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Invited Mini Review

Gut microbiota-generated metabolites: missing puzzles to hosts' health, diseases, and aging

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The gut microbiota, an intricate community of bacteria residing in the gastrointestinal system, assumes a pivotal role in various physiological processes. Beyond its function in food breakdown and nutrient absorption, gut microbiota exerts a profound influence on immune and metabolic modulation by producing diverse gut microbiota-generated metabolites (GMGMs). These small molecules hold potential to impact host health via multiple pathways, which exhibit remarkable diversity, and have gained increasing attention in recent studies. Here, we elucidate the intricate implications and significant impacts of four specific metabolites, Urolithin A (UA), equol, Trimethylamine N-oxide (TMAO), and imidazole propionate, in shaping human health. Meanwhile, we also look into the advanced research on GMGMs, which demonstrate promising curative effects and hold great potential for further clinical therapies. Notably, the emergence of positive outcomes from clinical trials involving GMGMs, typified by UA, emphasizes their promising prospects in the pursuit of improved health and longevity. Collectively, the multifaceted impacts of GMGMs present intriguing avenues for future research and therapeutic interventions. [BMB Reports 2024; 57(5): 207-215]

INTRODUCTION

The gut microbiota represents a complex community of bacteria inhabiting the human gastrointestinal tract that exert a pivotal influence over a multitude of physiological processes, such as digestion, immune regulation, and metabolic homeostasis (1). The gut microbiota transcends its role as a mere consortium of microorganisms within the body, as it profoundly influences the subject's health by producing various kinds of metabolites, referred to as GMGMs (2). The gut microbiota participates in nutrient metabolism and energy provision by absorbing glucose, amino acids, and lipids. During the utilization of these substrates, various metabolites are produced, such as phenolic compounds from protein and short-chain fatty acids from carbohydrate. These metabolites further exert positive or negative effects on human health (3, 4). GMGMs typically consist of small molecules that can impact the host through multiple pathways. Some of these metabolites can influence the host's metabolic processes, assisting in the maintenance of weight balance and blood glucose stability (5). Conversely, others may impact the immune system function, regulating inflammatory processes within the host (6). Research into the interplay between metabolites and their implications for health, disease, and the aging process is continually advancing. Remarkably, specific metabolites have been discovered to exhibit profound connections with overall health issues (7). For example, certain beneficial bacteria create antioxidants and anti-inflammatory chemicals that lower the risk of chronic diseases (8, 9). Other specific metabolites wield influence over brain health by altering cognitive performance and emotional regulation (10, 11). On the other hand, detrimental disturbances to the gut microbiota have been associated with the pathogenesis of different disorders. Recent investigations have elucidated links between gut microbiota dysbiosis and ailments, such as gastrointestinal inflammation, inflammatory bowel disease, and obesity (12-15). These explorations contribute significantly to our comprehension of the pathogenic mechanisms underlying specific disorders, providing valuable advances in corresponding therapeutic interventions. In this review, we provide an overview of recent investigations on GMGMs and their intricate interconnections with human health and disease. Furthermore,

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we have deliberated on the advanced techniques and clinical trials involving GMGMs, which may provide prospective insights for future research and therapeutic strategies.

GMGMs

GMGMs are highly diverse, and research in this domain remains in a perpetual state of evolution. In this section, we elucidate four metabolites currently under intensive investigation. Two of those, UA and equol, have been substantiated for their beneficial effects upon the human body, whereas the other two, TMAO and imidazole propionate, are generally recognized to have detrimental impacts (Fig. 1).

