively. With the application of high-throughput and high-sensitivity detection methods, more and more urolithins and their derivatives have been discovered and studied. Members of the urolithin family include uro-M5, uro-D, uro-M6, uro-E, uro-C, uro-M7, isouro-A, uro-B, uro-A, uro-M6R, uro-M7R, uro-CR and uro-AR (Figure 1) and their corresponding phase II metabolites (25,26). According to final metabolic products, it is divided into three metabo-types including UM-A (producing only uro-A conjugates), UM-B (producing uro-A, isouro-A and/or uro-B) and UM-0 (no urolithins) (27,28). Uro-AR exists in both UM-A and UM-B metabo-types (25). Studies have shown that the urolithin producing ability and the metabo-types are not closely related to food sources, age and health status (27), but are determined by the intestinal microorganisms that can metabolize ETs and EA. However, the analysis of the metabo-types of 839 healthy people aged from 5 to 90 showed that 70-80% of healthy young people aged 5-30 were UM-A, 10-20% were UM-B, while UM-B type increased in people aged 30-90 (29). In addition, individual health status such as obesity, colon cancer, hyperlipidemia, cardiovascular disease also affects metabo-types. Romo-Vaquero et al. analyzed the intestinal microflora of 249 healthy individuals by 16S rDNA sequencing. The results showed that bacteria Coriobacteriaceae may be the relationship between the level of UM and blood cholesterol. From the current research, UM-A may be more conducive to health, while UM-B may be associated with some diseases and flora disorders (30).

3. Urolithin-producing strains from intestinal microorganisms

Urolithins have been found in many animals such as mice, rats, beavers, sheep, cattle and humans after eating foods rich in ETs (31,32). Recently, urolithin-producing microorganisms have also been reported. The transformation of ETs and EA by fecal microorganisms of 6 volunteers in anaerobic environment was studied. Uro-A was detected in the fermentation products by fecal bacteria of different volunteers, which confirmed that uro-A is the metabolite of ETs in vitro for the first time, but the concentrations and yields were different, indicating that the composition of individual fecal flora was different (33). Studies have also been carried out on the isolation of microorganisms which can convert EA into urolithins from the feces of healthy people. Selma et al. confirmed for the first time that the new species Gordonibacter urolithinfaciens DSM27213 and G. pamelaeae DSM19378 have the ability to convert EA into urolithins in stationary culture under anaerobic conditions in vitro, and HPLC-DAD-MS analysis showed that pentahydroxy uro-M5, tetrahydroxy uro-M6 and trihydroxy uro-C were produced sequentially, but uro-A and uro-B were not detected in pure culture. It is suggested that the UM-A or UM-B

Figure 1. The presentative structures of urolithins.
metabolism may require the participation of other microorganisms or the regulation of culture conditions (34-36). Strains Ellagibacteris isouroolithinifaciens DSM104140 and G. urolithinfaciens DSM27213 could convert EA into uro-M5, uro-M6, uro-C and isouro-A (37); 48 strains of Bifidobacteria and 1070 strains of other bacteria were isolated from the feces of healthy women and their ability to produce urolithins were tested, only strain Bifidobacterium pseudocatenulatum INIA P815 could convert EA into uro-A in Brain-Heart Infusion (BHI) medium (38).

The number of bacteria in human intestinal tract is about 10 times that of human cells, obviously, the study of microorganisms (genes or enzymes) involved in the transformation of EA in vivo is not deep enough. The key rate-limiting steps of urolithin production in vivo have not been solved (20). The future research should combine the traditional microbial isolation and microbial culture technology together with metagenomics and culturomics to determine the key genes or enzymes involved in the production of urolithins and carry out in vivo investigation, and further to explore the role of these microorganisms and enzymes in the regulation of intestinal flora and their effects on human health (39).

4. Urolithins and intestinal health

ETs and EA are polyphenols present in a variety of fruits, vegetables, nuts and medicinal plants, with a variety of biological activities. Most of EA is metabolized by intestinal flora to produce a series of urolithins that are more easily absorbed, and their concentrations in different tissues ranging from 0.003 to 50 μM (40). Therefore, urolithins may be the real bioactive substances rich in ETs in organisms. Uro-A has been reported as a potential molecule in regulating metabolic diseases such as neuro-inflammation, cardiovascular disease and obesity (41). Anti-oxidation, anti-inflammation, anti-cancer, anti-obesity and neuroprotective activities have been reported (42). Also, uro-A showed protective potential in the gastrointestinal inflammatory diseases such as CRC and inflammatory bowel disease (IBD) (12). Here, we mainly summarize the role of urolithins in regulating intestinal flora and intestinal health.

