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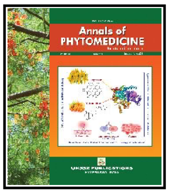

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Exploring modulatory mechanisms and therapeutic potential of dietary fatty acids on gut microbiome

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Abstract

Gut microbiota plays a crucial role in human health, influencing immune function, digestion, and metabolism. While much research has focused on the role of prebiotics and probiotics, recent attention has shifted to dietary fats, which also significantly impact gut microbial composition. High-fat diets, especially those prevalent in Western diets, can reduce gut microbial diversity, increase intestinal permeability, and contribute to systemic inflammation. The fats consumed, categorized into saturated, trans, monounsaturated, and polyunsaturated fats, differentially affect the gut microbiota. Polyunsaturated fats, like omega-3 and omega-6, show anti-inflammatory properties, while high saturated fat intake is linked to negative health outcomes such as obesity and cardiovascular disease. Though, animal studies demonstrate a clear link between fat intake and gut microbiota, research in humans, particularly young and healthy populations, remains limited. The nutrition transition in countries like India, driven by urbanization and globalization, has resulted in shifts towards high-fat diets, raising concerns about increasing obesity and non-communicable diseases. This review explores the existing evidence on the impact of dietary fats on gut microbiota as well explores the role of bioactive compounds present in omega-3-rich sources (oilseeds) in promoting gut health and highlights the need for further research, especially within human populations undergoing rapid nutritional changes.

1. Introduction

The relationship between the gut microbiota and dietary factors such as prebiotics and probiotics has been extensively studied. However, the amount and composition of total fat also give a significant contribution to shaping gut microbiota. Lipids are being paid special attention by researchers because of their ability to modulate several biological processes. Indeed, cell membranes are composed of phospholipids organized in bilayers, which act as precursors of lipid messengers such as the eicosanoids. Not coincidentally, an inadequate intake of polyunsaturated fatty acids is thought to be one of the most important shortcomings of inadequate diets and is probably more relevant than the reduction of a high dietary intake of saturated fatty acids (Poli *et al.,* 2023). While dietary fatty acids (FAs) have been found to demonstrate antimicrobial properties, their impact on the link between obesity and gut microbiota has been largely neglected (Bourdeau-Julien *et al.,* 2023). Instead, their antimicrobial activity has been primarily explored as a means to extend the shelf-life of food rather than influencing gut microbiota. The associations between dietary FAs intake and non-communicable diseases have been well studied and documented in the literature. Moreover, high-fat diets have been linked to a decrease in gut microbiota diversity, increased translocation of lipopolysaccharides (LPS), intestinal permeability, systemic inflammation, and disruptions to the immune system (Cândido *et al.,* 2018). The gut microbiota can regulate host nutrient absorption, energy metabolism, and intestinal barrier function, which has been confirmed to be crucially associated with obesity. Microorganisms, or their active components or metabolites, can modulate host metabolism. Among these, *Akkermansia muciniphila*, a resident bacterium in the intestine of humans, has been widely explored for its probiotic effects, primarily on metabolic regulation, immunity modulation, and intestinal barrier protection (Song *et al.,* 2023). *Fusimonas intestini*, a commensal species of the family Lachnospiraceae, is highly colonized in both humans and mice with obesity and hyperglycemia, produces long-chain fatty acids such as elaidate, and consequently facilitates diet-induced obesity. High fat intake altered the expression of microbial genes involved in lipid production, such as the fatty acid metabolism regulator FadR. The primary function of the bacterial transcription factor FadR is to control the metabolism of fatty acids in Escherichia coli. It functions as a positive regulator of the manufacture of unsaturated fatty acids as well as a negative regulator of the breakdown of fatty acids. FadR, frequently in conjunction with the regulatory protein FabR, inhibits the fatty acid breakdown mechanism in *E. coli* while promoting the synthesis of unsaturated fatty acids. Monocolonization with a FadR-over expressing *Escherichia coli* exacerbated the metabolic phenotypes, suggesting that the change in bacterial lipid metabolism is causally involved in disease progression (Takeuchi *et al.,* 2023). Though, the major site for the digestion and absorption of dietary FAs is the small intestine, the influence of fatty acid intake on gut microbiota has been extensively explored in animals. The human studies in this context are scarce.

