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INTERACTION OF PLANT-BASED DIETS AND GUT MICROBIOTA

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CONTENT

KEY POINTS

- The gut microbiota can influence and modulate the **health** of the individual, and its composition and metabolism are affected by **dietary patterns**.
- **Plant-based** dietary patterns **cover a spectrum** that includes vegan, vegetarian, flexitarian, Mediterranean, etc. Comparisons between individuals or populations consuming plant-based and Western diets have shown differences in composition and/or metabolic activity of gut microbiota. There are indications that these types of diets may be more **beneficial for the gut microbiota.**
- Plant-based diets are usually high in **dietary fibre**, leading to increases of short-chain fatty acid (SCFA)-producing bacteria in many studies. **SCFAs** have several identified benefits:
    - ✓ Strengthen the intestinal epithelial layer, helping the **immune system** against pathogenic bacteria.
    - ✓ May improve **energy metabolism** and leanness.
    - ✓ Increase **colonic motility** and decrease visceral pain.
    - ✓ Have antiproliferative properties in cancer cells with a potentially favourable role in colon **cancer**.
- Plant-based diets are also associated with high levels of **polyphenols**, which have been shown to beneficially modify the gut microbiota. They are extensively metabolized by the microbiota into potentially beneficial derivatives.
- Compared with 'Western diets', which are high in animal foods, consumption of plant-based diets is often associated with **reduced formation of deleterious bacterial metabolites** such as secondary bile acids, indole and p-cresol, and the proatherogenic compound trimethylamine oxide (TMAO).

INTRODUCTION

The concept that diet and health are strongly associated is today widely accepted and is the basis of dietary guidelines from health authorities worldwide. Indeed, the risk of diseases is strongly associated to food intakes: **diets lacking of certain foods, such as whole grains, nuts, fibre-rich foods, fruits and vegetables and legumes are more strongly associated to morbidity and mortality than diets rich in unhealthy foods and nutrients, such as sweetened beverages or trans-fatty acids (1).** Dietary patterns are thus today increasingly considered as more relevant than nutrient-focused approaches, especially when defining public health policies (2).

Dietary patterns have been evolving: observations in the past 30 to 100 years in different countries have revealed deeply modified food habits of most populations. Unfortunately, these alterations have occurred in a direction that has most often increased the risk of many chronic diseases (3), even though improvements over this period have been made regarding undernutrition and microbiological safety. Much more recently, other changes have appeared in a growing proportion of the population of Western countries, who favour lower intakes of meat and animal products, and a higher proportion of plant-based foods. These diets are linked to both health and sustainability benefits (4).

Independently, in the past 30 years, the importance of the gut microbiota in human health has become increasingly evident. It has been clearly demonstrated that the composition and the functionality of the gut microbiota affect many aspects of health, even though the understanding of what is a “healthy microbiota” is still unclear. In addition, numerous studies have shown that **diet is one of the most important lifestyle factors that affects the composition of the human gut microbiota**, even though many other features may interact (genotype, antibiotic use, smoking, …)(5–7).

Indeed, the gut microbiota is both known to be related to human health (8), and to interact with dietary components (9), which makes it a possible mediator in the relation between diet and health (10, 11).

In this overall context, this scientific update aims at reviewing the available evidence regarding the effects of plant-based diets on the composition and metabolism of gut microbiota.

A FEW DEFINITIONS AND POINTS TO CONSIDER

This scientific update will deal with broad concepts, such as “plant-based diets”, “gut microbiota” or “clinical studies”. Below, some critical points to take into account when reading the following sections of this update are highlighted, in order to help interpreting some conclusions.

“PLANT-BASED DIETS” refer to diets predominantly based on plant-based foods. As illustrated in Figure 1, **there is a spectrum of plant-based eating, varying in the proportion of animal products incorporated into the diet**: from vegan diets (exclude all animal products) to flexitarian or Mediterranean diets (12). Plant-based eating does not automatically exclude all animal products but puts plant-based foods at the core. The **flexitarian** diets include mostly plants, with occasional intakes of meat, dairy, and eggs. Flexitarian diets encourage variety rather than restriction and are typically followed by people who attempt to minimize animal products in their diets for health, ethical, or environmental reasons. The **Mediterranean** diet is a type of flexitarian diet which encourages consumption of vegetables, fruits, whole grain foods, legumes (incl. soya), nuts, and virgin olive oil, with moderate consumption of fish and dairy products. Vegetarian and vegan diets are focused on plant foods, including vegetables, fruits, soya, legumes, nuts and whole grains. **Vegetarian** diets exclude flesh meat and fish, but can include dairy and eggs (lacto-vegetarian or lacto-ovo-vegetarian). **Vegan** diets typically source all calories from plants and exclude all animal products.

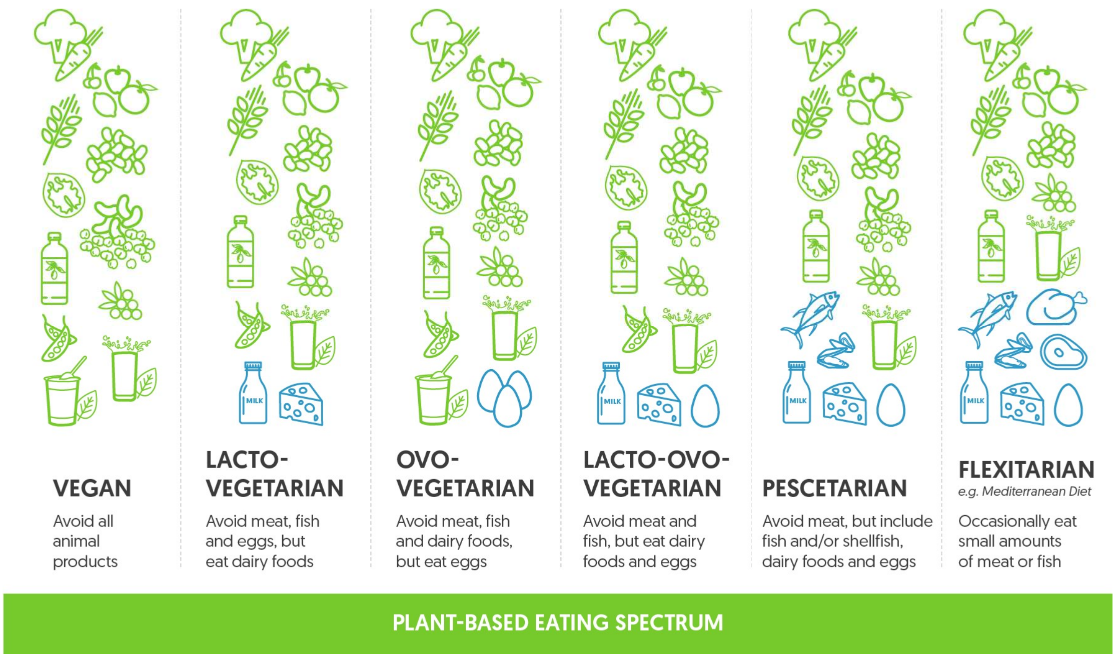


FIGURE 1. PLANT-BASED EATING SPECTRUM, varying in the proportion of animal products incorporated into the diet (from vegan to flexitarian). The Mediterranean diet is a type of flexitarian diet. Plant-based eating does not automatically exclude all animal products but puts plant-based foods such as fruits, vegetables, legumes, wholegrains, nuts, seeds, vegetable oils and plant-based alternatives to meat and dairy at the core of the diet.

Consequently, there are significant differences in composition within this broad range of plant-based diets, and this should be taken into account when interpreting and comparing clinical studies. Few tools exist to characterize plant-based diets: for example, the level of adherence to a **Mediterranean diet** can be **assessed using the 14-item scores** developed by the PREDIMED study group (13) or the **Mediterranean Diet score** (14), but similar evaluation may be more difficult for other plant-based diets. This is of importance, as the above definition of plant-based diets does not necessarily lead to "healthy diets". Some attempts have been made to create a **"plant-based diet index"** (PDI) to help characterize such diets and more recently the concept has been refined to **encompass healthy and unhealthy plant-based diet indices (hPDI and uPDI)**, with the former including whole grains, fruits, vegetables, nuts, soya, legumes, and vegetable oils, and the latter based around sweetened soft drinks, refined grains, sweets etc. PDI may thus reflect the **quality of a plant-based diet** and help linking it with functional or health outcomes (15, 16).

Unfortunately, this characterization of plant-based diets is seldom made in the studies that will be presented below and this often prevents an understanding of which characteristics of a plant-based diet are important.