UA, A FIRST-IN-CLASS MITOPHAGY ENHANCER

UA is a natural compound originally derived from the microbial metabolism of diets rich in ellagitannins, with its primary component, ellagic acid, undergoing a series of metabolic transformations into UA (Fig. 2A) (16, 17). Extensive research efforts spanning several decades have been dedicated to UA, with its potential health benefits attracting substantial scientific attention and continued exploration as research advances. Notably, UA has demonstrated the capacity to ameliorate diverse health conditions, with a particular emphasis on its role in promoting mitophagy, which has emerged as a focal point in the realm of disease recovery (18, 19). Mechanically, by stabilizing PTEN-induced kinase 1 (PINK1), and recruiting PARKIN, also known as PRKN/PARK2, UA triggers the recognition and labeling of damaged mitochondria. PINK1 accumulates on damaged mitochondria and activates PARKIN, leading to the ubiquitination of mitochondrial proteins and the recruitment of LC3. In a coordinated manner, UA also independently activates BNIP3/LC3 pathway, facilitating phagophore membrane formation and ultimate elimination via the cellular process of mitophagy, thereby preserving the health and functionality of mito-

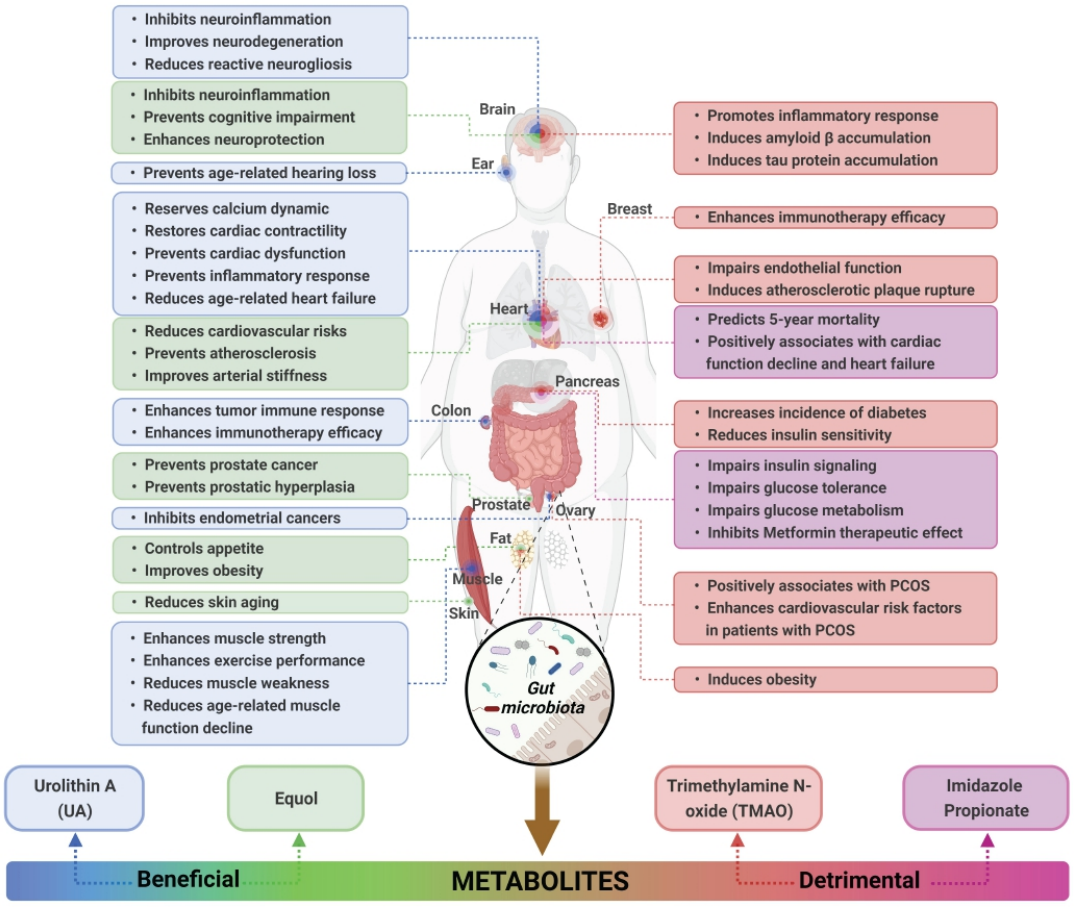

Fig. 1. The impacts of gut microbiota-derived metabolites (GMGM) in diverse tissues. GMGM, of both beneficial (urolithin A [UA] and equol) and detrimental (Trimethylamine N-oxide and imidazole propionate) influence, exhibit a diverse range of effects on various tissues, playing crucial roles in the regulation of human health. PCOS, polycystic ovary syndrome.