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Diet has been shown to shape the gut microbiome. So, there is an increase in attention towards understanding how different types of fat in our diet can contribute to obesity by altering the gut microbiome. There are evidence that high-fat diets, like what is typically consumed in Western countries, can significantly affect the genome and metabolic functionalities of the gut. Fats are generally categorized as either "healthy" or "unhealthy", with unhealthy fats including saturated (SFAs) and trans-fatty acids (TrFAs), and healthy fats including monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). As a result, health organizations recommend avoiding unhealthy fats and limiting the intake of saturated fats to less than 6 per cent of daily calories. PUFAs, specifically omega-3 (n3-FAs) and omega-6 fatty acids (n6-FAs), are thought to have anti-inflammatory properties and help protect against cardiovascular disease (Xu *et al.,* 2022). Research has also explored the mechanism of action of high-fat diets that lead to inflammation and disease. For example, some studies have found that a high-fat diet can trigger an inflammatory response in mice by activating certain immune receptors, while others have shown that long-term intake of a high-fat diet can lead to increased inflammation in the brain. Variations in the gut microbiome have been concomitant with high risk of systemic ailments such as cardiovascular disease and certain types of cancer (Kim *et al.,* 2012).

Dietary fatty acids significantly influence gut microbiota composition and diversity, with saturated fatty acids (SFAs) exerting the strongest effects. High SFA intake reduces α-diversity and the abundance of beneficial fiber-degrading bacteria, while monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) show weaker associations. Moreover, SFA and PUFA intake correlate positively with fatty liver index (FLI) in obese individuals, while gut microbiota diversity negatively correlates with liver fat, suggesting a protective role of microbial diversity against steatosis (Schoeler *et al.,* 2023). High-fat diets owing to an increase in consumption of western diets have established its gateway through nutrition transition in Indian traditional system (Wang *et al.,* 2024). The concept of nutrition transition refers to the significant changes in people's eating habits, physical activity, and lifestyle. In developing countries like India, factors such as globalization, urbanization, and economic growth are driving these changes (An *et al.,* 2022). As a result, people's diets are becoming more diverse and may include both local and global foods. Research has identified certain behaviours that are characteristic of this transition, including eating out and eating while watching television. Unfortunately, researchers suggest that young people aged between 10-19 years are more exposed to such behaviours (Shaikh *et al.,* 2016). As the dietary habits of India have changed, a worrying trend has emerged. Obesity and other chronic diseases are now affecting people of all ages, from children to adults, across all social classes. This is largely due to the increasing adoption of high-fat diets, which have replaced traditional Indian eating habits.

Dysbiosis of gut microbiota plays a major role in the pathogenesis of diet-induced obesity. Clinical studies have indicated that the alteration of the *Firmicutes/Bacteroidetes* ratio (F/B ratio) might be closely linked to the occurrence of obesity (Chen *et al.,* 2023). Evidence has shown that high-fat diets induce 'dysbiosis' in gut-associated microbial communities, that postulated as a major trigger of metabolic impairments associated with obesity. Most studies on humans have been observational, and while some short-term diet experiments have shown some benefits, the effects may be limited (Kang *et al.,* 2022). Unfortunately, there is a lack of research on healthy young people and Indian populations who are transitioning to diets that are associated with a higher risk of non-communicable diseases.

2. Gut microbiome

The wide array of microorganisms that live on the inside and exterior surfaces of the human body is referred to as the human microbiota. The gut microbiome, also termed commensal, refers to the entire microbial community that populates the mammalian gastrointestinal (GI) tract, with the majority residing in the colon. The human gut microbiome reaches 3.8×10^{13} microbes in a standard adult male, which outnumbers the human host cells (3.0×10^{13}). There are five major phyla for the human gut microbiota, namely *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobi*a, with the two dominating phyla, *Firmicutes* and *Bacteroidetes*, representing 90 per cent of the gut microbiota (Zhang, 2022). Numerous aspects of human health, such as the immunological system development, digestion, detoxification, and physiological activities, depend on these microbial populations. Interestingly, some gut microbes synthesize vitamins and create vital proteins for the host, like enzymes that break down otherwise indigestible food components. Because of this symbiotic relationship, people have two genomes: the microbiome, which we acquired, and the genome we received from our parents (D'Argenio, 2015). The gut microbiota largely derives its nutrients from dietary carbohydrates. Fermentation of the carbohydrates that escaped proximal digestion and indigestible oligosaccharides by colonic organisms such as *Bacteroides, Roseburia, Bifidobacterium, Fecalibacterium*, and *Enterobacteria* results in the synthesis of short-chain fatty acids (SCFA) such as butyrate, propionate, and acetate, which are rich sources of energy for the host. This host energy balance is believed to be mediated *via* a ligand-receptor interaction of the SCFAs with a G protein-coupled receptor Gpr41 (Jandhyala *et al.,* 2015). The gut, liver, and immune system have a symbiotic relationship with these gut microorganisms. Environmental factors, such as high-fat diets and alcohol consumption, can disrupt and alter microbial communities. This dysbiosis can lead to dysfunction of the intestinal barrier, translocation of microbial components to the liver, and development or progression of liver disease (Hsu and Schnabl, 2023). Dysbiosis of gut bacteria, fungi, viruses, and Archaea accompanies colorectal tumorigenesis, and these changes might be causative. Data from mechanistic studies demonstrate the ability of the gut microbiota to interact with the colonic epithelia and immune cells of the host via the release of a diverse range of metabolites, proteins, and macromolecules that regulate CRC development. Preclinical and some clinical evidence also underscores the role of the gut microbiota in modifying the therapeutic responses of patients with CRC to chemotherapy and immunotherapy (Wong and Yu, 2023). The microbes in the stomach have a big impact on the metabolism of the host and are becoming more and more known as possible sources of cutting-edge medicinal compounds. Over the past 15 years, developments in metagenomics and genetic techniques have made it possible to characterise in depth the microbiomes of different bodily areas, establishing connections between them and possible illnesses, hazards, and even distinct clinical symptoms. Microbes have mainly been used in the past several decades to develop particular diagnostics for a given condition; current research frequently describes the molecular mechanisms of interactions or defence against prospective pathogens. It is now known that some bacteria in the stomach can interact with human cells, especially by stimulating the immune system (Cani, 2018).