Although a diet is always more than the sum of the foods and the nutrients it contains, it should be remembered, particularly when dealing with gut microbiota, that **some components are especially important, such as dietary fibre, which is expected to be present in high amounts in plant-based diets, and specifically those with high nutritional value.**

The fibre content of plant-based foods varies markedly: whole grain cereals contain around 12 g fibre per 100 g, while refined cereals usually do not exceed 3 g/100 g. Similarly, there is a wide range of fibre content in fruits, vegetables and legumes, from 0.5 g/100 g up to 16 g/100 g, with legumes tending to have the highest contents (17). Therefore, **different plant-based diets may bring different quantities of dietary fibre, and omnivorous diets may contain high amounts of fibre, if these include large amounts of plant-based foods.**

Furthermore, dietary fibre consists of a variety of different components (Table 1) that are not digested in the upper gut and, when reaching the colon, often in the form of complex insoluble particles, need to be broken down before they can provide a major energy and carbon source for the microbiota. The difference in the chemical structures of fibre, including different glycosidic linkages, requires a large variety of enzymatic functions, which are collectively present in the gut microbiota. Some bacterial species (generalists) can use a wide range of carbohydrates as substrates, whereas specialist species have a much narrower substrate range (18). Thus, **different types of fibre will differently impact the composition of gut microbiota.**

TABLE 1. MAJOR PLANT DIETARY FIBRE TYPES AND EXAMPLES OF SOURCES.

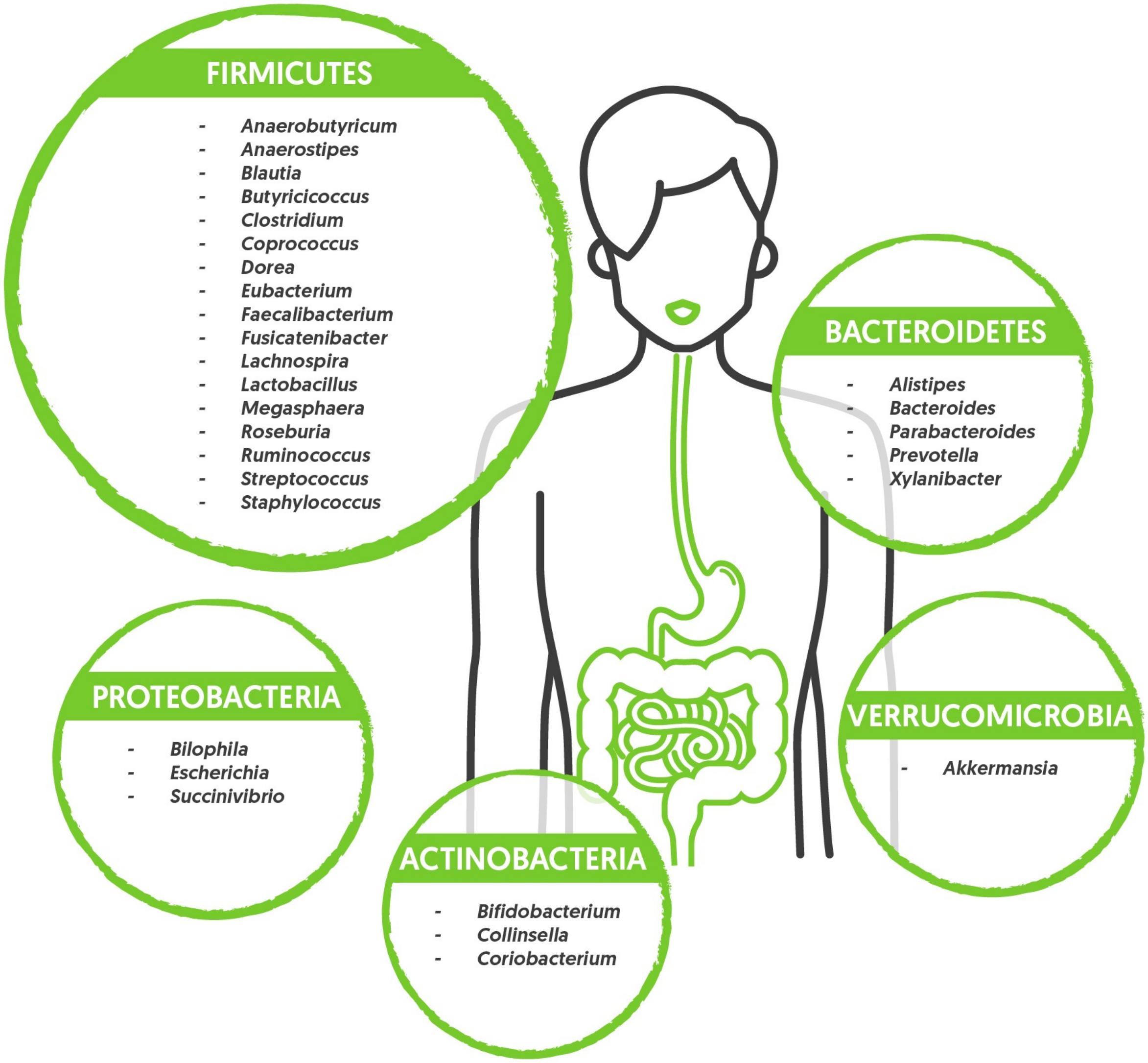
Adapted from: Stephen et al., 2017; Louis et al., 2021 (17–18).

FIBRE TYPE		EXAMPLES OF FOOD SOURCES
Plant cell walls *(non-carbohydrates)*		
Lignin		- Secondary cell walls of plants (carrots, celery, ...) - Red algae
Plant cell walls *(carbohydrates)*		
Cellulose		- Outer layers of cereal grains - Pulses
Hemicellulose	Xylans	- Psyllium - Outer layers of cereal grains
	Mannans	- Grain legumes - Guar gum
	Glucans	- Oat - Barley
Pectins	Galacturonans	- Fruits (apple, citrus, ...)
Storage carbohydrates		
Resistant starch		- High amylose wheat or maize - Cooked and cooled potatoes
Inulin-type fructans		- Artichoke - Chicory
Raffinose family of oligosaccharides		- Legumes (beans, chickpeas, lentils, ...)

“GUT MICROBIOTA” can be defined as the assemblage of the trillions of microorganisms (bacteria, archaea, fungi, and viruses) present in the gastrointestinal tract; the term “microbiome” also includes the surrounding environmental conditions, i.e., the tissues and biofluids of the host (19). The gut microbiome is a **complex and dynamic ecosystem that varies across and within individuals, because of genetics, age, lifestyle (including diet), hygiene, and other factors.**

Thanks to high throughput techniques, more than 1000 microbial species have been identified from the human gut (20, 21), and have been classified at different levels, such as phylum, genus and species. A list of some of the most important phyla and genera are shown in Figure 2. Each bacterium has its own genome and thus its own metabolic capacities, but the overall metabolism of the whole microbiota is also very important in fibre breakdown, as there is competition and cooperation between individual microbes (18).

F!IGURE 2: SOME IMPORTANT PHYLA AND GENERA IN THE HUMAN GUT MICROBIOTA



These multidimensional interactions make the interpretation of microbiota compositional data difficult. When addressing the relationships between diet, a health or well-being outcome, and microbiota features, the conclusion may be drawn that a **specific bacterium** or a limited number of microbes are causally linked with the **prevention or the onset of a symptom or a disease**. In fact, disease-related changes to the gut environment, as well as to the host, can influence the composition and functionality of the gut microbiota and all those factors interplay to produce the observed changes (22). Although the gut microbiota composition can differ between healthy and diseased subjects, being sometimes described as "dysbiotic" in the latter, this **does not necessarily infer that some kinds of microbiota composition are "healthier" than others**. Furthermore, the abundance of a given species or genus is not always linked to its importance in the gut ecosystem. **Some microbial species, referred to as 'keystone species', have a disproportionately large effect on the community, compared to their relative abundance** (23, 24). It is thus difficult to interpret changes in gut microbiota composition as beneficial, neutral or deleterious to health, and this should be kept in mind when reading the following pages.

Methodological drawbacks also exist in relation to the molecular techniques used to assess the microbiota, that may limit the conclusions that can be drawn from many studies. For example, sample processing can introduce errors: certain microbes appear to be more vulnerable to being lost during frozen storage and different DNA extraction methods can lead to different results (25). The way data on gut microbiota composition is reported also needs careful consideration: sequence-based community analyses, which are by far the most frequent in the studies presented in this scientific update, report proportions of the different bacteria. Other methods inform on the absolute numbers, which may appear more relevant in the context of dietary changes, as the total number of bacteria may be affected (i.e., increased following plant-based, high fibre diets). Absolute numbers are also preferable from a metabolic perspective, as they can more easily be connected to metabolic fluxes or concentrations (24).

These methodological drawbacks do not prevent attempts to examine changes in the overall gut microbiota composition, using the few indicators that have been proposed to characterize the gut microbiota. Below are a few definitions, as well as cautionary notes, to keep in mind regarding this domain.

Diversity of the microbiota refers to the variety and abundance of microbial species and is composed of both **richness**, which refers to the total number of species within a microbiota sample, and **evenness**, which describes the relative distribution of bacterial species and indicates if a few species quantitatively dominate the other ones (26). One also distinguishes between α-diversity (microbiota within a sample) or β-diversity (across different samples). Although a greater diversity is usually considered as associated with a balanced microbiota (27), the diversity of microbes is not fully reflected in their functions, as **different microbes can provide the same function**. This phenomenon, known as "functional redundancy" has, for example, been largely documented for production of short-chain fatty acids (SCFA) (28), and it exists for other functions (5).