4.1. Urolithins inhibit bacterial infection

The virulence factor of bacteria is the guarantee for the stable existence of intestinal flora in human digestive tract and against human immunity (43). Inhibiting virulence is an important way to control pathogen infection. Four μM uro-A and uro-B can reduce the levels of N-hexanoyl-L-homoserinelactone (C6-HSL) and N-(3-oxohexanoyl)-L-homoserinelactone (3-oxo-C6-HSL) in Yersinia enterocolitica, thus inhibit the formation of quorum sensing-related biofilm and the movement ability of bacteria, and maintain the balance of intestinal flora (44). Uro-M5 is an inhibitor of type three secretion system of Salmonella and can protect the host by reducing virulence and inflammation (45). Moreover, uro-A, uro-B and uro-D have certain antibacterial activity, but their antibacterial activities are weak, coupled with their weak cytotoxicity, the researchers attributed their antibacterial activity to high intake (46). The above results suggested that urolithins are inhibitors of bacterial virulence without killing pathogens, and are substitute antibiotics without producing drug resistance (47).

4.2. Urolithins regulate intestinal flora

The intestines of human contain 100 trillion viable bacteria, including beneficial and harmful to human health. After feeding uro-A to colitis mice for 10 days, the abundance of the beneficial bacteria Lactobacillus, Bifidobacterium and Clostridium in fecal samples significantly increased (48). Also, the abundance of Akkermansia and Gordonibacter in intestinal flora of uro-A producers was higher than that of non-uro-A producers (49). The body weight of high-fat diet induced obese mice was greatly reduced by treatment with 2.5 mg/kg uro-A or uro-B. 16S rDNA sequencing analysis showed that the anti-obesity effects of uro-A or uro-B may play an important role in weight loss by regulating intestinal flora (50). Uro-A can also help restore colon tissue damage and regulate intestinal flora, thereby reducing inflammation (51). Food-derived metabolites can also regulate intestinal flora (24). Medicinal edible plants rich in ETs have different metabo-types (UM-A, UM-B and UM-0) after intestinal flora metabolism, and different metabo-types also reflect the differences of intestinal flora. Foods rich in ETs can increase the abundance of urolithin-producing Gordonibacter in fecal microorganisms (52). High-fat diet caused intestinal flora disorder in mice, in which Ruminococcus increased significantly. Compared with high-fat diet, foods rich in polyphenols increased the abundance of Roseburia and decreased the abundance of Mogibacteriaceae, while the polyphenols in red raspberry seeds increased the abundance of Bifidobacterium (53). Therefore, ET-containing food and urolithins can increase the beneficial bacteria such as Akkermansia and Bifidobacteria, and can restore normal intestinal balance and produce beneficial effects to maintain intestinal homeostasis.

4.3. Urolithins enhance the function of intestinal barrier

Uro-A showed anti-inflammatory activity against mouse Raw264.7 macrophages induced by lipopolysaccharide (LPS). Uro-A pretreatment and post-treatment of DSS induced colitis mice can reduce
inflammatory signals and up-regulate the expression of tumor suppressor genes, thus alleviating colonic injury and playing an important role in regulating the balance of intestinal flora in mice (48). Aromatic hydrocarbon receptor (AhR) plays an important regulatory role in enteritis. Recent studies on inflammatory cell models have shown that uro-A can improve the biosynthesis of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) by activating the AhR-Nrf2 dependent pathway, up-regulate the expression of tight junction proteins, prevent inflammation, and improve the barrier function of intestinal epithelium in IBD disease (54). Uro-A and its synthetic analog UAS03 enhance the barrier function of intestinal epithelial cells by up-regulating Nrf2 dependent epithelial cell connexin, and protect IBD by reducing inflammatory response (53).