3. Fats/oils sources and requirements-

The various fatty acids that make up dietary fats are divided into three categories: saturated, monounsaturated (MUFA), and polyunsaturated (PUFA). In addition, the unsaturated ones are categorised into omega series, which are ω-9, ω-3, and ω-6. A major source of these fatty acids in daily diets is vegetable oils. The healthiest oil is characterized by its high content of polyunsaturated fatty acids, including 46.26 per cent linoleic acid, 38.84 per cent oleic acid, and 0.9 per cent arachidonic acid (Subrahmaniyan *et al.*, 2024). The fatty acid compositions, bioactive compounds, and health benefits of different vegetable oils are summarised in Table 1. Certain omega-3 and omega-6 polyunsaturated fatty acids (PUFA) are regarded as essential fatty acids since they are not manufactured by the human body and are obtained through diet, however, omega-9 is not necessary for human health (Ivanova *et al.,* 2016). Humans require the omega-3 fatty acid alpha-linolenic acid (ALA) and the omega-6 fatty acid linoleic acid (LA). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two significant long-chain omega-3 fatty acids, are precursors of ALA. Major sources of vegetable origin of ALA are walnuts, flaxseed, flaxseed oil, canola oil, and soybean oil, while EPA and DHA are found predominantly in fatty fish. LA was founded on unaltered vegetable oils derived from sunflower, corn, cotton, and sesame seeds. ALA and LA metabolism are linked to interactions with certain enzymes and are thus altered in two ways. First, they are unsaturated due to the loss of hydrogen atoms, hence increasing the number of double bonds within. Second, by adding carbon atoms to the chain, these fatty acids can become longer. This kind of reaction makes it possible to convert ALA into EPA and DHA. These long-chain fatty acids eventually combine to generate eicosanoids, which are chemical molecules (Riediger *et al.,* 2009).

The World Health Organisation (WHO) suggests that the optimal diet should have a ratio of 0.8 to 1 for polyunsaturated fatty acids and 5 to 10 for ω-6 to ω-3 fatty acids. To guarantee a sufficient intake of important fatty acids and to promote the absorption of lipid-soluble vitamins A, D, E, and K, individuals should consume a total fat intake that falls between 20 and 35 per cent of energy (E). At very low-fat consumption, the absorption of these vitamins is compromised. Indian adults' fat and oil requirements were set at 15-30 per cent E (or 35% E for active and in energy balance), at least 20 per cent E for women of reproductive age, and 30-40 per cent E for children under two years old. Fatty acid recommendations are based on the idea that high serum LDL cholesterol is a significant risk factor for coronary heart disease. The ALA (alpha-linolenic acid) is also an important source of antioxidants, which possess anti-inflammatory traits (Singh *et al*., 2022). A maximum of 10 per cent E SFAs and less than 300 mg/d of dietary cholesterol were advised, along with optimal intakes of LA ranging from 4-10 per cent E and a ratio of LA: ALA between 5:1 and 10:1. To attain a ratio of LA: ALA between 5:1 and 10:1, consumption of green vegetables, legumes, fish, and sea foods had to be promoted (ICMR-NIN, 2020).