At phylum level, the ratio of Bacteroidetes to Firmicutes has sometimes been considered as indicative of more or less favourable to health. For example, the **Firmicutes/Bacteroidetes ratio** has been said to be a marker of obesity risk; however, this view has been heavily challenged, due to numerous contradictory findings across studies, and it is today more generally believed that this ratio cannot be associated with a determined health situation (29).

At lower taxonomic levels, some linking attempts can be made for a few specific genera and species, that are most often seen associated with healthy or unhealthy situations, although as mentioned above, these associations may not be causal.

For example, **pathobiont** species are commensal bacteria, that **under environmental or host pressure, may overgrow and cause disease**, as can be the case for some strains of *Escherichia coli*, *Enterococcus faecalis* or *Clostridioides difficile* (30). Lowering their presence can thus be seen as favourable to health. To the opposite, several additional species have been consistently associated with health status, although with weaker evidence. While this does not mean that these bacteria are themselves causally related to health, they might be seen, with caution, as health indicators. This may for example be the case for the genus ***Roseburia***, which contains five species able to produce butyrate, with the **potential to affect colonic motility, immune status, and exert anti-inflammatory properties**. Reduction in *Roseburia* species representation in the gut microbiota may affect various metabolic pathways and has been associated with several diseases (31). Similarly, ***Akkermansia muciniphila***, a mucin degrader, is thought to **stimulate mucosal microbial networks and to improve intestinal barrier function**. It is inversely correlated to inflammatory conditions and may provide **protection against the development of intestinal and metabolic disorders** (32). The abundance of *Faecalibacterium prausnitzii,* which exerts anti-inflammatory properties, has also been found reduced in different intestinal disorders (33). In contrast, some gut bacteria have been **associated with cancer, through their virulence factors or toxins**, such as ***Bacteroides fragilis*** (34)

In addition, enterotypes correspond to specific profiles of bacterial compositions, and several have been identified, driven by the high proportions of one taxon, such as *Bacteroides* or *Prevotella* (35). Although enterotypes may appear as a relevant frame for analysis, controversies have arisen regarding the number of enterotypes or their specificity and this may question their usefulness to predict functions (36). Caution is thus needed and no clear correlation has been ascertained between one enterotype and health status.

“CLINICAL STUDIES” included in this scientific update have been selected among the investigations that have been undertaken in the past ten years to understand how dietary intakes and gut microbiota features are connected in humans. The original studies were either observational or interventional and only those with a reasonable methodological quality have been retained (e.g., randomized controlled trials have been included, studies using only cultivation methods have been discarded, etc.).

Ecological studies are observational studies that **compare the gut microbiota** of populations **from different countries, with different lifestyle and eating habits.** They sample subjects in their real, everyday life, but without assessing in detail the content of the individual diets by means of dietary surveys, and other lifestyle features are usually poorly characterized. Although these studies provide relevant insights, data analysis cannot be adjusted for these multiple confounding factors and this may significantly affect the meaning of some of the observed associations, which should thus be viewed with caution.

Cross-sectional observational studies compare the **gut microbiota** in a **population consuming a plant-based diet** with the microbiota of **populations following other types of diets**. A major difference with ecological studies lies in a more precise definition of the compared diets, which are usually characterized, at least roughly. Although confounding factors are still present and analyses are often not fully adjusted, the associations can be evaluated more accurately than in ecological studies. Interestingly, the observed populations usually have stable eating habits and it is thus possible to **assess differences in the microbiota** of subjects who have consumed a certain type of diet **over a long time**.

Intervention studies are experimental trials during which subjects are asked to **modify their diet**, either from an omnivorous/Western type diet to a plant-based diet (most frequently encountered situation), or vice-versa, and usually for a **limited duration** (a few days or weeks). Such studies can provide information about **how**, and at **what speed**, the **changes in these bacterial populations occur and can analyse the accompanying metabolic changes.** They do not reflect the long-term steady-state situation, but rather the changes occurring when one shifts from an omnivorous to a vegetarian/vegan diet. Although such designs are usually considered as the only ones able to **establish a causal relationship between diet and microbiota changes**, the dietary changes explored do not always concern only the plant or animal source of foods: substituting an animal food for a plant one will be associated with changes in macro- and micronutrients, and the variations observed may be due, for example, to an enhanced fibre intake, but also to a reduced fat or sugar intake.

PLANT-BASED DIETARY PATTERNS AND COMPOSITION OF THE GUT MICROBIOTA

ECOLOGICAL STUDIES

Findings from ecological studies clearly demonstrate that **healthy subjects in different geographical areas display a different composition of their gut microbiota and consequently, differences in microbial genes and metabolites**. In the five studies briefly summarized in Table 2, faecal samples were compared across small to medium-size populations, that had either a traditional, plant-based diet or a Western, omnivorous diet (37–41). The **higher presence of *Prevotella* in plant-based/traditional diet** eaters was observed consistently in most studies, as well as higher concentrations of SCFAs.

While the findings from these studies are consistent with the hypothesis that traditional, plant-based diets are important drivers for the observed changes, many other features, such as genetics, level of hygiene and medical care, environmental exposure, physical activity and so on, distinguish the compared populations, even though they were usually matched for age and gender.

TABLE 2: ECOLOGICAL STUDIES. Overview of gut microbiota composition and microbial metabolites in healthy populations living in areas where plant-based (usually traditional) diets are common, compared with areas where omnivorous (usually Western-type) diets are common. Abbreviations: Ref., reference; SCFA, short-chain fatty acid.

REF.	POPULATIONS COMPARED	MICROBIOTA COMPOSITION IN POPULATIONS EATING PLANT-BASED VS OMNIVOROUS-WESTERN DIETS	MICROBIAL FAECAL METABOLITES IN POPULATIONS EATING PLANT-BASED DIETS	ADDITIONAL DATA OR COMMENTS FROM AUTHORS
(37)	School-aged children from rural Burkina Faso (BF; n=14) vs Italian children (n=15).	In Burkina Faso children, less *Enterobacteriaceae (Shigella* and *Escherichia);* significant enrichment in Bacteroidetes and depletion in Firmicutes with a unique abundance of bacteria from the genera *Prevotella* and *Xylanibacter,* (absent in Italian children).	More SCFA in Burkinabe than in Italian children.	Results support the hypothesis that gut microbiota has coevolved with the polysaccharide-rich diet of Burkinabe children, allowing them to maximize energy intake from fibre.
(38)	Adults aged 50-65: 12 African Americans and 12 native rural South Africans.	In native Africans, total bacteria and major butyrate-producing groups were significantly more abundant, with a predominance of *Prevotella* (enterotype 2); more *Succinivibrio* and *Oscillospira, more Faecalibacterium prausnitzii* and *Clostridium* cluster IV and XIV. In African Americans predominance of *Bacteroides* (enterotype 1) was seen.	Higher SCFA and lower secondary bile acids; more microbial genes encoding for methanogenesis and hydrogen sulphide production and less encoding for secondary bile acids.	Authors relate their observations to the different risk of colon cancer in both populations, which could be influenced by the balance between microbial production of health-promoting metabolites such as butyrate and potentially carcinogenic metabolites such as secondary bile acids.
(39)	28 Egyptian vs 14 US teenagers, all from urban location. Little information on their diet, assumed to be "Mediterranean" and "Western", respectively.	In Egyptians, higher abundance of *Prevotella, Succinivibrio* and *Treponema (Prevotella* enterotype*)* and lower abundance of *Bacteroides, Ruminococcus, Blautia, Bilophila, Akkermensia* and *Faecalibacterium.* In US teens *Bacteroides* enterotype was more frequent.	Higher level of SCFAs and of microbial polysaccharide degradation-encoding genes; lower levels of microbial genes encoding protein degradation, vitamin biosynthesis, and iron acquisition pathways.	The differences in faecal microbiota structure and functions and metabolite profiles between Egyptian and US teenagers are consistent with the nutrient variation between Mediterranean and Western diets.
(40)	110 Indians from two regions with either an omnivorous or a vegetarian (carbohydrate)-based diet.	In the carbohydrate-based diet, the Bacteroidetes to Firmicutes ratio was higher; there was more *Prevotella* and *Megasphaera* and less *Faecalibacterium, Akkermansia, Ruminoccoccus* and *Roseburia.*	Enrichment of branched chain amino acids in carbohydrate (plant-based) diet. Lower levels of SCFA than in omnivorous diet.	Findings highlight the importance of the overall diet quality: here, the plant-based diet is also rich in sweets, fried snacks and fats whereas the omnivore diet consisted of meat, fish and rice.
(41)	147 adults from Cameroon, from different urban settings (rural, small town, large town) with increase of Western-type diet with urbanisation.	In rural vs urban areas: more *Succinivibrio, Faecalibacterium, Prevotella, Roseburia* and *Coprococcus* and less *Alistipes, Bacteroides, Clostridium* and *Butyricicoccus.*	Not assessed.	The urbanization gradient is characterized by differences in diet, but also in habitat and socio-cultural conditions that can contribute to the observed differences.

