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Comparative Observation of the Impact of Ultra-Processed Foods on Gut Microbiota

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***Abstract*—*Diet significantly influences energy metabolism and intestinal microbiota, closely linked to human health. The increased consumption of ultra-processed foods, high in calories, fat, and salt, has contributed to rising obesity rates and the prevalence of chronic diseases. This study explores the regulatory effects of processed foods on intestinal microbiota by comparing the impact of an ultra-processed diet with a whole-grain diet. A controlled experiment measured the fecal microbiota's number, species, size, and color under each diet. The results confirm that ultra-processed foods lead to notable alterations in gut microbiota composition and metabolism, reinforcing the potential health risks. However, the study also highlights the potential benefits of a whole-food diet in promoting healthier gut microbiota, offering a hopeful solution to the health risks posed by ultra-processed foods. These findings could inform public health policy and inspire optimism about the potential of whole foods to improve health*.

***Keywords*—** *Ultra-processed Foods (UPF), Gut Microbiota, Health*.

I. INTRODUCTION

Among the several existing food and beverage classification systems, the NOVA classification is currently the most widely used in nutritional investigations and etiological studies (Monteiro CA et al., 2016). According to the degree of food processing, NOVA classifies foods into four categories: "unprocessed or minimally processed foods," "processed culinary ingredients," "processed foods," and "ultra-processed foods (UPF)" (Monteiro CA et al., 2016). As economic living standards improve, the abundance of food types and the emergence of ultra-processed foods (UPF) have increasingly changed human eating habits. This shift has led to a high incidence of non-communicable chronic diseases, such as diabetes, hypertension, and hyperlipidemia, attracting attention to dietary patterns and nutritional recommendations that are closely related to health. It has become crucial to identify dietary strategies that benefit the body (Armet et al., 2022).

Generally, a healthy diet ensures adequate nutrient intake, while some foods also contain natural bioactive ingredients that offer antioxidant, anti-inflammatory, and anti-aging benefits, which promote overall health (Tang & Tsao, 2017). Unhealthy eating habits, on the other hand, increase the risk of chronic diseases such as diabetes and cardiovascular conditions. Numerous studies have revealed that high-fat diets can induce oxidative stress and inflammation in the body, potentially leading to cognitive impairment (Chareonrungrueangchai et al., 2020; Tan & Norhaizan, 2019). Globally, the consumption of UPF has exceeded that of healthy foods (Baker et al., 2020). UPFs contain additives, such as hydrogenated oils and modified starches, to improve taste or mask low-quality raw materials (Monteiro et al., 2019). UPFs are generally high in calories, sugars, and fats while lacking dietary fiber (Pagliai et al., 2021), contributing to chronic conditions like type II diabetes (Pagliai et al., 2021). Additionally, UPFs have been linked to mental illness and gut microbiota imbalances. A recent study showed that increased consumption of UPF is associated with a higher risk of dementia while replacing UPFs with minimally processed foods can significantly reduce this risk (Li et al., 2022).

The gut microbiota plays an essential role in the body's physiological and metabolic processes, including immune regulation, metabolism, and digestion (Fung et al., 2017). In recent years, many studies have examined the role of gut microbes in governing metabolic disorders in humans (Wu et al., 2021). Researchers have highlighted the close connection between gut microbes and their human hosts from a genomic perspective (Gilbert et al., 2018). Most gut microbes coexist in harmony with the intestinal environment for the host, although some are recognized as pathogens by the immune system. Gut microbiota is vital for metabolizing exogenous substances and drugs and maintaining the integrity of the intestinal barrier (Jandhyala et al., 2015).

Laboratory-based pre-clinical research, epidemiological studies, and clinical trials suggest that UPFs impact human health by modifying gut microbiota composition and function (Srour et al., 2022). A study from 2006-2008 was one of the first to associate diet-induced changes in microbiota with host metabolism changes. The researchers transferred gut microbiota from obese mice to normal mice, increasing fat accumulation in the recipients (Turnbaugh, Peter J et al., 2008). UPFs have been shown to promote gut microbial changes that can lead to metabolic disorders, such as obesity and insulin resistance (Cani et al., 2007).