4.4. Urolithins inhibit CRC and IBD

Urolithins are mainly produced in the small intestine and colon. Therefore, urolithins are expected to play a role in the intestine and intestinal wall (56). In vitro studies have proved that uro-A has the inhibitory effects on CRC and IBD. The anticancer effect of uro-A may come from autophagy induction, because autophagy will be triggered after eating polyphenols, thus inhibiting the growth and metastasis of CRC cells (57). The mixture of uro-A and uro-B acts on colorectal adenocarcinoma Caco-2 cells both in the long and short term, and the drug reaching the intestinal cavity helped to reduce oxidative stress, and prevented the damage caused by reactive oxygen species (58). Uro-A has a significant inhibitory effect on the growth of colon cancer cell line HCT116 with IC_{50} of 19 μM (72 h) and has a synergistic effect with oxaliplatin, which can induce the stability of p53 and the expression of p53 target gene, resulting in p53/p21 dependent aging like growth arrest (59). At an achievable concentration in the human colon and rectum, uro-A can enhance the sensitivity of 5-fluorouracil to the anticancer effect of human colon cancer cells, block the cells at G2/M and cause the activation of caspases 8 and 9 (60).

IBD is a chronic disease that causing inflammation in the small or large intestines, and is thought to increase the risk of CRC. Uro-A has been reported to prevent the intestinal inflammation by attenuating the inflammatory signaling and upregulating of the tumor suppressor genes (61), to increase the permeability of tight junctions (62), and to prevent the detrimental effect of inflammation on the cells' viability (63). Those finding have given evidence of urolithins, especially uro-A, in the protection of intestinal diseases such as CRC and IBD.

5. The safety of urolithins

Urolithins are the metabolites of tannic polyphenols in vivo, which exist in blood, urine and feces in a free form or phase II conjugation, and have extensive biological activities in vivo and in vitro. Therefore, the experiments based on direct oral administration can verify their safety. The genetic and toxicological toxicity of oral uro-A in rats were studied. The results suggested that high-dose oral synthetic uro-A did not show any toxicity to the target organs at the histopathological level, indicating the clinical safety of uro-A (64). Andreux et al. recruited 60 elderly people and randomly divided them into four groups: placebo group, uro-A 250 mg, 500 mg and 1,000 mg daily for 28 days. The effects of uro-A on the body were evaluated by the levels of health biomarkers of cells and mitochondria in blood and muscle tissue. The results indicated that uro-A can help slow down the aging process by improving the function of cell mitochondria.

6. Conclusions

Intestinal microbiota regulates the material and energy metabolism of the host, "You are what you eat" (66). Different eating habits have a great impact on the types of intestinal microorganisms in human. At the same time, the types of intestinal microorganisms also determine the metabo-types. At present, the production process of urolithins in vivo, the mechanism of intestinal diseases and the interaction with intestinal flora are still in the exploratory stage. It is of great significance to analyze the pharmacological effect and mechanism of urolithins in vivo through metabolomics, culturomics and microbiomics, to explore the development of relevant microbial preparations and drugs, and promote its application in the prevention and treatment of intestinal diseases.

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References

1. Yang W, Yu T, Huang X, et al. Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. Nat Commun. 2020;
2. Yao Y, Cai X, Fei W, Ye Y, Zhao M, Zheng C. The role of short-chain fatty acids in inflammation, immunity, and metabolism. Crit Rev Food Sci Nutr. 2022; 62:1-12.

3. Postler TS, Ghosh S. Understanding the holobiont: how microbial metabolites affect human health and shape the immune system. Cell Metab. 2017; 26:110-130.

4. Guevara-Cruz M, Godinez-Salas ET, Sanchez-Tapia M, et al. Genistein stimulates insulin sensitivity through gut microbiota reshaping and skeletal muscle AMPK activation in obese subjects. BMJ Open Diabetes Res Care. 2020; 8:e000948.

5. Amrein M, Li XS, Walter J, et al. Gut microbiota-dependent metabolite trimethylamine-N-oxide (TMAO) and cardiovascular risk in patients with suspected functionally relevant coronary artery disease (iCAD). Clin Res Cardiol. 2022; 111:692-704.

6. Barrea L, Annunziata G, Muscogiuri G, Di Somma C, Laadisio D, Maisto M, de Alteriis G, Tenore GC, Colao A, Savastano S. Trimethylamine-N-oxide (TMAO) as novel potential biomarker of early predictors of metabolic syndrome. Nutrients. 2018; 10:1971.