OBSERVATIONAL STUDIES

The findings from six such studies are summarized in Table 3 (42–47). Although most studies highlight **some differences in the composition of the gut microbiota, no clear pattern potentially associated to diet type can be ascertained from these data**.

TABLE 3: OBSERVATIONAL CROSS-SECTIONAL STUDIES. Overview of gut microbiota composition (16S rRNA genes and microbial metabolites) in healthy populations living in the same area and with different diets. Abbreviations: 16S rRNA, 16S ribosomal ribonucleic acid; O, omnivorous; Ref., reference; SCFA, short-chain fatty acid; TMAO, trimethylamine oxide; V, vegetarian; VG, vegan.

REF.	STUDY POPULATION	MICROBIOTA COMPOSITION IN SUBJECTS WITH HIGHER ADHERENCE TO PLANT-BASED DIETS	MICROBIAL FAECAL METABOLITES IN SUBJECTS WITH HIGHER ADHERENCE TO A PLANT-BASED DIET
(42)	153 Italian adults usually following an O, V or VG diet. Most V & VG and 30% of O had high adherence to Mediterranean diet.	No difference in α-diversity; A small cluster of genera was associated with the plant-based diet, with the strongest correlations found for *Roseburia, Lachnospira* and *Prevotella*; *Ruminococcus* and *Streptococcus* correlated negatively with the plant-based diet patterns.	Faecal content in SCFA increased with adherence to a Mediterranean diet, in all subjects. *Lachnospira* and *Prevotella* genera correlated with SCFAs; Urinary TMAO levels were significantly lower in VG and V and, in a dose-dependent way, in subjects with higher adherence to Mediterranean diet.
(43)	15 American VG (at least for 6 months) and 6 O.	No difference in diversity. Only a few taxa had differential presence (all below 1% of total bacterial counts), including *Ruminococcus, Holdemania, Dialister, Actinomyces* and *Proteobacteria RF32*, but not *Prevotella*.	No increase in faecal SCFA, despite higher fibre intake in vegans (35 vs 18 g/d); benzoate or hippurate, and other plasma metabolites produced by the gut microbiota from polyphenolic compounds from grain, nuts and berries were present at higher levels in subjects under a VG diet.
(44)	66 VG, 102 V, and 100 O Brazilian adults.	Less Firmicutes and more Bacteroidetes in VG vs V and O. VG had higher *Prevotella* abundance than V and O. *Faecalibacterium* low in VG, higher in V, highest in O. *Succinivibrio* & *Halomonas* overrepresented in O.	Not assessed.
(45)	74 healthy Spanish adults, > 50, with various adherence levels to a Mediterranean diet.	Higher levels of *Clostridium* cluster XIVa and *Faecalibacterium prausnitzii*.	Higher faecal concentrations of metabolites from polyphenols (benzoic, syringic and 3-hydroxyphenylacetic acids).
(46)	27 Spanish adults, among which 11 had a good adherence to Mediterranean diet.	No difference in α-diversity but trend for a greater richness; some isolated differences were seen at family or genus level (more *Christensenellaceae, Catenibacterium, less Clostridium)* and species level (lower *Bacteroides uniformis* and *Bacteroides ovatus).*	Higher level of SCFA with better adherence to Mediterranean diet.
(47)	26 VG, 32 V, 43 O Italian adults.	Microbiota richness was higher in O vs V (trend only vs VG; no difference between V and VG). Greater amounts of Bacteroidetes in V and VG, compared to O, but at genus level, only 4 taxonomic units (affiliated to *Bacteroides, Lachnospiraceae*, and *Ruminococcaceae* and each accounting for less than 1% of total counts) differed. No difference in β diversity or in enterotype distribution.	Not assessed.

The studies presented above have been carried out in urban settings of countries where Westernized diets are predominant and where plant-based diets are likely to be different from the traditional diets addressed in ecological studies. Most studies provide some evaluation of dietary and nutrient intakes, but few attempts were made to consider the potential impact of the nutritional quality of the diet or the presence of specific components, such as fibre or polyphenols.

This gap has been addressed in a recent observational study (48) of the faecal microbiota of 1,098 British and American subjects with detailed information on the habitual diet, obtained by food frequency questionnaire. The study found segregation of microbial species between distinct clusters associated with the healthy PDI foods (whole grains, fruits & vegetables, legumes, nuts, vegetable oils) and unhealthy PDI foods (refined grains, fruit juices, potatoes, sugar sweetened beverages, sweets and desserts). This study went one step further by determining that **the diversity of healthy plant-based foods in the habitual diet shapes gut microbiome composition, with microbial species being segregated into groups associated with more or less healthy plant-based foods** (Figure 3). Taxa linked to healthy plant-based foods mostly included butyrate producers, such as *Roseburia hominis*, *Agathobaculum butyriciproducens*, *F. prausnitzii* and *Anaerostipes hadrus*. Several *Clostridium* species (*C. innocuum*, *C. symbiosum*, *C. spiroforme*, *C. leptum*, C. *saccharolyticum*) correlated with several less healthy plant-based foods.

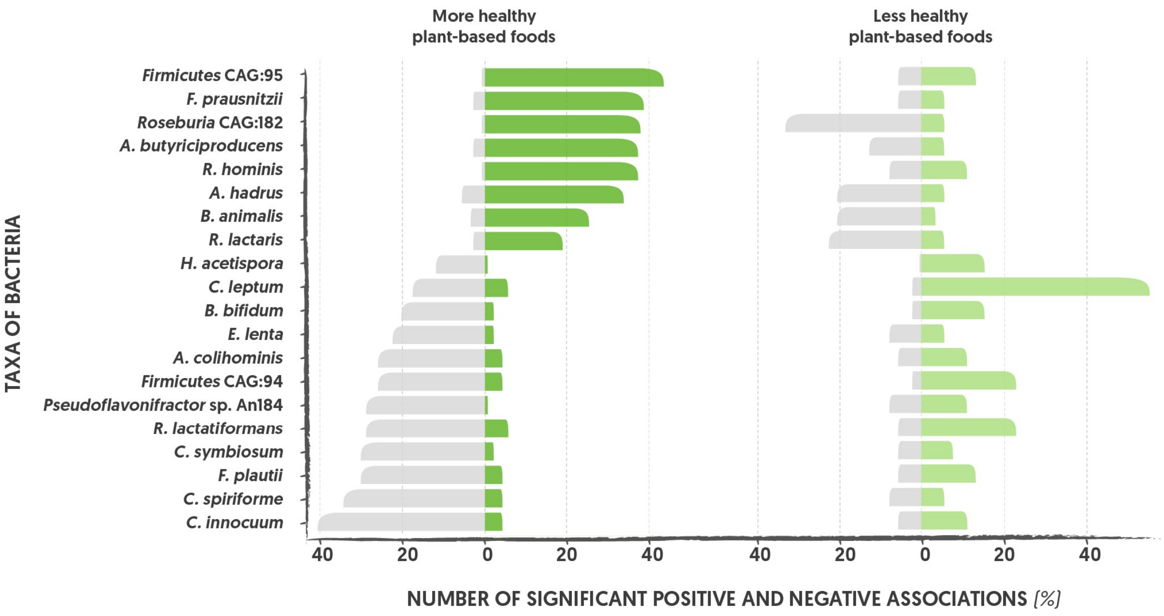


FIGURE 3 FOOD QUALITY (ACCORDING TO PDI) IS LINKED TO THE COMPOSITION OF GUT MICROBIOME. Number of significant positive and negative associations in % between taxa and foods categorized by more and less healthy plant-based foods. The taxa shown are the 20 species with the highest total number of significant associations. Coloured bars indicate positive associations; grey bars indicate negative associations. An example for clarification: *F. prausnitzii* is positively associated with around 40% of heathy plant foods and is positively associated with less than 15% of less healthy plant foods. To the opposite, *C. leptum* is positively associated with more than 50% of less healthy plant food and is positively associated with less than 10% of healthier plant foods.

Abbreviations: PDI, plant-based diet index. *Adapted from: Asnicar et al., 2021 (48).*

INTERVENTION STUDIES

Table 4 summarises the findings of seven controlled trials, illustrating the variability of populations, diets and designs (49–55). Confirming the conclusions of the observational studies, **diet-induced differences in the composition of the gut microbiota were detected, even after short-term dietary changes**. Furthermore, species known to be producers of butyrate (see next section) were generally increased on plant-based diets.

Several studies included subjects suffering from metabolic dysfunctions, such as metabolic syndrome or obesity, who were compared to healthy counterparts. At baseline, i.e., before dietary intervention began, the gut microbiota in unhealthy subjects was different from the microbiota of healthy subjects. After a few weeks or months of plant-based diet, a **partial restoration towards a more balanced microbiota was observed in metabolically-diseased subjects** (51, 52).