Food ingredients that are not nutrients but have biological activity in the body and affect health and disease are known as bioactive substances. They include a wide range of chemicals found in plant-based foods such as fruits, vegetables, grains, legumes, nuts, and teas, such as polyphenols, dietary fibres, carotenoids, phytosterols, and alkaloids. These substances have a major impact on the composition and activity of the gut microbiota, which in turn influences host metabolism and immunological response. For instance, by blocking bacterial enzymes, rupturing cell walls and membranes, and altering quorum sensing pathways, bioactive substances like polyphenols can have a direct impact on microbial development (Duda-Chodak *et al*., 2015). By blocking bacterial DNA gyrase and dihydrofolate reductase, catechins can stop the growth of harmful germs. Bioactive substances have the ability to indirectly affect the gut microbiota by modifying the immunological responses of the host. Certain polyphenols have the ability to modify the gut microbial ecology by influencing cytokine production and immune cell activity. For instance, quercetin reduces the generation of pro-inflammatory cytokines *via* altering signalling pathways like NF-κB and mitogen-activated protein kinase (MAPK) in intestinal epithelial cells (Wang *et al.*, 2024).

Table 1: Fatty acid composition, bioactive compounds, and health benefits of different oils

Fats/oils	Percentage of different fatty acids*	Bioactive compounds#	Health benefits
Coconut	SFA- 92MUFA-6LA-2	Contains lauric acid, capric acid, and myristic acid. Phenolic compounds such protocatechuic, vanillic, caffeic, syringic, ferulic, and p-coumaric acids.	Acts as an antioxidant, antidiabetic, antimicrobial, and anticancer agent, and supports cholesterol management.
Palm kernel	SFA-83MUFA-15LA-2	High in catechin, vanillic acid, luteolin, tyrosol, anthocyanins, and carotenoids.	Supports cardiovascular and immune health, helps manage obesity, and may reduce cancer risk.
Groundnut	SFA-19MUFA-41LA-32ALA-<0.5	Rich in vitamin E, ferulic acid, chlorogenic acid, caffeic acid, and coumaric acid. Several phenolic acids, flavonoids, and stilbenes (*e.g.*, resveratrol)	Provides antioxidant, antidiabetic, anti-inflammatory, antimicrobial, and pain-relief properties.
Rice bran	SFA-17MUFA-43LA-38ALA-1	High in tocopherols, λ-oryzanol, and tocotrienols	Acts as an antioxidant and supports antibacterial, antifungal, anticancer, antidiabetic, liver-protective, and heart-protective effects.
Sesame	SFA-16MUFA-41LA-42ALA-<0.5	Contains lignans like sesamolin, sesamin, pinoresinol, and lariciresinol. High in antioxidants, vitamin E, and phytosterols.	Helps lower cholesterol, reduces inflammation, supports brain and liver function, and protects against damage from low oxygen (hypoxia).

Cottonseed	SFA-24MUFA-29LA-48ALA-1	Sterols, resins, phospholipids, pesticides, carbohydrates, and gossypol	Known for anti-inflammatory and cardio-protective properties.
Corn	SFA-12MUFA-35LA-50ALA-1	Phenolic acids (ferulic acid, coumaric acid, and syringic acid), carotenoids, and flavonoids (anthocyanins)	Offers antioxidant, antibacterial, anticancer, heart-protective, and liver-protective benefits.
Safflower	SFA-9MUFA-13LA-75	Contains sterols, especially in concentrations of 0.24-0.26%.	Helps manage cholesterol, has anti-inflammatory and antidiabetic effects.
Sunflower	SFA-12MUFA-22LA-62	Contains bioactives like pinoserol, vanillin, tyrosol, and naringin.	Supports healthy blood parameters, reduces cholesterol, and helps in bone health and skin disorders.
Soybean	SFA-14MUFA-24LA-53ALA-7	Isoflavones (daidzein and genistein), tocopherols, carotenoids, phytosterols	Offers antioxidant, antimicrobial, anticancer, liver-protective, heart-protective, anti-inflammatory, and antidiabetic effects.
Canola	SFA-6MUFA-60LA-22ALA-10	Sterols, tocopherols, polyphenols, phospholipids (PLs), and flavonoids	Offers antioxidant, liver-protective, anticancer, and antidiabetic benefits.
Mustard/ rape seed	SFA-4MUFA-65LA-15ALA-14	Erucic acid, glucosinolates (sinigrin and gluconasturtiin), isothiocyanates, and thiocyanates	Provides antioxidant, antimicrobial, anticancer, antifungal, antidiabetic, antiobesity, and cholesterol-lowering effects.
Flax seed	SFA-10MUFA-21LA-16ALA-53	Rich in ferulic acid, chlorogenic acid, gallic acid, secoisolariciresinol, and other compounds like lignans, flavonoids, and pinoresinol.	Helps prevent chronic diseases, including cardiovascular issues, obesity, and cancer.
Olive	SFA-17MUFA-71LA-10ALA-<0.5	Includes small amounts of free fatty acids, glycerol, phosphatides, pigments, flavor compounds, sterols, and microscopic olive solids.	Known for anticancer, anti-inflammatory, and antiageing properties.