7. Dalmasso G, Couognoux A, Delmas J, Darfeuille-Michaud A, Bonnet R. The bacterial genotoxin colibactin promotes colon tumor growth by modifying the tumor microenvironment. Gut Microbes. 2014; 5:675-680.

8. Kallio T, Kallio J, Jaakkola M, Maki M, Kilpelainen P, Virtanen V. Urolithins display both antioxidant and pro-oxidant activities depending on assay system and conditions. J Agric Food Chem. 2013; 61:10720-10729.

9. Piwowarski JP, Granica S, Kiss AK. Influence of gut microbiota-derived ellagitannins' metabolites urolithins on pro-inflammatory activities of human neutrophils. Planta Med. 2014; 80:887-895.

10. Xia B, Shi XC, Xie BC, Zhu MQ, Chen Y, Chu XY, Cai GH, Liu M, Yang SZ, Mitchell GA, Pang WJ, Wu JW. Urolithin A exerts antiobesity effects through enhancing adipose tissue thermogenesis in mice. PLoS Biol. 2020; 18:e3000688.

11. Zhao W, Wang L, Haller V, Riitsch A. A novel candidate for prevention and treatment of atherosclerosis: Urolithin B decreases lipid plaque deposition in apoE(-/-) mice and increases early stages of reverse cholesterol transport in ox-LDL treated macrophages cells. Mol Nutr Food Res. 2019; 63:e1800887.

12. Kujawska M, Jourdes M, Witucki L, Karazniewicz-Lada M, Szulc M, Gorska A, Mikolajczak PL, Teissedre PL, Jodynis-Liebert J. Pomegranate juice ameliorates urolithin phenotypes in intervention trials, independent of human gut microbiota: consistent observation of three urolithin metabotypes revisited: the human gut microbiota urolithin metabotypes revisited: the evidence so far. Evid Based Complement Alternat Med. 2013; 2013:270418.

13. Tomas-Barberan FA, Garcia-Villalba R, Selma MV, Espin JC, Tomas-Barberan FA. Time course production of urolithins from ellagic acid by human gut microbiota. J Agric Food Chem. 2013; 61:8797-8806.

14. Maim Rekdal V, Nol Bemadino P, Luessencher MU, Kiarne S, Le C, Bisanz JE, Turnbaugh PJ, Bess EN, Balskus EP. A widely distributed metalloenzyme class enables gut microbial metabolism of host- and diet-derived catechols. eLife. 2020; 9:e50845.

15. Dalmasso G, Couognoux A, Delmas J, Darfeuille-Michaud A, Bonnet R. The bacterial genotoxin colibactin promotes colon tumor growth by modifying the tumor microenvironment. Gut Microbes. 2014; 5:675-680.

16. Nawwar MAM, Hussein SAM, Merfort I. NMR spectral analysis of polyphenols from Punica-Granatum. Phytochemistry. 1994; 36:793-798.

17. Huang X, Xu M, Shirahata T, Li W, Koike K, Kojima-Yusa A, Yusa I, Kobayashi Y. Anti-steinos compounds from leaves of Mallotus furetianus. Nat Prod Res. 2018; 32:1459-1462.

18. Totiger TM, Srinivasan S, Jala VR, et al. Urolithin A, a novel natural compound to target PI3K/AKT/mTOR pathway in pancreatic cancer. Mol Cancer Ther. 2019; 18:301-311.

19. Garcia-Villalba R, Beltan D, Espin JC, Selma MV, Tomas-Barberan FA. Time course production of urolithins from ellagic acid by human gut microbiota. J Agric Food Chem. 2013; 61:8797-8806.

20. Maim Rekdal V, Nol Bemadino P, Luessencher MU, Kiarne S, Le C, Bisanz JE, Turnbaugh PJ, Bess EN, Balskus EP. A widely distributed metalloenzyme class enables gut microbial metabolism of host- and diet-derived catechols. eLife. 2020; 9:e50845.