TABLE 4 INTERVENTION STUDIES. Effect of plant-based diets on microbiota composition in healthy and non-healthy populations, compared to omnivorous, Western-type diets. Abbreviations: BMI, body mass index; CVD, cardiovascular disease; IgA, immunoglobulin A; MedD, Mediterranean diet; MetS, metabolic syndrome; O, omnivorous; OB, obese; RCT, randomized clinical trial; Ref., reference; SCFA, short-chain fatty acid; V, vegetarian; VG, vegan.

REF.	STUDY POPULATION	DESIGN & DIETS	MICROBIOTA	ADDITIONAL DATA (INCL. ABOUT MICROBIAL FAECAL METABOLITES), OR COMMENTS FROM AUTHORS
(49)	10 healthy American subjects.	Cross-over intervention, strict V vs strict animal-based (no plant food at all) 5 days.	Animal-based diet increased the abundance of bile-tolerant microorganisms (*Alistipes, Bilophila,* and *Bacteroides*) and decreased the levels of Firmicutes that metabolize dietary plant polysaccharides (*Roseburia, Eubacterium rectale,* and *Ruminococcus bromii*). No change in α diversity, increase in β-diversity.	More acetate & butyrate in plant-based diet; more isovalerate & isobutyrate in animal-based diet; animal-based diet increased the levels of faecal bile acid (deoxycholic acid). Microbial activity appears to reflect trade-offs between carbohydrate and protein fermentation.
(50)	20 African Americans (Western diet-O) vs 20 rural South Africans (traditional, plant-based diet).	Cross-over intervention, 2-week periods: African American switch to an African traditional diet and vice-versa.	Focus on butyrate producers, which were in greater abundance in Africans consuming traditional diet or in Americans when switched to this diet. At baseline, *Bacteroides vulgatus* and related species dominated in the Americans and *Prevotella melaninogenica* and related species dominated in Africans.	At baseline: more SCFA in Africans than Americans; SCFA increase in Americans on plant-based diet and decrease in Africans on O; Africanization reduced colonic evacuate secondary bile acids by 70%, and Westernization increased them by 400%. Fibre intake in diets of rural Africans was approx. 66 g/d, vs 14 g/d for African American diets.
(51)	138 subjects with MetS and 101 without; all medically treated CVD patients, with coronary event over 6 months before enrolling.	MedD: 35% fat (22% olive oil), 50% carbohydrate (max.) Control group: low fat (less than 30%), high carbohydrate (min. 55%) diet. 2 year duration.	At baseline, *Bacteroides fragilis* group, *Parabacteroides distasonis, Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Fusobacterium nucleatum, Bifidobacterium longum, Bifidobacterium adolescentis, Ruminococcus flavefaciens* subgroup, *and E. rectale* were depleted in MetS patients. MedD partially restored the population of *P. distasonis, B. thetaiotaomicron, F. prausnitzii, B. adolescentis*, and *B. longum* in MetS patients, but had no effect in non-MetS volunteers.	A Mediterranean diet appears more effective than a low-fat diet at restoring the alteration found in the microbiota of MetS patients.

TABLE 4 (continued).

REF.	STUDY POPULATION	DESIGN & DIETS	MICROBIOTA	ADDITIONAL DATA (INCL. ABOUT MICROBIAL FAECAL METABOLITES), OR COMMENTS FROM AUTHORS
(52)	106 CVD male patients (same inclusion criteria as in ref (51) above): 33 obese + MetS, 32 obese non-MetS and 41 non-obese non-MetS.	Same experimental protocol as in ref (51) above.	No difference in bacterial diversity. *Bacteroides*, *Prevotella* and *Faecalibacterium* genera increased in the MetS-OB group with both diets compared with baseline. Thus, the differences between the MetS-OB group and the non-MetS- groups found at baseline disappeared in MetS group, MedD increased the abundance of *Bacteroides*, *Prevotella*, *Faecalibacterium*, *Roseburia* and *Ruminococcus* genera and *P. distasonis* and *F. prausnitzii* species. No changes were seen in groups without MetS at baseline.	Not assessed.
(53)	22 healthy omnivorous volunteers. 15 switched for 3 months to a V diet (O→V group) and 7 remained on O diet; 7 volunteers were on a long-term V diet.	O→V compared before and after change and with long term V and long-term O groups.	O→V change at 3 months: Phylum level, no change. Genus level: Reduction in *Alistipes*, *Mobiluncus*, *Histophilus*, *Leeuwenhoekiella* and increases in *Moorella*, *Proteus* and *Leptospira*. Species level: reduction in *Streptococcus thermophilus*, *Proteus mirabilis*, *Alistipes shahii*, *Alistipes putredinis*, unclassified *Alistipes*, and *Bacteroides ovatus*, increases in *Roseburia inulinivorans*, *Ruminococcus lactaris*, *Lactobacillus plantarum*, *Bacteroides uniformis*. Others: No difference in enterotypes, gene count, α diversity. The β diversity, showed a reduction at genus and species levels.	Some species (*Megasphaera micronuciformis* and *Prevotella multisaccharivorax*) were associated with the expression level of IgA, an immunoglobulin class present in the gut.
(54)	Overweight subjects randomized to a VG (84) or an O diet (84).	Parallel design, 16 weeks.	Increased *F. prausnitzii* in VG group; *B. fragilis* decreased in both groups, but less in VG; trend for an increase in Bacteroidetes in the VG group. The α-diversity unchanged in VG and increased in the control group.	Intake of total dietary fibre was 33 vs 23 g/d in V and O groups, respectively; lower caloric intake in the V vs O group (400 kcal less). Changes in microbial diversity correlated positively with changes in body weight (minus 5.9 kg in VG) and negatively with changes in predicted insulin sensitivity.
(55)	Subjects (BMI>25), with low initial intake of fruits & vegetables.	Parallel 8-week RCT; subjects consumed a MedD or maintained usual diet (control group); both diets are isocaloric.	When comparing the MedD group with the control groups: - During the 4 first weeks, but not after 8 weeks: the similarity of the microbiota was decreased and *Ruthenibacterium lactatiformans*, *Flavonifractor plautii*, *Parabacteroides merdae*, *S. thermophilus*, *Ruminococcus torques*, and *Ruminococcus gnavus* were significantly reduced. - *F. prausnitzii*, *Roseburia and Lachnospiraceae* were enriched at 4 and 8 weeks.	The switch towards a Mediterranean diet increased by two-fold the intake of dietary fibre. Gut microbiota composition was associated with the level of adherence to MedD.

Although this scientific update is dealing with dietary patterns and not with single foods or nutrients, it is interesting to briefly focus on soya-based foods (as an example of foods more specifically encountered in plant-based diets, especially in vegan ones) and on dietary fibre, a nutrient of outmost importance to support an effect of plant-based diets on the gut microbiota.

- In a randomised trial on 64 Americans with **overweight or obesity**, those who **consumed soya drinks saw an increase in the proportion of *Prevotella* and a decrease in *Roseburia*,** while **opposite changes** were seen in the group consuming **cow's milk**. Subjects receiving **soya drinks**, but not cow's milk, also exhibited a **decrease in the Firmicutes/Bacteroidetes ratio (56).**
- However, in a different cross-sectional observational study, the increase in *Prevotella* was not confirmed as subjects who had higher soya intakes had decreased *Prevotella* levels (57).
- In another study, an increase in the *Bifidobacterium* genus and individual *Bifidobacterium* species was observed after a 2-week intake of 100 ml of soya drink daily; however, the lack of a control group diminishes the strength of this finding (58).

The significant impact of dietary fibre on the composition of the human gut microbiota has been synthesized in a meta-analysis gathering 64 intervention studies, encompassing more than 2000 subjects who received either a placebo or dietary fibre for a duration varying from five days to three months in amounts ranging from 1.2 to 50 g/day (59).

In spite of the high heterogeneity in experimental conditions, the **dietary fibre interventions**, compared to placebo/low fibre conditions, resulted in **higher abundance of *Bifidobacterium* species and *Lactobacillus* species, as well as higher faecal butyrate concentrations, illustrating a favourable modification to the gut microbiota.**

Individual studies provided more detailed results, as did a small, but well-controlled trial, in which 19 healthy subjects were given a standard Western for three days (399 g/day carbohydrates, 28 g/d fibre), followed by two diets with reduced amounts of carbohydrates (164 and 24 g/day) and fibre (12 and 6 g/day) for four weeks, each in a cross-over design. Major groups of faecal bacteria were monitored, and no significant change was seen in the relative counts of the genus *Bacteroides* or the clostridial cluster XIVa, IX or IV. In contrast, the *Roseburia* species and *Eubacterium rectale* subgroup of cluster XIVa and *Bifidobacterium* species decreased as carbohydrate and fibre intakes decreased (60).

PLANT-BASED DIETARY PATTERNS AND MICROBIAL METABOLITES

Although both are closely linked, the metabolic capacities of the gut microbiota might be even more relevant to the host biology than its detailed bacterial composition.

Several studies have addressed these metabolic functionalities and have monitored the gut microbiota capabilities either at the genomic level or by assessment of the production of metabolites within the gut. Only microbial metabolites which have been studied in humans in relation to plant-based diets are mentioned below.

