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Firmicutes/Bacterioidetes Ratio as an Indicator of Intestinal Microflora

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Annotation: Today, advances in fundamental and clinical microbiology show that the correct formation of intestinal microflora from the earliest days of human development serves as the basis for subsequent stages of development. This article presents information about representatives of two main groups of intestinal microflora, their relationship, factors influencing them, as well as changes in the biological characteristics of representatives of this group in various pathological conditions of the body. A prospective target for the treatment or avoidance of inflammatory and metabolic diseases in humans is the gut flora. The identification of particular microbial signatures, particularly those linked to obesity, type 2 diabetes, and cardiovascular illnesses, is the focus of a large portion of current research efforts. According to certain research, the gut microbiota of obese humans and animals has a greater Firmicutes/Bacteroidetes ratio than that of people of normal weight, suggesting that this ratio may one day be used as a biomarker. As a result, a common indicator of obesity in the scientific literature is the Firmicutes/Bacteroidetes ratio. Given the large number of conflicting findings documented in the literature, the purpose of this review was to examine the validity of this putative marker. These disparities could be explained by the presence of interpretative bias brought about by methodological variations in DNA sequence analysis and sample processing, or by the generally subpar characterization of the subjects recruited and, more specifically, the failure to take lifestyle-related factors that are known to influence microbiota diversity and/or composition into account. Because of these factors, it is currently challenging to regard the Firmicutes/Bacteroidetes ratio as a sign of obesity and to link it to a specific health condition.

Key words: Intestine, microflora, Firmicutes, Bacterioidetes, biological properties, LPS

Relevance. A complex community of bacteria that live in the gastrointestinal system and have developed a tight symbiotic relationship with their human host is known as the gut microbiota. It is essential for maintaining health because it permits the synthesis of certain vitamins and the metabolism of indigestible food components, inhibits the colonization of pathogens, and aids in the development and training of the immune system. The two dominating bacterial phyla, *Firmicutes and Bacteroidetes,* which make up over 90% of the entire community, and several subdominant phyla, such as *Proteobacteria, Actinobacteria*, and *Verrucomicrobia,* make up the majority of the human gut microbiota. Because of its plasticity, which enables it to quickly revert to its original composition, this composition is largely unaffected by acute perturbations [1,2,3]. Nonetheless, it is constantly exposed to a number of stressors linked to contemporary lifestyles, such as drinking chlorinated water and consuming food additives and contaminants such heavy metals, pesticides, antibiotics, organic pollutants, and mycotoxins. These elements may alter its composition over time (dysbiosis), favoring more pathogenic microbes and having a negative impact on the host's health. Even though it is still unclear whether these changes are the cause or result of these disorders, gut dysbiosis is also linked to a number of pathologic conditions that affect the gastrointestinal tract (diarrhea, irritable bowel syndrome), the immune system (allergy, multiple sclerosis, type 1 diabetes, inflammatory bowel diseases, rheumatoid arthritis), the central nervous system (Alzheimer and Parkinson diseases, autism), and the host's energy metabolism (obesity, type 2 diabetes, atherosclerosis) [4,5,6]. The intestinal microbiota is a set of interconnected, commensal, symbiont, and pathogenic microorganisms inside the body [7]. Although the microbiota of every person in the world is determined by their genotype, a

number of changes may occur in later life due to factors such as diet, lifestyle, physical activity, and mental state [5]. Considering that the specific ratio of *Firmicutes to bacterioidetes* (or F/B ratio) in the intestinal microflora is an important indicator in determining the degree of risk of diseases and health status, the study of the mechanisms of their biological action is especially important.considering that the specific ratio of *Firmicutes to bacterioidetes* (or F/B ratio) in the intestinal microflora is an important indicator in determining the degree of risk of diseases and health status, the study of their mechanisms. Discussing the applicability of the *Firmicutes/Bacteroidetes* ratio as an indicator of obesity is the main goal of this paper. First, we will discuss the research that either supports or refutes the link between obesity and the *Firmicutes/Bacteroidetes* ratio [8,9,10]. We will then reveal the potential causes of these discrepancies, including variations in the techniques used to analyze the microbiota, the management of the interfering variables (diet, antibiotics, etc.), and potential biases in the subject recruitment procedure. Finally, in order to enable direct comparisons between their *Firmicutes/Bacteroidetes* ratio, we re-analyzed the 16S rRNA gene sequence data from nine published research. In light of the high level of gut microbiome variety shown in the healthy population, we will demonstrate that obesity is linked to a number of taxonomic markers [11,12,13]. The analysis of the literature devoted to the study of intestinal microflora using the search engines PubMed, GoogleScholar, cyberleninka. The results included publications in English, Russian, and Uzbek. There were no restrictions on the length of publication. The articles were selected based on the relevance of the answer to the research question under consideration. In addition, any article that the author considered relevant was added. the literature devoted to the study of intestinal microflora using a search area review.