SFA- Saturated Fatty Acids, MUFA- Monounsaturated fatty acids, LA- Linoleic acid, ALA- Linolenic acid *ICMR-NIN Expert Group (2020), #Choudhary *et al.* (2014), ^Rahim *et al.* (2023)

4. Nutrition transition and its effect on gut microbiome

The interactions of the changing food systems, diet transition, and environment have resulted in the alteration of microbiomes, leading to epigenetic changes and a global burden of health challenges. Long-term alterations in the gut microbiota transition that result in dysbiosis are connected to changes in the food system and some elements of the diet transition. Dysbiosis, a change in the composition of the intestinal microbiota, has been linked to a variety of chronic diseases, such as metabolic disorders (obesity and obesity-associated metabolic diseases such as type 2 diabetes mellitus and non-alcoholic fatty liver disease), immune-mediated diseases such as inflammatory bowel diseases and colorectal cancer (Elechi *et al.,* 2023). There is an abundance of scientific evidence associating refined carbohydrates and lipids in excess with metabolic syndrome and obesity. Recent investigations indicate that certain food ingredients also significantly affect the gut biodiversity of the host. Shifts in macronutrients, including dietary fat, can cause rapid alterations in the bacterial and fungal communities that inhabit the gut. These alterations can have a significant impact on human health by influencing the diversity of the gut microbiota and causing low-grade systemic inflammation. Diets high in fat have been associated with altered species, genus, and family composition, as well as a decrease in the diversity of the gut microbiota and an increase in the ratio of *Firmicutes* to *Bacteroidetes*. Gut microorganisms share vital pathways for immune system activation or inhibition with saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), and conjugated linolenic fatty acids. The mechanisms linking dietary fat, gut microbiota, and obesity involve increased intestinal permeability, systemic endotoxemia, and activation of the endocannabinoid system (Cândido *et al.,* 2018).

India is in the middle of a nutrition transition, which implies profound changes in the consumption of foods in a relatively short span of twenty years due to economic liberalization, urbanization, and the communication revolution. Unfortunately, this change of fate has taken a negative trend toward unhealthy diets, thus becoming a major issue of concern to health practitioners. Surprisingly, the Indian diet is still not adequate in fruits and vegetables and essential micronutrients, while the intake of salt, sugar-sweetened beverages, and trans fats has become more prominent (Raza *et al.,* 2018). During human existence, there have been some dramatic changes in diet and food availability, with recent changes advancing at a faster rate because of socioeconomic and demographic transitions in many parts of the world. With post-modernization in most nations, diets also evolve from traditional high fiber diets to more sugar, animal products, processed foods, and saturated fats, known as the nutrition transition. This change, along with increasing cases of physical inactivity, was accompanied by a great number of people who are overweight or become obese, and non-communicable diseases as well (Al-Shamsi *et al.,* 2018).

Raskind *et al.* (2018) emphasized that the nutrition transition is not merely a top-down process; rather, global forces intersect with local practices at multiple levels of social ecology. The family environment, a critical site for transmitting local customs and norms, remains largely unexplored in India. Their research examines the extent to which opposite-gender siblings and mother-child pairs exhibit concordance or discordance in body weight and whether aspects of the family environment, such as food practices, food-related gender norms, and household resources, are associated with unhealthy weight patterns within and between families. Thus, the variation in microbial composition can be normally observed in the healthy gut-brain microbiota axis, and such variation has been observed to be determined by factors like age, ethnicity, lifestyle, and diet. Still, these differences in gut microbial profile have important consequences for both intestinal and extra-intestinal diseases. Dysbiosis, by which it is possible to describe a change in the composition of the gut microbiota, can be present as a primary factor, consequence, or a combination of these two, which means that it is difficult to define whether the alterations of the microbiota are positive or negative (Rinninella *et al.,* 2018).