SHORT-CHAIN FATTY ACIDS FROM DIETARY FIBRE

Short-chain fatty acids (**SCFAs**) are major microbial metabolites, produced through **fermentation of dietary fibre** (61). Fermentable fibre reaches the colon where it is depolymerized thanks to a wide variety of bacterial enzymes which use these undigestible carbohydrates as a source of carbon and energy. The released sugars are fermented by a process which produces a range of metabolites, including lactate and SCFAs (acetate, propionate and butyrate). **A large variety of gut bacteria can be involved in this process**, as evidenced from in vitro investigations with cultured isolates in the laboratory, and in silico analyses of their genomes (18).

Their molecular properties (61) explain how SCFAs, may favourably affect numerous cellular and physiological processes, resulting in a **lower risk for disorders and diseases.**

For example:

- SCFAs **strengthen the intestinal epithelial layer**, which contributes to an efficient barrier that prevents passage of toxic and potential pro-inflammatory molecules into the submucosa and the systemic circulation (62). SCFAs also help maintaining a balanced gut immunity, enabling both a tolerance to commensals and an immunity to pathogenic bacteria (61).
- SCFAs have been associated with **metabolic benefits and leanness**. Preclinical and clinical studies have shown that SCFA act on peripheral neurons, such as on the gut-brain neural axis responsible for an improved energy metabolism. These studies have also shown that SCFAs may affect thermogenesis and energy expenditure, as well as the level of enteroendocrine hormones, with possible consequence on appetite and glucose homeostasis (61).
- SCFAs may **induce colonic motility and decrease visceral pain**. Animal and *in vitro* studies have shown that butyrate significantly increased the proportion of choline-responsive neurons in the muscle layer that surrounds the gut and allow for bowel movements (63, 64).
- SCFAs have **antiproliferative properties** which may support a **favourable role in colon cancer**. *In vitro* studies have shown that in abnormal or pre-cancerous colon cells, butyrate selectively inhibits cell proliferation and migration and induces apoptosis (65).

PLANT-BASED DIETS AND PRODUCTION OF SCFAS

Overall, consumption of plant-based diets is most often associated with increased levels of faecal SCFA [Tables 1 to 3], independent of the clinical studies' design [37, 38, 49, 50]. For example:

- When comparing baseline diets between rural Africans and African Americans, or when monitoring the effect of a switch from a Western to a traditional plant-based diet [50], **higher levels of butyrate** in faeces and in colonic evacuates from colonoscopies have been observed in subjects who had habitual consumption of, or who had recently switched to, a plant-based diet.
- When the faecal concentration of SCFA was monitored in relation to the adherence to **Mediterranean diet** in 120 Greek adults, those who had the **higher adherence** also had the **highest amounts of total SCFA** [66].
- This observation has been reproduced in an Italian population, where those with the highest score for Mediterranean diet had the highest level of faecal SCFA, irrespective of whether their basic diet was vegan, vegetarian or omnivorous [42].

This is consistent with the **expected higher content of dietary fibre in plant-based diets**. In one of the above-described studies, where the effects of different amounts of total carbohydrates and non-starch polysaccharides on the gut microbiota of 19 healthy subjects were compared, the concentrations of total faecal SCFAs were 114 mM, 74 mM, and 56 mM ($P < 0.001$) for diets that provided, respectively, 28, 12 and 6 g non-starch polysaccharides/day. With the decrease of fibre intake, there was also a disproportionate reduction in faecal butyrate [18 mM, 9 mM, and 4 mM, respectively]. The abundance of butyrate-producing bacteria related to *Roseburia* species and *E. rectale* correlated well with the amount of faecal butyrate [60].

However, in other studies, the increase in SCFA with higher plant-based intakes was not seen. For example, in a 10-day controlled feeding experiment, a higher consumption of fermentable substrate in vegans was not associated with higher levels of faecal SCFA vs omnivores [43]; a similar observation has been made following an 8-week, Mediterranean diet, without caloric restriction, in women with obesity [55].

The most often encountered explanation for such discrepancy is that faeces may not be the ideal material to determine SCFA, which are produced and absorbed in high amounts in the colon [55], making faeces a poor indicator of SCFA production in the gut. This is in line with multiple observations that plant-based, fibre-rich diets are promoting gut bacterial species known to be producers of SCFAs, even in studies which did not find increased faecal SCFA levels [48, 67–69]. **However, some authors also suggest that individuals residing in Westernized communities are constrained by a more 'restrictive' gut microbiota with a limited ability to engage in energy harvest via fermentation of indigestible carbohydrates** [43]. This is in agreement with the hypothesis of the **disappearance of gut microbial diversity due to Westernization of diets and lifestyle** [70], which would render human hosts unable to fully adapt to an increase in undigestible carbohydrates.

MICROBIAL METABOLISM OF PLANT SECONDARY METABOLITES

Phenolic acids and polyphenols are plant secondary metabolites, belonging to several chemical classes, including anthocyanins, procyanidins, flavanones, flavanols, tannins and isoflavones. They are synthesized by plants to exert a wide range of effects on living organisms, including the plant itself, to make it competitive in its own environment (71).

Current thoughts highlight a “bidirectional” relationship between polyphenols and the gut microbiota, as **phenolics may affect the composition of gut microbiota** (some polyphenols being sometimes qualified as “candidate prebiotics”), while many gut bacteria have the capability of transforming polyphenols into simple phenolic compounds that can be absorbed by colonic cells and **exert anti-inflammatory and signalling effects at local and systemic levels** (72–74).

In clinical studies that compare plant-based and omnivorous diets, it is difficult to ascertain that the observed effect is due to a specific component such as polyphenols. **The main polyphenol dietary sources are fruits, fruit juice, wine, tea, coffee, chocolate and beer, and to a lesser extent vegetables, legumes and cereals** (71). Therefore, omnivorous and even Western-type diets may contain significant polyphenol amounts from these sources.

Several human intervention studies with various polyphenols have shown that polyphenol intake may **enhance the abundance of potentially beneficial bacterial taxa**. For example, intake of high levels of cocoa polyphenols or red wine extracts stimulated levels of *Bifidobacterium* and *Lactobacillus* (75), with red wine polyphenols also increasing numbers of *F. prausnitzii*, and *Roseburia*, and decreasing abundance of the less desirable *E. coli* and *Enterobacter cloacae* (76).

There is some **direct evidence that dietary polyphenols may affect gut health in humans**. For example, following an 8-week long trial providing 1,391 mg polyphenols/day (polyphenol-rich diet) versus 812 mg polyphenols/day (control diet), an increase in fibre-fermenting and butyrate-producing bacteria such as the family *Ruminococcaceae* and members of the genus *Faecalibacterium* was observed together with an improvement in a marker of **intestinal permeability** and a reduction in **diastolic blood pressure** (77). The intervention consisted of 3 daily servings of specific polyphenol-rich foods (such as berries, chocolate, green tea, pomegranate) which likely also contained other active ingredients. For example, dietary fibre and polyphenols often co-exist in plant-based foods and their molecular interactions can affect their relative bioaccessibility and bioavailability (78).

A higher occurrence of polyphenol metabolites has been observed following plant-based diets: Spanish middle-aged subjects with a higher (vs a lower) adherence to a Mediterranean diet had greater faecal concentrations of benzoic and 3-hydroxyphenylacetic acids, positively correlated with the intake of the principal classes and subclasses of polyphenols and fibre, and higher levels of *Clostridium* cluster XIVa and *F. prausnitzii* (45). In another study, subjects who received a Mediterranean diet for 8 weeks excreted more urolithins than those who consumed their usual diet. **Urolithins,** which have been shown to **improve intestinal barrier function** in a preclinical model, are gut microbial metabolites of ellagitannins, polyphenols found in berries, pomegranate and walnuts (55). These findings are in good agreement with dietary specificities of the Mediterranean diet, which incorporates many polyphenol-rich foods, such as red wine, fruits and vegetables.

Interactions between polyphenols and gut microbiota are of specific relevance when considering soya foods, often part of plant-based diets. **Soya** has been suggested to have **beneficial effects on health such as on menopause symptoms, cardiovascular diseases and cancer.** The naturally present plant compounds **daidzein** and **genistein**, both polyphenols (isoflavones), could play a role. Equol is a metabolite produced from daidzein by a relatively limited number of gut bacteria, most of them belonging to the family *Coriobacteriaceae* [79]. While **only 30% of adults in Western populations are able to produce equol from daidzein, nearly 60%–70% of residents in Asia are equol producers**, a variability with unknown reasons so far, although different microbial composition may be a relevant explanation. However, equol production appears as a stable trait over time, which seems rather insensitive to dietary changes: in long-term American vegans with a regular soya consumption, only 40% had detectable equol in plasma, suggesting 60% of them lacked equol-producing bacterial species [43].