Moreover, studies indicate that UPFs influence the gut microbiota's secretion of virulence factors, enhancing inflammatory manifestations (Viennois, Emilie et al., 2020). Common food additives like artificial sweeteners, food dyes, and emulsifiers also affect gut microbiota. For example, a high-fructose diet has been shown to disrupt gut microbiota and reduce mucus layer thickness (Montrose, David C et al., 2021). Food colorants, such as Yellow 6 and Red 40, have been linked to colitis in animal models through microbial

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metabolism (He, Zhengxiang et al., 2021). Emulsifiers have been found to promote inflammation in the body by altering gut microbiota (Chassaing, Benoit, et al., 2022).

In summary, UPFs can disrupt the immune system's stability and metabolic balance, induce inflammatory and metabolic diseases, and ultimately affect human health. More importantly, this process is closely related to intestinal microbiota. Therefore, three controlled experiments were designed to compare the impacts of UPF foods on the population makeup and living status of gut microbiota and to provide suggestions for future research directions. However, studies explicitly focusing on the impact of UPF on gut microbiota composition and function remain limited, highlighting the urgent need for further investigation in this area. This study aims to contribute to this emerging field by examining the effects of UPF consumption on gut microbial populations and underscores the importance of continued research in this critical area.

II. Materials and Methods

Preparation of Experimental Materials

To investigate the impact of a UPF (ultra-processed food) diet and a whole-food diet on gut microbiota, bacterial culture and molecular methods were employed to characterize alterations in the gut microbiota. The experimental setup required agar plates (suitable for anaerobic bacteria), sterile swabs or inoculation loops, Petri dishes, and warm incubators set at approximately 37°C for bacterial growth. Additionally, sterile stool collection kits, pipettes, pH strips, sterile phosphate-buffered saline (PBS), and sterile water were used. Essential tools such as sterile gloves, labels, markers, and containers were prepared to maintain sterile conditions throughout the experiment and ensure accuracy during sample collection and analysis.

Study Design and Grouping

The participants were 16-year-old high school students recruited for the experiment. Each participant was randomly assigned to one of three dietary groups: baseline (control), ultra-processed food, and whole food. The study utilized a crossover design, with the same participants rotating through each dietary condition. Washout periods between each diet phase were included to eliminate carryover effects, allowing for unbiased comparisons between the diets. Each group consisted of 10 participants, and the study was conducted over three stages, with each stage lasting 48 hours to accommodate the dietary conditions.

Experimental Procedure

Baseline (Control) Group: Participants followed a neutral diet consisting of balanced meals without excessive processed foods. Fecal samples were collected after 48 hours and streaked onto labeled agar plates using sterile swabs or inoculation loops. The plates were incubated in a dark, controlled environment (37°C) for 48 to 72 hours. To ensure consistency, three plates were prepared per sample for colony counting, and the average colony count was calculated.

Ultra-Processed Food Group: After the baseline phase, participants consumed a diet of ultra-processed foods, including potato chips, soda, and instant noodles, for 48 hours. Fecal samples were then collected, streaked onto a new set of agar plates, and incubated under the same conditions. As with the baseline group, three plates were prepared per sample, and the average colony count was calculated.

Whole Food Group: Following a washout period, participants transitioned to a whole-food diet, including fresh fruits, vegetables, and whole grains, for 48 hours. Fecal samples were collected at the end of this period, streaked onto labeled agar plates, and incubated under the same conditions. Three plates were used for colony counting, and the average count was recorded for analysis.

Fecal Sample Collection and Inoculation

Fecal samples were collected under standardized conditions for all participants. Each participant collected a sample at the same time following their final meal in each dietary phase. The collected samples were placed in sterile containers to prevent contamination and maintain sample integrity.

A standard amount of fecal matter (approximately 1 gram) was used for each participant's sample to ensure consistency across all experimental groups. The samples were diluted in sterile PBS at a ratio of 1:10 (1 gram of fecal matter per 10 mL of PBS) to create a uniform suspension of bacterial cells. Each diluted sample was vortexed for 30 seconds to ensure an even distribution of bacteria. Then, 100 microliters of the diluted sample were inoculated onto each agar plate, with the solution spread evenly using a sterile inoculation loop to allow for uniform colony growth. This inoculation process was replicated consistently across all dietary groups to ensure accuracy in comparing bacterial growth.