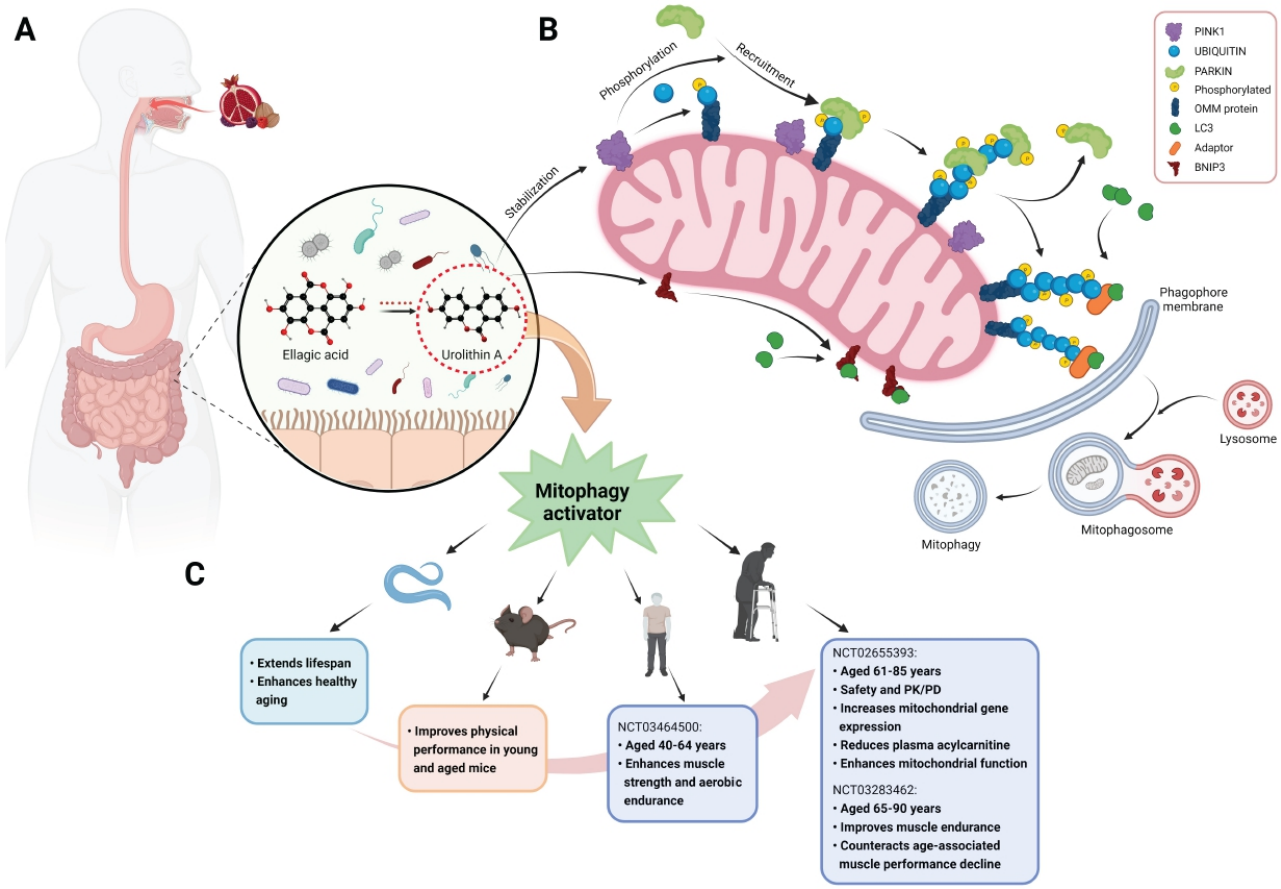

Fig. 2. The mitophagy activator UA demonstrates evident therapeutic benefits and efficacy in experimental models and humans. (A) After the consumption of diets rich in ellagitannins (such as pomegranate, berries, and nuts), the primary component, ellagic acid, is metabolized and conversed to UA by the gut microbiota. (B) UA activates mitophagy via PINK1/PARKIN and BNIP3/LC3 pathways, respectively. UA stabilizes PINK1 kinase, then phosphorylating Ubiquitin while recruiting PARKIN. PARKIN subsequently ubiquitinates mitochondrial proteins, which facilitates ubiquitin chains formulation and interaction with mitophagy adapter proteins (such as p62) to bind with LC3. Additionally, UA activates the BNIP3 protein, resulting in the recruitment of LC3 and the formulation of phagophore membrane. Ultimately, via the concerted fusion of lysosomes, dysfunctional mitochondria form mitophagosomes, leading to their eventual degradation. PINK1, PTEN-induced kinase 1: OMM, outer mitochondrial membrane. (C) UA has demonstrated multiple therapeutic benefits in experimental models (*C. elegans* and rodents) and clinical trials (middle-aged adults and elders), indicating its promising potential for future research and therapeutic interventions. PK/PD, pharmacokinetic/pharmacodynamic.

chondria (Fig. 2B) (17).

As a first-in-class mitophagy enhancer, UA improves mitochondrial quality control by assisting in the clearance of damaged or aging mitochondria, contributing to the mitigation of various pathological processes in a wide range of species, spanning from *C. elegans* and rodents to humans (Fig. 2C). UA has been proven to extend lifespan and enhance healthy aging in *C. elegans*, with notable improvements of respiratory capacity, mobility, and pharyngeal pumping (16). In view of these findings, researchers have extended investigations into mammals to clarify the therapeutic effects of UA on muscle-related aspects. Muscle health is crucial for preserving mobility, particularly with aging. During the aging process, a decline in mitophagy in muscles can be considered a contributing factor to muscle weakness (20). UA has been demonstrated to effectively improve physical performance, characterized by muscle strength and exercise endurance in young and aged mice (16). Notably, UA supplementation can improve mitochondrial metabolic efficiency, lower levels of inflammation-related biomarkers in the blood, and significantly improve muscle endurance in human. This is critical for preventing age-related muscular weakening and functional decline, highlighting the profound prospects and applications of UA in improving human health (21-23). In addition to its remarkable muscular benefits, recent studies also identified UA as a highly promising therapeutic candidate for the maintenance of cardiovascular health,