MICROBIAL METABOLITES DERIVED FROM PROTEIN

Protein catabolism by gut bacteria yields a great diversity of end products, including amines, phenols, indoles and thiols, which are often seen as being **potentially deleterious to health** [80]. It should be noted that little protein reaches the colon, as most protein is broken down and absorbed earlier in the gastrointestinal tract. However, dietary fibre plays an indirect, but important role on protein microbial fermentation. Indeed, when large amounts of fibre are readily available in the colon, protein fermentation is very low in the proximal parts of the colon, because bacteria preferentially metabolise fibre. Protein fermentation will thus be limited to the distal part of the colon, where the slow transit time also facilitates proteolysis. Nevertheless, **when dietary fibre is limited due to insufficient intakes, proteolysis may begin earlier in the colon and this may result in a higher accumulation of protein-derived end products** [81].

Therefore, the content of proteins in a diet may not be of primary importance – within reasonable ranges – when the amount of accompanying dietary fibre is sufficient [82].

Another study pointed out that animal and plant proteins, which differ in their amino acid composition, may provide the gut microbiota with different amounts of individual amino acids as substrates, thus potentially resulting in different faecal bacterial metabolite compositions. However, this theory has not been deeply explored [82].

SHORT-CHAIN BRANCHED-CHAIN FATTY ACIDS

Although it also produces SCFA [83], protein fermentation mostly favours short-chain branched-chain fatty acids (BCFAs) such as isobutyrate, 2-methylbutyrate, and isovalerate, exclusively originating from branched-chain amino acids valine, isoleucine, and leucine. BCFA and the products of protein fermentation are not well characterized and are generally considered deleterious to the gut, although this has been poorly studied [84]. Even though few studies have investigated how BCFAs were affected by the type of diet, lower levels of faecal BCFA have been found after a Mediterranean diet intervention, in a way that correlated with an increased intake of plant-based foods [55].

PHENOLIC COMPOUNDS

Tryptophan is an essential aromatic amino acid present in all protein sources and the unique precursor for the neurotransmitter serotonin. Its catabolism in the gut by many species of *Bacteroides* and *Enterobacteriaceae* can produce **tryptamine**, a neurotransmitter that **plays a role in regulating intestinal motility and immune function** [84], as well as a large range of indole-derivatives, with multiple actions. Indoles are signalling molecules for enteroendocrine cells and for bacteria, and they can inhibit the colonization capabilities of pathogens such as *Salmonella enterica*. However, **indole overproduction can increase its export to the liver**, where it is metabolized to indoxyl sulphate, a uremic toxin **associated with chronic kidney disease** [84, 85].

No data are available in humans about the interaction between plant-based diets and production of tryptamine, but some information exists regarding indole derivatives, with results that depend on the indole derivative considered. In a study comparing subjects following a vegan, vegetarian or omnivorous diet, 3-methyl indole faecal amounts were lower in vegans and vegetarians compared to omnivores or those with a lower adherence to a Mediterranean diet [42]. On the other hand, a small (n=10) intervention trial found that **4 days of a Mediterranean diet** was able to **increase** the amount of acid 3-derivatives of indole-3, thought to have **anti-inflammatory and neuroprotective properties, while other indole derivatives were decreased** [86].

The phenolic compound **p-cresol** is produced by microbial catabolism of the aromatic amino-acid tyrosine; its production can be strongly inhibited by the presence of fermentable carbohydrates [81]. p-cresol may cause **DNA damage and alter the cell cycle, and it has been identified as a genotoxic agent toward colonocytes, making it a potential enhancer of colon cancer** [81, 87].

Predictive metabolite profiling (i.e., based on genes rather than on metabolites) has suggested that a **12-month Mediterranean diet** intervention in elderly people was associated with a **lower production of p-cresol** when compared with people remaining on their usual omnivorous diet [88]. In a study comparing subjects following a vegan, vegetarian or omnivorous diet, each with different levels of adherence to a Mediterranean diet, higher content of phenolic derivatives, produced by colonic deamination of aromatic amino acids, was found in omnivorous subjects and in those with low adherence to the Mediterranean diet [42].

TRIMETHYLAMINE

Trimethylamine (TMA) is a proatherogenic molecule that can be produced by the gut microbiota through metabolism of choline, phosphatidylcholine, and L-carnitine (present in animal foods, especially in meat, eggs and fish); it is further oxidized in the liver into trimethylamine oxide (TMAO), which is associated with the **development of cardio-vascular diseases**, although its relevance as a new non-traditional risk factor for cardio-vascular diseases is still debated [89]. TMAO measured in serum or in urine is a reflection of TMA production in the gut.

Prevotella enterotype is prevalent in patients with high systemic TMAO levels. Of note, several genera and species, such as *Peptostreptococcaceae incertae sedis,* are significantly associated with an omnivorous diet and increased TMAO levels [89].

A recent sophisticated study using stable isotopes has addressed how L-carnitine was metabolized by American vegans, vegetarians and omnivores. Firstly, the role of the gut microbiota was ascertained: TMA production was abolished by a one-week antibiotic treatment. Secondly, it was shown that **omnivores generated significantly more TMAO than vegans/vegetarians** when given a single oral dose of labelled L-carnitine. Thirdly, a chronic (daily for 2 months) dietary L-carnitine supplementation enhanced gut microbiota–dependent generation of TMA. Interestingly TMA formation plateaued after one month of L-carnitine supplementation in omnivorous subjects, whereas it kept on increasing in vegan/vegetarians, suggesting that **changes in the gut microbiota that could enable the most efficient TMA formation took more time in vegans/vegetarians than in omnivores**, in whom dedicated species may have already been present in larger amounts at baseline. Finally it was shown that the transformation of L-carnitine into TMA involved several species, including *Escherichia fergusonii*, *Edwardsiella tarda*, *Proteus penneri*, and *Emergencia timonensis* (90). Using similar L-carnitine challenge tests, another study, carried out in China, also found that omnivores were more likely to be TMAO producers than vegetarians, but some long-term vegetarians could still demonstrate substantial TMAO production from oral L-carnitine by their gut microbiota (91).

Observational studies provide variable findings. Some studies found that levels of TMAO were lower in subjects following plant-based diets, such as in American vegans/vegetarians compared to omnivores (92), or in those with a higher adherence to a Mediterranean diet (93). A higher level of urinary TMAO was also found in Italians with lower adherence to the Mediterranean diet and associations were found between TMAO levels and a number of microbial genera that have been linked to the intake of animal protein (42).

However, no associations were found in other studies, such as in Canadian cardio-vascular patients advised to follow a Mediterranean diet who had similar levels of serum TMAO, whatever their level of adherence to the Mediterranean diet (94). No change in TMAO was found after 4 days of a Mediterranean diet as compared to a Western-type, fast-food based diet (86). In American healthy adults at risk of colon cancer, a 6-months intervention comparing Mediterranean diet to a Healthy-Eating-Diet did not result in different levels of plasma TMAO. However, the difference between both diets may have been too small, as the Healthy-Eating-Diet included 5 daily servings of fruits & vegetables and 3 daily servings of whole grains, and is thus likely high in dietary fibre (95).

MICROBIAL METABOLITES DERIVED FROM BILE ACIDS

Primary bile salts (cholic and chenodeoxycholic acids) are produced from cholesterol in liver cells and can be conjugated with glycine or taurine; they contribute to several important functions, including **regulation of cholesterol levels, intestinal fat absorption and bile flow. Secondary bile salts** (lithocholic acid, deoxycholic acid, etc.) are **produced from primary bile salts by the gut microbiota**, which can also deconjugate primary bile acids (96, 97).

Around twelve different secondary bile acids have been identified and most of them seem to have both a **physiological role in a healthy gut but also pathophysiological properties**. On the positive side, they can exhibit antimicrobial activity and carry out endocrine functions. On the other hand, some of them have been associated with development of **colorectal cancer, reduced intestinal barrier function and increased inflammation** (98).

These secondary bile acids may also enter the systemic circulation, and have been implicated in **atherosclerosis, diabetes, and other cardiometabolic diseases** (85, 99).

Primary and secondary bile acids affect the composition and function of the gut microbiota. This mutual interplay contributes to shaping mucosal colonization resistance as well as local and distant immune responses. Therefore, **disrupted interactions of bile acids and gut bacteria are associated with many pathophysiological processes (98).**

BILE ACIDS AND PLANT-BASED DIETS

Dietary intakes may affect the faecal concentrations of bile acids, as suggested by the observation that faecal bile acids, in particular secondary and glycine- and taurine-conjugated ones, were significantly lower in long-time vegans than omnivores with at least three servings of meat per week. (100). Variance analysis showed that **intakes of fish, margarine, fried potatoes and processed meat** were associated with **higher level** of faecal **bile acids**, while **muesli intake was negatively correlated**.

This confirms the results of a former cross-over trial that compared a Western, a vegetarian and a vegan diet provided for 20 days to 12 young healthy subjects. When under a vegan or vegetarian diet, subjects had a lower level of total faecal bile acids when compared to omnivorous diet. Vegan values were lower than vegetarian ones, however, the difference did not reach statistical significance for total acids, except for deoxycholic and chenodeoxycholic acids (101).