21. Leather L. Chemistry and biochemistry of some mammalian secretions and excretions. J Chem Soc; 1949; 2115-2125.

22. González-Sarrías A, Gimenez-Bastida JA, Nunez-Sanchez MA, Larrosa M, Garcia-Conesa MT, Tomas-Barberan FA, Espin JC. Phase-II metabolism limits the antiproliferative activity of urolithins in human colon cancer cells. Eur J Nutr. 2014; 53:853-864.

23. Kujawska M, Jourdes M, Witucki L, Karazniewicz-Lada M, Szulc M, Gorska A, Mikolajczak PL, Teissedre PL, Jodynis-Liebert J. Pomegranate juice ameliorates dopamine release and behavioral deficits in a rat model of Parkinson's disease. Brain Sci. 2021; 11:1127.

24. Tomas-Barberan FA, Selma MV, Espin JC. Interactions of gut microbiota with dietary polyphenols and consequences to human health. Curr Opin Clin Nutr Metab Care. 2016; 19:471-476.

25. Garcia-Villalba R, Selma MV, Espin JC, Tomas-Barberan FA. Identification of novel urolithin metabolites in human feces and urine after the intake of a pomegranate extract. J Agric Food Chem. 2019; 67:11099-11107.

26. Garcia-Villalba R, Espin JC, Tomas-Barberan FA. Chromatographic and spectroscopic characterization of urolithins for their determination in biological samples after the intake of foods containing ellagitannins and ellagic acid. J Chromatogr A. 2016; 1428:162-175.

27. Tomas-Barberan FA, Garcia-Villalba R, Gonzalez-Sarrias A, Selma MV, Espin JC. Ellagic acid metabolism by human gut microbiota: consistent observation of three urolithin phenotypes in intervention trials, independent of food source, age, and health status. J Agric Food Chem. 2014; 62:6535-6538.

28. Espin JC, Larrosa M, Garcia-Conesa MT, Tomas-Barberan FA. Urolithins, a novel natural compound to target PI3K/AKT/mTOR pathway in pancreatic cancer. Mol Cancer Ther. 2019; 18:301-311.

29. Cortes-Martín A, Garcia-Villalba R, Gonzalez-Sarrias A,romo-Vaquero M, Loria-Kohen V, Ramirez-de-Molina A, Tomas-Barberan FA, Selma MV, Espin JC. The gut microbial urolithin metabotypes revisited: the evidence so far. Evid Based Complement Alternat Med. 2013; 2013:270418.

30. Romeo-Vaquero M, Cortes-Martín A, Loria-Kohen V, Ramirez-de-Molina A, Garcia-Mantrana I, Collado
MC, Espin JC, Selma MV. Deciphering the human gut microbiome of urolithin metabotypes: association with enterotypes and potential cardiometabolic health implications. Mol Nutr Food Res. 2019; 63:e1800958.

31. Cerda B, Tomas-Barberan FA, Espin JC. Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: identification of biomarkers and individual variability. J Agric Food Chem. 2005; 53:227-235.

32. Mertens-Talcott SU, Jilma-Stohlwpez P, Rios J, Hingorani L, Derendorf H. Absorption, metabolism, and antioxidant effects of pomegranate (Punica granatum L.) polyphenols after ingestion of a standardized extract in healthy human volunteers. J Agric Food Chem. 2006; 54:8956-8961.

33. Cerda B, Periago P, Espin JC, Tomas-Barberan FA. Identification of urolithin A as a metabolite produced by human colon microflora from ellagic acid and related compounds. J Agric Food Chem. 2005; 53:5571-5576.

34. Selma MV, Beltran D, Luna MC, Romo-Vaquero M, Garcia-Villalba R, Mira A, Espin JC, Tomas-Barberan FA. Isolation of human intestinal bacteria capable of producing the bioactive metabolite isourolithin A from ellagic acid. Front Microbiol. 2017; 8:1521.

35. Martinez-Blanch JF, Ramon D, Beltran D, Romo-Vaquero M, Garcia-Villalba R, Espin JC, Tomas-Barberan FA, Codoner FM, Selma MV. Complete genome sequence of the new urolithin-producing bacterium Gordoniabacter urolithinfaciens DSM 27213(T). Genome Announce. 2017; 5:e01120-01117.

36. Selma MV, Tomas-Barberan FA, Beltran D, Garcia-Villalba R, Espin JC. Gordoniabacter urolithinfaciens sp. nov., a urolithin-producing bacterium isolated from the human gut. Int J Syst Evol Microbiol. 2014; 64:2346-2352.