as it effectively reduces age-related heart failure, as well as reserving cardiac function (24). Mitochondrial dysfunction in aging hearts is a contributing factor to the decline of heart function, which is closely associated with the downregulation of mitophagy and reduced clearance rates in aging hearts (25). UA administration significantly counteracted mitochondrial and cardiac dysfunction, as evidenced by the improvements in cardiac contractility and calcium dynamics (26, 27). In addition, UA has also demonstrated curative effects in tumor immunotherapy. T-Stem cell memory (TSCM) cells play a key role in preventing T cell exhaustion and promoting anti-tumor T-cell responses (28). UA activates tumor immune response by inducing the formation of TSCM cells, and its involvement in this process is intricately linked to mitophagy, which enhances T-cell function and the effectiveness of immunotherapy (29). Likewise, UA exhibits considerable benefits in addressing neurological disorders, primarily owing to its remarkable antioxidant properties and its potential to mitigate neuroinflammation, thus exerting neuroprotective effects (30, 31). Specifically, UA provided neuroprotection in Parkinson's disease (PD) by triggering mitophagy in microglial cells, thereby restoring mitochondrial function and reducing the activation of NLRP3 inflammasomes, which, in turn, alleviates the inflammatory response and neurological symptoms (32). In this regard, UA has emerged as a valuable strategy for ameliorating neurodegeneration and inflammation driven by microglial cells. Consistently, UA is implicated in regulating reactive neurogliosis and inflammation signaling pathways, and proving to be protective against the pathology of Alzheimer's disease (AD) (33). Additional study has shown the mitophagy induction of UA in auditory cells as well, thereby preventing age-related hearing loss (34). Beyond its induction in mitophagy, UA has demonstrated therapeutic efficacy in preventing oocyte aging (35), as well as inhibiting endometrial cancer proliferation via the regulation of estrogen receptor-α dependent gene expression (36). Collectively, decades of research have unveiled that UA holds significant potential in the treatment of multiple diseases, especially age-related disorders. Meanwhile, the investigation of UA at the molecular levels has remained comparatively limited, underscoring the necessity for more comprehensive and in-depth studies in this area.