Similar findings arose from two-week food exchanges in which African Americans and rural Africans went from a high-fat low-fibre Western-style diet to a high-fibre, low-fat African-style diet, and vice-versa (50). Before the intervention there was already a strikingly lower concentration of total bile acids in the faeces of rural Africans (5.5 µmol/g) vs African Americans (64.4 µmol/g). The intervention led to an increase of faecal bile acids in rural Africans (up to 9.5 µmol/g) and a decrease in African Americans (down to 40.7 µmol/g). When colonic evacuates obtained by coloscopy were analysed, the same changes were observed. Interestingly, the microbial genes that encode for the enzymes responsible for deconjugation of bile acids were more abundant in faeces from African Americans at baseline (38).

In a different study, a decrease in primary and secondary faecal bile acid levels occurred in subjects with excessive body weight after 4 weeks on a Mediterranean diet. Subjects showing the highest reduction in bile acids were the ones with the highest baseline levels of *Bilophila wadsworthia*, which is involved in bile acid metabolism. After the Mediterranean diet intervention, the levels of *B. wadsworthia* significantly decreased (55).

Some studies however failed to show changes in faecal bile acids levels, such as in a small (n=10) and very short term (4 days) trial (86) or following a one-year trial with a Mediterranean diet (102). However, in this last study, the low amount of fat and high amount of fibre in the comparator diet may have skewed the effect of the Mediterranean diet.

CONCLUSION

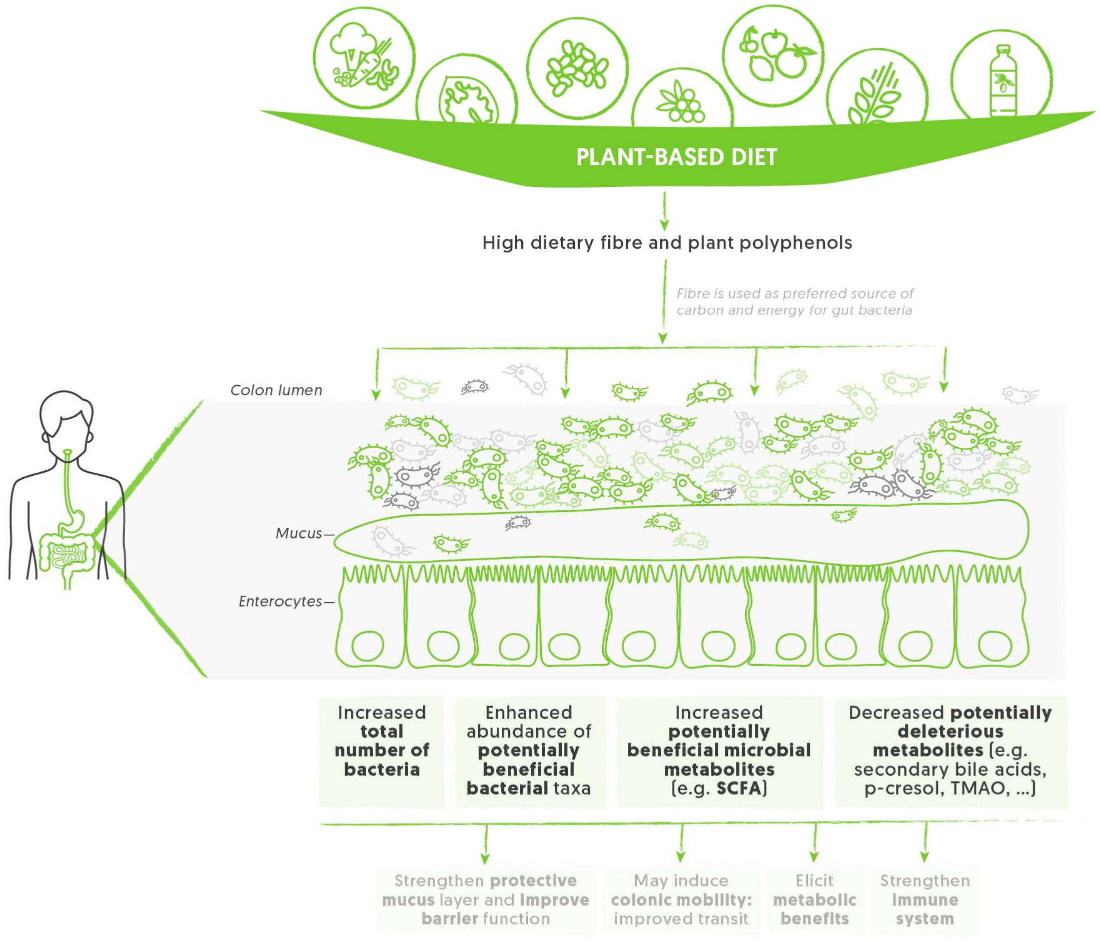


FIGURE 4. PLANT-BASED DIETS AND GUT MICROBIOTA: A FAVORABLE INTERPLAY.

Abbreviations: SCFA, short-chain fatty acid; TMAO, trimethylamine oxide.

Observational studies comparing omnivores and vegetarians/vegans, or subjects with various levels of adherence to a Mediterranean diet, indicate that **consumption of plant-based diets is associated with differences in composition of gut microbiota.**

Data from controlled intervention trials with various plant-based diets, in healthy subjects and those with metabolic dysfunctions, similarly showed changes in microbiota composition, **even after short intervention periods**.

There is, however, little consistency in the specific types of bacteria involved. This is quite likely, in part, due to the variability in composition or characterization of plant-based diets used, and also, due to methodological differences in assessing microbiota, as well as the large variation in microbiota composition between individuals.

Perhaps the only exception to this lack of consistency is that many studies indicate an **increase in abundance** of at least some **bacteria able to produce the SCFA butyrate**, such as *Roseburia* and *Faecalibacterium,* **in subjects consuming plant-based diets** (Figure 4).

There is some consensus that the increased **fibre** intake associated with many plant-based diets is an important factor in the changes seen in microbiota composition and metabolism. Intervention studies with plant **polyphenols** also suggest that these plant-derived secondary metabolites can modify microbiota composition.

Although both are closely linked, **the metabolic capacities of the gut microbiota might be even more relevant to the host biology than its detailed bacterial composition**. Several studies have addressed these metabolic functionalities and have monitored the microbiota capabilities either at the genomic level or by assessment of the production of metabolites within the gut.

The major microbial metabolites of relevance for gut health are **SCFAs**, predominantly derived from fermentation of dietary fibre. SCFAs, especially butyrate, exert key roles, including **providing energy to colonic cells and regulating the integrity of the epithelial barrier**, and have also been associated with **metabolic benefits and energy expenditure**. In most, though not all, observational and clinical studies, consumption of plant-based diets is associated with increased levels of faecal SCFAs. As plant-based diets are likely to provide more dietary fibre than most omnivorous diets, this is probably a major reason for the observed higher faecal SCFA levels.

Correlation analyses often suggest that faecal metabolite changes observed after plant-based dietary intervention occur through changes in microbiota composition, most notably in higher butyrate levels and enrichment of butyrate-producing groups.

Studies exploring the impact of plant-based diets on microbial metabolites other than SCFA are much fewer in number. **However, on the basis of this limited evidence, plant-based diets seem to have overall favourable effects.** They are associated with **lower amounts of potentially deleterious metabolites** such as **secondary bile acids, indole and p-cresol, and the proatherogenic compound TMAO.** In addition, there is some evidence of **higher amounts of potentially beneficial metabolites of plant polyphenol derivatives**, such as urolithins, in subjects on predominantly plant-based diets.

In the future, there is a clear need for well-controlled human studies that pay attention to the characterization of the plant-based diets used and focus on functional aspects of the microbiota, for example, by metabolite identification or relevant microbial genes that enable more direct assessment of health consequences.

PRACTICAL TAKEAWAYS FOR NUTRITIONAL ADVICE

When it comes to which diet is best for the gut microbiota, this scientific update shows that we are still far from being able to provide definitive advice. However, **balanced diets** focused on a variety of plant-based foods have the potential to **positively modify gut microbiota composition and metabolism** through **increased intake of fibre and polyphenols and reduced intake of animal products.**

Some practical tips to achieve a healthy balanced diet:

- Go for a balanced plate with different plant-based foods, exploring a **variety** of fruits, vegetables, nuts, seeds, legumes, pulses, and wholegrains.
- Set a **fibre target** of at least 25 grams per day.
- Look for **specific swaps** in the diet:
  - ✓ Reduce the intake of red or processed meats.
  - ✓ Use more lentils, beans, legumes and (partly) replace meat in dishes, e.g. chili con carne, stews, pasta sauces.
  - ✓ Prefer wholegrain pasta, cereals, or bread rather than the refined versions, as much as you can.
  - ✓ Add extra grains to stews or soups, e.g. bulghur, pearl barley, or brown rice.
  - ✓ Include fruits, nuts, seeds, and wholegrains at breakfast and snacks.
  - ✓ Add extra vegetables to your meals.

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