5. Role of dietary fats and bioactive compounds of oilseeds on gut microbiome

The impact of a diet high in fat was examined in a study where mice were fed a hyperlipidic diet for three months, resulting in a decrease in *Bacteroidetes* and an increase in *Firmicutes*, *Proteobacteria*, and *Actinobacteria*. While long-term observations provide a concise view of microbiota changes, short-term interventions (five weeks) have also been shown to modify the microbial ecosystem of mice. Moreover, it has been observed that the diversity and composition of the microbiota, once altered by a high-fat diet, can be restored with a low-fat diet (Candido *et al.,* 2018). The effects of a high-fat diet (HFD) on body parameters were analyzed at different time points in mice. Body weight significantly increased after 12 weeks of HFD feeding and continued to rise at 24 weeks. Notably, HFD-fed mice at 24 weeks were significantly heavier than those at 12 weeks ($p<0.05$). The difference in Lee's index, indicating obesity in mice, was more pronounced at 24 weeks compared to 12 weeks. Lee's index of HFD-fed mice at 24 weeks tended to be higher than at 12 weeks (He *et al.,* 2018). In a cross-over randomized controlled trial (RCT) with identical total fat intake but different MUFA-rich oil treatments, high MUFA diets showed no effect on diversity indexes, phylum distribution, or the *Bacteroidetes* to *Firmicutes* ratio. MUFA-rich diets were positively correlated with the genera *Parabacteroides, Prevotella*, and *Turicibacter*, and the family Enterobacteriaceae. Body mass index (BMI) had no significant association with richness (Chao1, ACE) and alpha diversity (Shannon, Simpson), although rarefaction curves showed higher richness and diversity in overweight/obese participants compared to those with normal weight. Additionally, beta diversity revealed differences in gut microbiota between normal-weight and obese participants, with a higher proportion of the phylum *Firmicutes* in obese individuals compared to the combined normal weight/overweight group. Triglyceride levels were negatively correlated with the phylum *Aquificae* and positively with *Cyanobacteria*, while LDL was positively correlated with *Proteobacteria* and HDL with *Verrucomicrobia* (Wolters *et al.,* 2019).

The study conducted by Lu *et al.* (2016) showed that mice administered the high-fat diet gained weight steadily over time and showed an increase in body weight that was 56.12 per cent higher than that of the control diet group after 16 weeks of feeding ($p<0.05$). Dietary supplementation of acetate, propionate, butyrate, or their admixture significantly suppressed the HFD-induced weight gain, with acetate having the greatest effects at 72.31 per cent ($p<0.05$). Two weeks after the feeding intervention, both food and energy intake were increased in the HFD-fed mice ($p<0.05$), and thereafter, energy intake remained higher ($p<0.05$) with no differences in food intake at both 8 and 12 weeks compared to that of the control lean mice. Dietary supplementation of acetate, propionate, butyrate, or their admixture did not change the intakes of both food and energy at 8 and 12 weeks after the feeding intervention.

The review by Machate *et al.* (2020) found that medium chain fatty acids showed an increase in *Bacteroidetes* and a decrease in *Firmicutes* and *Proteobacteria* in the mouse gut reduces inflammation and obesity. A higher *Bacteroidetes*-to-*Firmicutes* ratio and the abundance of *Ruminococcaceae*, *Bifidobacteri*a, and *Lactobacillus* are linked to SCFA production and reduced risks of obesity, IBD, T2DM, and CVD. *Bifidobacteri*a and *Lactobacillus*, abundant in early life, produce lactate and acetate to protect against pathogens. MCFAs are abundant in human milk (9-15%) and virgin coconut oil (61%) compared to infant formula (8-42%). However, diets with $\geq$ 25 per cent coconut oil in female animal models increased obesity, dysfunction, and the *Firmicutes*-to-*Bacteroidetes* ratio.

According to the study conducted by Bourdeau-Julien *et al.* (2023), the effects of short-term Med Diet interventions, with and without a lead-in Can Diet stabilization phase, on plasma metabolites. Both Med Diet phases increased polyunsaturated fatty acids and several eCBome mediators (NAEs, DHEA, EPEA, 2-MAGs, 2-DHG, 2-EPG) compared to baseline and Can Diet. BCFAs and SCFAs showed no significant changes after the first Med Diet. However, propionate, valerate, isobutyrate, and isovalerate increased with CanDiet and decreased during the second Med Diet. While bioactive lipid levels were comparable between Med Diet phases (V2, V4), stabilization enhanced metabolite response reproducibility.

According to the study conducted by Wang *et al.* (2020) the initial composition of the gut microbiota of mice fed on (High fat diet) HFD or (Low fat diet) LFD consisted of *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria phyla*. No significant differences were observed in the abundance of *Firmicutes* or the *Firmicutes*: *Bacteroidetes* ratio between the HFD and LFD groups. Finally, the Operational Taxonomic unit (OTU) analysis revealed that 96 OTUs were present in the HFD group and 61 OTUs in the LFD group, and that Faith's phylogenetic diversity (PD) was significantly higher in the HFD group than in the LFD group. In a similar study conducted by Cao *et al.* (2020), it was stated that while comparing the fatty (F) and lean (L) groups, both had a high concentration of *Firmicutes* and *Bacteroidetes*. The relative abundance of each of the two phyla was also less in the lean group compared to the fatty group, although the changes did not reach the level of statistical significance. The *Proteobacteria* and *Verrucomicrobia phyla* relative abundance were higher compared to the fatty group, although these changes were also not statistically significant. Nor did the *Firmicutes/Bacteroidetes* (F/B) ratio. The F/B ratio for lean and fatty mice was 1.11 ± 0.33 and 1.04 ± 0.34, respectively.