EQUOL, A GUT MICROBIOTA-GENERATED ISOFLAVONOID

Equol is produced by specific bacterial biotypes in the human and animal guts through the metabolism of soy isoflavones. It exists in two diastereoisomeric forms, S-equol and R-equol, with S-equol having received more attention (37). Equol is thought to provide potential health benefits, but not everyone can generate equol after diet consumption. Only 20-30% of individuals in Western populations produce S-equol, whereas among Asians, the percentage rises to approximately 50-70% (38). Due to its structural similarity to estrogen, a significant portion of research focuses on its binding to estrogen receptors, and its effects in cells and tissues. This resemblance has raised concerns regarding its potential association with breast cancer risk (39). It is reported that appropriate equol supplementation may serve as an effective alternative to hormone therapy in controlling appetite, obesity and metabolic syndrome, as well as alleviating symptoms of perimenopause (40). Furthermore, in the context of male prostate-related diseases, equol may contribute to the prevention of prostate cancer and benign prostatic hyperplasia (41).

The protective effects of equol on heart and brain are gradually gaining recognition. Recent study has indicated its neuroprotective properties in PD (42). S-equol has shown potential benefits in treating atherosclerosis and may have the potential to prevent dementia and coronary heart disease (43, 44). Meanwhile, S-equol supplementation has been shown to ameliorate emotion dysregulation in females with premenstrual symptoms (45), which might be contributed by its estrogenic actions in the brain (46). In line with this, its antidepression effect has also been observed in obese individuals, implying its curative promise in 'metabolic-mood syndrome' (47). In addition, S-equol displays protective potential in addressing cognitive decline and dementia, suggesting that S-equol may effectively penetrate the blood-brain barrier, allowing its excellent antioxidant and anti-inflammatory effects to be exerted within the brain (48). Meanwhile, its antioxidant properties are also clarified in relation to skin, with the potential of reducing skin aging (39). Evidence of equol's effects on female health presents a nuanced panorama. A cross-sectional investigation reveals a positive correlation between equol levels and the risk of female uterine leiomyomata (49), in accord with observations implicating equol in the induction of uterine tissue hyperplasia (50). Equol has also exhibited the capacity to impede growth and induce atresia in cultured murine ovarian follicles (51). Nevertheless, an interventional study involving isoflavone supplementation among female polycystic ovary syndrome (PCOS) patients yielded intriguing outcomes, with enhancements noted in microbial alpha diversity and glucose homeostasis. Additionally, heightened equol production exhibited an association with favorable fertility markers (52). As such, it is worth noting that as research continues to validate the curative effects of equol, its specificity ought to be taken into consideration. There are significant individual differences, and when considering its effects, the influence of genetics and diet, as well as other factors, should not be overlooked.

TMAO, AN AMINE OXIDE AND OSMOLYTE

TMAO is an organic osmolyte methylamine compound synthesized in human and various animals through a series of metabolic processes. After consuming a diet rich in choline compounds, the gut microbiota catalyzes these substances into trimethylamine (TMA) through a series of biochemical reactions. TMA is subsequently absorbed into the bloodstream via