Three agar plates per sample were prepared for colony counting, providing replication and improving the reliability of results. After inoculation, the plates were incubated at 37°C for 48-72 hours to allow bacterial colonies to grow.

Microbial Identification

In addition to colony morphology analysis, microbial species were identified using 16S ribosomal RNA (rRNA) gene sequencing. After incubation, DNA was extracted from the cultured bacterial colonies using a commercial DNA extraction kit. The 16S rRNA gene, a widely used marker for bacterial identification, was amplified using polymerase chain reaction (PCR). The resulting PCR products were sequenced, and the sequences were compared with known microbial databases to accurately identify the bacterial species present in the fecal samples. This approach allowed for a more detailed understanding of the microbial diversity and specific species composition under each dietary condition.

III. Results and Discussion

As indicated in Table 1, statistics were conducted on the bacterial culture plates from all three experiments. The control group exhibited an average of 70 colonies per plate, signifying a highly diverse and balanced intestinal microbiota.

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Additionally, the average colony size was 1.8 mm. Bacteroides and Faechubacterium prausnitzii were the dominant species, presenting various shapes and colors. In comparison, the colony count in the ultra-processed food group showed a remarkable increase, with an average of 150 colonies per plate, indicating the overgrowth of certain bacterial species. The average colony size was 3.5 mm, significantly larger than in the control group. The dominant species included E. coli, C. difficile, and Bacteroides, with colonies appearing dense and mostly uniform. The whole food group had an average of 80 colonies per plate, slightly higher than the control group but with similar diversity characteristics. The average colony size was 2.0 mm, comparable to the control group, and the dominant species were Bacteroides, Faechubacterium, and Bifidobacterium, with diverse colony shapes and colors.

TABLE 1. Comparison Table of Intestinal Microbiota Characteristics among Different Diet Groups.

Sample group	Average Number of Colonies per Plate	Average Colony Size (mm)	Dominant Bacterial Species	Colony Appearance
Baseline (Control)	70	1.8	Bacteroides spp., Faecalibacterium prausnitzii	Diverse in shape and color, indicating a healthy balance of gut microbiota.
UPF	150	3.5	Escherichia coli, Clostridium difficult, Bacteroides spp.	Large, dense, and primarily uniform in appearance, with fewer distinct colony types, suggesting reduced microbiota diversity.
Whole Foods	80	2.0	Bacteroides spp. Faecalibacterium prousnitzii, Bifidobacterium spp.	Medium-sized, with various shapes and colors, indicates a more balanced and diverse gut microbiota.

The results clearly illustrate the substantial impacts of diverse dietary patterns on intestinal microbiota. The control group exhibited a high degree of diversity in balanced intestinal microbiota. Bacteroides and Faecrovectelli were the dominant species in this group, and the colony shapes and colors were varied. This indicates that a relatively healthy intestinal environment is maintained by a variety of beneficial bacteria working together to sustain the normal physiological function of the intestinal tract. In line with previous research, the results of the UPF group are concerning. The significant increase in colony numbers, the high occurrence of potentially harmful E. coli and C. difficile, and dense and uniform colonies imply a deficiency in gut microbiota diversity. This phenomenon suggests that a highly processed diet may disrupt intestinal microbiota balance, increase the risk of intestinal unhealthiness, and subsequently affect the human body's digestion, immunity, and other functions.

In contrast, the whole food group presented more positive results. Parameters such as average colony number, colony size, and colony species were similar to those of the control group. The dominant bacteria included Bacteroides, Faectelli, and Bifidobacterium, indicating that a whole-food diet is beneficial in maintaining the diversity and stability of the intestinal microbiota and is advantageous for overall health.

The above results will offer valuable guidance for people's healthy living.

Due to objective limitations, this study has several drawbacks. For instance, the study sample size is relatively small, the study period is brief, and the analysis of micro microbiota indicators is simplistic. These factors may reduce the persuasiveness and universality of the results and hinder further promotion of the findings.

Therefore, future research will concentrate on addressing these shortcomings. The study sample size will be expanded to observe the longer-term trends of gut microbiota under different dietary patterns. Simultaneously, advanced biotechnologies such as genomics, molecular biology, and cytochemistry will be incorporated to comprehensively and deeply investigate the influence of diet on intestinal microbiota, which will facilitate the pursuit of a healthy life for humans.

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