In vitro fermentation of flaxseed polysaccharides results in the release of monosaccharides such as xylose, arabinose, and rhamnose. These sugars are further metabolized into short-chain fatty acids (SCFAs), including butyrate and propionate, which serve as key energy sources and exhibit anti-inflammatory properties within the gastrointestinal tract. SCFAs such as acetate, propionate, and butyrate are synthesized in the gut through the metabolic activity of beneficial microbes like *Bifidobacterium longum* and *Eubacterium rectale*. Additionally, flaxseed lignans undergo microbial biotransformation into bioactive metabolites such as secoisolariciresinol, enterodiol, and enterolactone. This transformation is facilitated by various bacterial genera, including

Bacillus, *Clostridium*, *Klebsiella*, *Eubacterium*, *Peptostreptococcus*, *Ruminococcus*, *Nocardia*, and *Streptomyces*. These metabolites are known to contribute significantly to the body's defense mechanisms against a range of diseases. Flaxseed-derived compounds have been shown to play a beneficial role in preventing obesity by modulating the gut microbiota. One key indicator is the *Firmicutes*-to-*Bacteroidetes* (F/B) ratio in the intestinal microbiome; an elevated F/B ratio has been associated with enhanced energy harvest from food and increased triglyceride accumulation in adipose tissues. Flax seed mucilage has been reported to lower this ratio by influencing the microbial composition, promoting the relative abundance of *Bacteroidetes*, and supporting glycemic regulation (Mueed *et al.*, 2022).

6. Microbiota profiling using 16S rRNA

High-fat diet (HFD) effects on gut microbiota were examined using bacterial 16S rRNA gene sequencing of fecal samples, yielding 1,171,031 sequences across 12 samples, with reads per sample ranging from 13,908 to 191,289. For analysis, sequence numbers were normalized to the minimum read count per sample. Beta diversity, evaluated using principal coordinate analysis (PCoA) on weighted Bray-Curtis distance matrices, demonstrated that HFD significantly altered gut microbiota composition (PC1, 37.29%). Although, obesity typically reduces bacterial diversity, this study found no significant differences in alpha diversity indices observed species, Shannon, Simpson, and Chao1 between the control and HFD groups (Jo *et al.,* 2021). Transplantation of HFD gut microbiota into normal diet-maintained (THD) mice significantly increased susceptibility to atrial fibrillation (AF). Gut microbiota analysis revealed a notable rise in Desulfovibrionaceae, leading to metabolic endotoxemia in THD mice. This microbial transplant also elevated circulating lipopolysaccharide (LPS) levels, disrupted intestinal histologic architecture, and increased pro-inflammatory cytokines in the left atrium, suggesting atrial inflammation as a contributor to AF susceptibility. RNA sequencing showed enhanced activation of ferroptosis and the TLR4/NF-κB/NLRP3 inflammasome signaling pathway in the THD group. Inhibiting ferroptosis or NLRP3 inflammasome signaling significantly improved atrial fibrosis and reduced AF susceptibility linked to obesity-related gut dysbiosis (Kong *et al.,* 2022).

The number of operational taxonomic units (OTUs) showed a decrease in the diversity of gut microbiota with increasing body weight. The alpha diversity indices showed that the normal weight group had higher abundance and observed species than the obese group (Chao1: $p<0.001$; observed species: $p<0.001$; PD whole tree: $p<0.001$; Shannon index: $p=0.008$). Principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS) revealed significant differences in gut microbial community structure between the normal weight group and the obese group (Chen *et al.,* 2020). The richness and diversity of the microbial community (alpha diversity) in obese individuals was lower than that in normal weight individuals, albeit no significant difference in the Shannon index was observed (OB = 2.299 ± 0.338, NW = 2.334 ± 0.175, $p = 0.833$). Similarly, no statistically significant differences in the Shannon index across different BMI categories and between metabolic syndrome discordant obese patient subgroups were observed (p=0.780 and 0.873, respectively). The principal coordinates analysis (PCoA) based on Bray-Curtis distance matrix showed a marked separation between the GM communities of OB and NW confirmed by PERMANOVA analysis, adjusted for sex, age and smoking status, that indicated a significant difference in beta diversity between the two cohorts (sum of squares = 0.5492, mean of squares = 5.0297, F = 0.0533, R = 0.047, $p = 0.002$) (Palmas *et al.,* 2021).