the intestinal mucosa and undergoes conversion into TMAO in the liver (53, 54). TMAO can protect proteins inside deep-sea organisms from being damaged by high pressure, enabling them to adapt to the high osmotic pressure environment (55). Correspondingly, accumulating investigations have progressively revealed the adverse implications of TMAO in specific aging disorders. Elevated TMAO levels promote the formation of foam cells, impair normal endothelial function, and render atherosclerotic plaques more prone to rupture (56). In AD, TMAO leads to the accumulation of amyloid-β peptides and tau proteins, activates astrocytes, and promotes inflammatory responses (57, 58). Additionally, abnormal TMAO levels are further associated with insulin resistance, and contribute to metabolic dysfunction, which holds substantial significance in the context of both diabetes and obesity (59, 60). Increased levels of circulating TMAO in plasma have also been linked to the etiology of PCOS without hyperandrogenism, exhibiting a concomitant correlation with heightened systemic inflammation, while elevated TMAO could further lead to an exacerbation of cardiovascular risk factors in patients with PCOS (61, 62). As such, TMAO is generally regarded as a detrimental metabolite, while recent studies have revealed intriguing contradictions whereby patients with higher plasma TMAO levels achieve enhanced immunotherapy efficacy by evoking endoplasmic reticulum and enhancing antitumor immunity (63). This paradox underscores the intricate crosstalk between metabolite and immunity, while also highlighting the gaps in our current comprehension of TMAO and possibly other metabolites as well, indicating the necessity for further mechanistic studies and research endeavors.

IMIDAZOLE PROPIONATE, A HISTIDINE METABOLITE

Imidazole propionate is a metabolite that was initially found to be elevated in individuals with diabetes. It was subsequently identified as a microbially produced histidine-derived metabolite, and its inhibitory role in insulin signaling has been revealed (64). Accumulating evidence has gradually unmasked the link between imidazole propionate levels and diet, gut microbiota composition, and obesity. The levels of imidazole propionate are typically elevated in obese individuals, and lower in those with normal weight (65, 66). Due to its impact on glucose metabolism and insulin sensitivity, the influence of imidazole propionate in diabetes has been corroborated in several studies. It has the potential to influence the efficacy of diabetes medications, such as metformin, as well as being correlated with diabetic complications (67-69). Recent research has also confirmed an association between the levels of imidazole propionate and heart failure, with imidazole propionate serving as a predictive marker for overall survival following heart failure (70-72). Despite these insights, the specific mechanisms of imidazole propionate in diseases and its associations with different health conditions have not yet been fully understood.

ADVANCED RESEARCH ON GMGMs

In clinical treatments, the discovery of new candidates and demonstrating their efficacy are of paramount importance. However, in addition to the inherent effectiveness of the drug, the efficient delivery of candidates and the precise control of their release to align with the desired outcomes pose significant and formidable challenges. In current research, various technologies for supplementary drugs delivery and release have emerged. For instance, nanoparticles are widely utilized in the development of drug delivery systems. By encapsulating drug carriers within nanoscale particles, it is possible to enhance the solubility, stability, and targeting of drugs (73, 74). Researchers have converted UA into biodegradable nanoparticles for oral administration in the treatment of acute kidney injury in mice, thereby enhancing the oral bioavailability of UA, and ultimately improving the survival rate of mice (75). Additionally, the application of hydrogels has also contributed to this direction, evidenced by the locally delivered NECA, an agonist of adenosine, with hydrogel implant enhancing neovascularization in islet transplantation (76). These existing studies make clear that the advanced biomaterials and delivery technologies possess multifaced benefits, pointing to their extensive potential applications in the realm of GMGMs, with the UA example being a good illustration. With more flexible control over drug targeting, the beneficial or harmful effects of metabolites may be reevaluated. For instance, as previously mentioned, TMAO has consistently emerged as a risk factor in human, while it also promotes antitumor immunity in breast cancer (63). In this case, exploring novel techniques that enhance the safety and targeting of TMAO via advanced biomaterials may represent a promising avenue for breast cancer therapy. Similarly, the application of other metabolites can also be considered to maximize their benefits, while minimizing potential risks. Such approaches may hold potential for optimizing the therapeutic effects of specific metabolites and ensuring their safety.