37. Garcia-Villalba R, Beltran D, Frutos MD, Selma MV, Espin JC, Tomas-Barberan FA. Metabolism of different dietary phenolic compounds by the urolithin-producing human gut bacteria Gordoniabacter urolithinfaciens and Ellagibacter isourolithinfaciens. Food Funct. 2020; 11:7012-7022.

38. Gaya P, Peiroten A, Medina M, Alvarez I, Landete JM. Bifidobacterium pseudocatenulatum INIA P815: The first bacterium able to produce urolithins A and B from ellagic acid. J Funct Foods. 2018; 45:95-99.

39. Amrane S, Raoult D, Lagier JC. Metagenomics, culturomics, and the human gut microbiota. Expert Rev Anti Infect Ther. 2018; 16:373-375.

40. Garcia-Villalba R, Gimenez-Bastida JA, Cortes-Martín A, Avila-Galvez MA, Tomas-Barberan FA, Selma MV, Espin JC, Gonzalez-Sarrias A. Urolithins: a comprehensive update on their metabolism, bioactivity, and associated gut microflora. Mol Nutr Food Res. 2022; e2101019.

41. Toney AM, Fox D, Chaidze Y, Ramer-Tait AE, Chung S. Immunoeducational role of urolithin A on metabolic diseases. Biomedicines. 2021; 9:192.

42. Zhang M, Cui S, Mao B, Zhang Q, Zhao J, Zhang H, Tang X, Chen W. Ellagic acid and intestinal microflora metabolite urolithin A: A review on its sources, metabolic distribution, health benefits, and biotransformation. Crit Rev Food Sci Nutr. 2022.

43. Chen L, Wang D, Garmaeva S, Kurilshikov A, Vich Vila A, Gacesa R, Sinha T, Lifelines Cohort S, Segal E, Weersma RK, Wijmenega C, Zhernakova A, Fu J. The long-term genetic stability and individual specificity of the human gut microbiome. Cell. 2021; 184:2302-2315 e2312.

44. Gimenez-Bastida JA, Truchado P, Larrosa M, Espin JC, Tomas-Barberan FA, Allende A, Garcia-Conesa MT. Urolithins, ellagitannin metabolites produced by colon microbiota, inhibit Quorum sensing in Yersinia enterocolitica: Phenotypic response and associated molecular changes. Food Chem. 2012; 132:1465-1474.

45. Lu C, Shen Y, Li T. Application of 3,4,8,9,10-pentahydroxyxibenzo [b, d] pyran-6-one in the preparation of antibiotics. In: China Intellectual Property Office, 2019; pp.1.

46. Diop EHA, Queiroz EF, Marcourt L, Kicka S, Rudaz S, Diop T, Soldati T, Wolfender JL. Antimycobacterial activity in a single-cell infection assay of ellagitannins from Combreutum aculeatum and their bioavailable metabolites. J Ethnopharmacol. 2019; 238:111832.

47. Cheng G, Hao H, Xie S, Wang X, Dai M, Huang L, Yuan Z. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front in Microbiol. 2014; 5:217.

48. Larrosa M, Gonzalez-Sarrias A, Yanee-Gascon MJ, Selma MV, Azorin-Ortuno M, Totsi S, Tomas-Barberan F, Dolara P, Espin JC. Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. J Nutr Biochem. 2010; 21:717-725.

49. Garcia-Villalba R, Vissenaeakens H, Pitarti J, Romo-Vaquero M, Espin JC, Grootaert C, Selma MV, Raes K, Smagge G, Possemiers S, Van Camp J, Tomas-Barberan FA. Gastrointestinal simulation model TWIN-SHIME shows differences between human urolithin-metabotypes in gut microbiota composition, pomegranate polyphenol metabolism, and transport along the intestinal tract. J Agr Food Chem. 2017; 65:5480-5493.

50. Abdulrahman AO, Alzuabaidy MI, Nadeem MS, Khan JA, Rather IA, Khan MI. Effects of urolithins on obesity-associated gut dysbiosis in rats fed on a high-fat diet. Int J Food Sci Nutr. 2021; 72:923-934.