Significant differences in BMI and body-fat percentage were observed between the two groups. The Ace and Chao1 indices were significantly lower in the obese group than those in the control group, whereas differences were not significant in the Shannon and Simpson indices. Kruskal-Wallis tests indicated significant differences in unweighted and weighted UniFrac distances between the gut microbiota of normal-weight and obese children ($p<0.01$), suggesting substantial disparities in both the species and quantity of gut microbiota between the two groups. *Prevotella*, *Firmicutes*, *Bacteroides*, and *Sanguibacteroides* were more abundant in the obese and control groups, respectively. Heatmap results demonstrated significant differences in the gut microbiota composition between obese and normal-weight children.

A high-fat and cholesterol diet was found to drastically modify the gut microbiota of mice in a study employing 16S rRNA gene sequencing, which also reduced the number of amplicon sequence variants (ASVs) across multiple taxonomic levels. But the addition of highland barley (HB) and especially its bran (HBB) reversed the pattern by boosting the abundance of *Lactobacillus* and *Akkermansia muciniphila*, two beneficial bacteria, and decreasing the *Bacteroidetes/Firmicutes* ratio. Furthermore, supplementing with HBB increased alpha-diversity indices, indicating a potential restoration of the microbial community's previously disrupted richness, evenness, and diversity due to the HFCD. These results suggest that HB, particularly HBB, significantly improves the makeup of the gut microbiota and may help to mitigate dysbiosis brought on by diet (Li and Wang, 2023). In another study, *Lycium barbarum* polysaccharide (LBP) supplementation in healthy mice led to notable changes in the composition and function of the gut microbiota. While alpha diversity indices remained unchanged, LBP intervention altered beta diversity, with significant shifts in microbial community structure. At the phylum level, there was an increase in *Firmicutes* and *Proteobacteria* and a decrease in *Bacteroidetes.* At the genus level, LBP significantly increased the relative abundance of beneficial bacteria, such as *Lactobacillus* and *Lactococcus*, while reducing *Ruminococcus*. Functional predictions suggested that LBP also influenced metabolic pathways, particularly by enriching the arachidonic acid metabolism pathway, indicating potential health benefits from the modulation of gut microbiota (Liang *et al.,* 2023).

Mice that were fed a high-fat diet (HFD) together with a sucrose solution had substantial changes in their gut microbiome, which decreased the diversity of bacteria as seen by reduced Shannon and Pielou's indices. In contrast to beneficial taxa like *Rikenellaceae* and *Alistipes*, the combination diet increased the abundance of harmful bacteria linked to inflammatory bowel disease (IBD), such as *Prevotellaceae, Bacteroidaceae*, and *Enterobacteriaceae.* This change in the gut microbiota was linked to higher colitis susceptibility and pro-inflammatory gene expression, indicating that co-consuming sucrose and HFD contributes to gut dysbiosis and stimulates inflammation (Shon *et al.,* 2023). Furthermore, it has been demonstrated that rats on a high-fat diet had a significantly altered gut microbiota when exposed to hazelnut soluble dietary fibre (SDF). Higher ACE and Chao1 indices revealed an increase in alpha diversity, and hazelnut-SDF supplementation improved microbial richness and diversity, which were diminished by the high-fat diet. More specifically, the presence of *Lactobacillus, Alloprevotella, Fusicatenibacter,* and *Roseburia* was enhanced by hazelnut-SDF. These modifications were linked to benefits in lipid metabolism, such as lowered blood cholesterol and decreased buildup of liver fat. This suggests that by modifying the makeup of the gut microbiota, hazelnut-SDF may be able to lessen the adverse consequences of a high-fat diet (Lin *et al.,* 2024).

7. Conclusion and prospect

Dietary fats are powerful modulators of gut microbiota, with distinct effects based on fat type and intake levels. High-fat diets, particularly rich in saturated and trans fats, can negatively alter gut microbiota composition, promoting dysbiosis, intestinal inflammation, and metabolic disorders. In contrast, polyunsaturated fats, particularly omega-3 fatty acids, are associated with beneficial anti-inflammatory effects and improved gut health. As more populations transition to Westernized diets, there is an urgent need for human-focused research to clarify the mechanisms by which dietary fats impact gut microbiota and related health outcomes. Understanding these interactions will be critical in addressing rising global rates of obesity, metabolic syndrome, and other diet-related diseases. Future studies should prioritize long-term dietary interventions in diverse human populations to establish clearer guidelines on fat consumption and its role in maintaining gut and overall health.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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