GMGMs IN CLINICAL STUDIES

The studies of GMGMs are growing more in-depth, and their performance in clinical trials is receiving widespread attention. In a randomized clinical trial conducted with elderly participants, individuals who are supplemented with UA show evident improvements in muscle endurance, as well as mitochondrial health, compared to the placebo group (21). In another clinical trial involving middle-aged adults, researchers found that UA could enhance muscle strength, and have a positive impact on aerobic endurance and physical performance (22). Furthermore, Nestlé Health Science is currently conducting two randomized, double-blind, placebo-controlled Phase II clinical trials aimed at quantifying the effects of UA on muscle health and function, as well as evaluating its impact on other parameters. Apart from UA, a 24-week randomized, double-blind, placebo-controlled clinical trial on Equol is investigating its

effects on 24-hour dynamic blood pressure, vascular function, and other cardiovascular risk factors, including lipid profiles, glucose metabolism control, and inflammatory markers, in 207 postmenopausal women who do not produce Equol (77). However, there appears to be limited reporting on clinical trials targeting TMAO and imidazole propionate. While latest clinical investigations have revealed that high level of TMAO is considered a risk factor for multiple aging-related diseases (78). For instance, a latest case-control clinical study revealed that TMAO, as well as its nutrient precursor choline, independently corelated with the incident risk for CVDs development (79). TMAO concentration was also reported to be closely corelated with age and positively associated with higher systolic blood pressure and arterial stiffness (80). Additionally, elevated TMAO expression was linked to the incidence of diverse metabolic syndrome, such as type 2 diabetes, obesity, and dyslipidemia (81). Notably, several observational and prospective clinical studies have highlighted TMAO as a potentially predictive biomarker indicating the diagnosis of CVDs and metabolic syndromes (82, 83), as well as the prognosis of conditions including acute coronary syndrome, myocardial infarction, chronic kidney disease, and AD (84-86). Moreover, in studies related to HIV infection, imidazole propionate has been found to be associated with carotid atherosclerosis (87). The latest study further links circulating imidazole propionate plasma levels to coronary artery disease in people with HIV (88). GMGMs are garnering growing attention in the realms of health and disease, prompting expectations for a surge in related research and clinical trials in the future. Advances in the field of research could lead to further studies to delve into their biological functions and potential medical applications.

PERSPECTIVES

Due to the increasing severity of global population aging, there is a growing investment in the prevention and treatment of chronic diseases, with a desire for safer and long-term use medications. In this context, research on GMGMs holds significant benefits and huge potential. With technological advances, more metabolites are being identified, and their importance in various diseases is becoming increasingly evident, including gastrointestinal disorders, CVDs, obesity, diabetes, and cancer (89-92). GMGMs exert their effects through multiple mechanisms, including the regulation of cellular metabolism, modulation of the immune system, and influence on mitochondrial function, among others. While more research is needed to fully understand their specific mechanisms in the human body and the molecular pathways involved, it is worth recognizing that their complex and diverse effects encompass multi-level and multi-stage regulation in both healthy and diseased states. Moreover, while conducting the related research, it is essential to consider the following issues: First, their involvement in biological activities is diverse, and the relationships among them, as well as with the gut microbiota that mediates their production, are highly intricate. Current research usually focuses on the role of individual metabolites in specific diseases. However, exploring their interactions, and whether there are competitive or synergistic effects, should be another research focus. Furthermore, substantial individual variations exist in the levels of metabolites within the human body, and in the ways in which these metabolites impact the body. These differences arise not only from diet and geographical factors, but also need to consider genetic factors, as previously noted with Equol. Even when people consume the same foods, they can produce completely different metabolites. When considering the development of these metabolites as pharmaceuticals or dietary intervention strategies for humans, this factor needs to be considered, or else it may be challenging to guarantee the effectiveness. In summary, our understanding of GMGM has been evolving for several decades, but many of the mechanisms involved require further in-depth research. Additionally, there may well be new metabolites identified in the future, and gaining a deeper comprehension of their biological properties can provide new clues for disease prevention and therapeutic strategies.

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AUTHOR CONTRIBUTIONS

H.J.L and D.R. conceived the study. Y.Z. and S.W. contributed to the primary writing and organization of the manuscript. Y.Z., S.W., and H.Z. conducted the literature review. Y.J., J.S.K., K.T.H., J.J., H.J.L., and D.R. provided overall supervision and revision on the final manuscript. All authors have read and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors have no conflicting interests.

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