51. Zhao R, Long X, Yang J, Du L, Zhang X, Li J, Hou C. Pomegranate peel polyphenols reduce chronic low-grade inflammatory responses by modulating gut microbiota and decreasing colonic tissue damage in rats fed a high-fat diet. Food Funct. 2019; 10:8273-8285.

52. Li Z, Henning SM, Lee RP, Lu QY, Summanen PH, Thames G, Corbett K, Downes J, Tseng CH, Finegold SM, Heber D. Pomegranate extract induces ellagitannin metabolite formation and changes stool microbiota in healthy volunteers. Food Funct. 2015; 6:2487-2495.

53. Xian YB, Fan R, Shao J, Toney AM, Chung S, Ramer-Tait AE. Polyphenolic fractions isolated from red raspberry whole fruit, pulp, and seed differentially alter the gut microbiota of mice with diet-induced obesity. J Funct Foods. 2021; 76:104288.

54. Hering NA, Luettel J, Jebautzk B, Schulzke JD, Rosenthal R. The punicagin metabolites ellagic acid and urolithin A exert different strengthening and anti-inflammatory effects on tight junction-mediated intestinal barrier function in vitro. Front Pharmacol. 2021; 12:610164.

55. Singh R, Chandrashekarappa S, Bodduluri SR, et al. Enhancement of the gut barrier integrity by a microbial metabolite through the Nrf2 pathway. Nat Commun. 2019; 10:89.

56. Kawabata K, Yoshioka Y, Terao J. Role of intestinal bacteria in the bioavailability and physiological functions of dietary polyphenols. Molecules. 2019; 24:370.
57. Zhao W, Shi F, Guo Z, Zhao J, Song X, Yang H. Metabolite of ellagitannins, urolithin A induces autophagy and inhibits metastasis in human SW620 colorectal cancer cells. Mol Carcinog. 2018; 57:193-200.

58. Kojadinovic M, Arsic A, Petovic-Oggiano G, Gavrovic-Jankulovic M, Gibetic M, Popovic M. Effect of urolithins on oxidative stress of colorectal adenocarcinoma cells-Caco-2. Int J Food Sci Nutr. 2017; 68:952-959.

59. Norden E, Heiss EH. Urolithin A gains in antiproliferative capacity by reducing the glycolytic potential via the p53/TIGAR axis in colon cancer cells. Carcinogenesis. 2019; 40:93-101.

60. Gonzalez-Sarrias A, Tome-Carneiro J, Bellesia A, Tomas-Barberan FA, Espin JC. The ellagic acid-derived gut microbiota metabolite, urolithin A, potentiates the anticancer effects of 5-fluorouracil chemotherapy on human colon cancer cells. Food Funct. 2015; 6:1460-1469.

61. Larrosa M, Gonzalez-Sarrias A, Yanez-Gascon MJ, Selma MV, Azorin-Ortuno M, Toti S, Tomas-Barberan F, Dolara P, Espin JC. Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. J Nutr Biochem. 2010; 21:717-725.

62. Singh R, Chandrashekharappa S, Boddukutty SR, et al. Enhancement of the gut barrier integrity by a microbial metabolite through the Nrf2 pathway. Nat Commu. 2019; 10:89.

63. Gimenez-Bastida JA, Larrosa M, Gonzalez-Sarrias A, Tomas-Barberan F, Espin JC, Garcia-Conesa MT. Intestinal ellagitannin metabolites ameliorate cytokine-induced inflammation and associated molecular markers in human colon fibroblasts. J Agric Food Chem. 2012; 60:8866-8876.

64. Heilman J, Andreux P, Tran N, Rinsch C, Blanco-Bose W. Safety assessment of urolithin A, a metabolite produced by the human gut microbiota upon dietary intake of plant derived ellagitannins and ellagic acid. Food Chem Toxicol. 2017; 108:289-297.

65. Andreux PA, Blanco-Bose W, Ryu D, Burdet F, Ibberson M, Aebischer P, Auwerx J, Singh A, Rinsch C. The mitophagy activator urolithin A is safe and induces a molecular signature of improved mitochondrial and cellular health in humans. Nat Metab. 2019; 1:595-603.

66. Moszak M, Szulinska M, Bogdanski P. You Are what you eat-the relationship between diet, microbiota, and metabolic disorders—a review. Nutrients. 2020; 12:1096.

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