This review's main aim is to talk about the Firmicutes/Bacteroidetes ratio's applicability as an indicator of obesity. The evidence that either supports or refutes the link between obesity and the Firmicutes/Bacteroidetes ratio will be discussed first. The potential causes of these discrepancies will then be revealed, including variations in the techniques used to analyze the microbiota, the management of the interfering variables (diet, antibiotics, etc.), and potential biases in the subject recruitment procedure.

The association between an elevated *firmicutes/bacterodites* ratio and obesity. Numerous factors, such as the host's genetic background, a decrease in physical activity, and excessive food consumption, contribute to the complex and multifaceted nature of obesity. The gut microbiota has been suggested as an additional component that promotes weight gain, insulin resistance, and fat storage in recent decades. In fact, by obtaining energy from food through fermentation and the production of short-chain fatty acids (SCFAs), the gut microbiota contributes to energy balance. Additionally, it promotes villous vascularization, which enhances food absorption and lowers ß-oxidation and AMPK levels in muscle tissue. Triglycerides are therefore stored in the liver and adipose tissue as a result of the microbiota's modulation of the release of fasting induced adipose factor (Fiaf), an inhibitor of lipoprotein lipase (LPL) activity [1-4]. Lastly, it affects the emergence of low-grade inflammation and metabolic endotoxemia. Additionally, by transferring the gut microbiota of typical obese mice to normal-weight germ-free animals, it was demonstrated that the obesity phenotype in mice is transmissible. The identification of bacterial taxa implicated in the development of obesity was the subsequent focus of scientific efforts. Changes that impact the dominant phylum. The initial reports of *Firmicutes* and *Bacteroidetes* were made in obese animals and subjects that had higher *Firmicutes abundances* at the expense of *Bacteroidetes*. In tandem with weight loss, these participants exhibited an increase in Bacteroidetes abundance and a normalization of their *Firmicutes/Bacteroidetes* ratio after a year of calorie restriction. Studies in animals given diets high in fat or fiber demonstrated greater abundances of *Firmicutes and Bacteroidetes*, respectively, supporting these findings [5-9]. Children from rural African communities who ate a traditional, high-fiber diet had higher proportions of *Bacteroidetes* and lower proportions of *Firmicutes* than children from western nations whose diets were high in protein, fat, sugar, and starch. These findings imply that changes in the metabolic profile of the microbiota, which also affect host health, are typically linked to changes in the variety and composition of bacteria.

Consequently, the *Firmicutes/Bacteroidetes* ratio has been widely regarded as a potential indicator of obesity within the past ten years [10,11,12].

Debates Regarding Obesity's Modified Firmicutes/Bacteroidetes Ratio. Nevertheless, some investigations contradicted these findings by finding no changes in this parameter or even a lower *Firmicutes/Bacteroidetes* ratio in obese humans and animals. The presence of other compositional changes at the family, genus, or species level, which may be more significant than the *Firmicutes/Bacteroidetes* ratio, is suggested by the fact that, in the majority of the studies, the obese patients displayed less bacterial diversity than the lean ones. Regarding this, the metabolic endotoxemia hypothesis suggests that long-term exposure to lipopolysaccharide (LPS), a pro-inflammatory molecule produced by Gram-negative bacteria, may be the cause of increased adiposity and the emergence of systemic inflammation. LPS would enter the bloodstream from the gut lumen [1,3,4,5]. Given that *Bacteroidetes* is the predominant phylum of Gram-negative bacteria in the gut microbiota, this theory is incompatible with the lower abundance of these bacteria that has been linked to obesity. The endotoxic activity of LPS from bacteria in the *Bacteroidetes phylum* is thought to be lower than that of other Gram-negative bacteria, such as those in the Proteobacteria phylum, which may account for this disparity. It's interesting to note that Proteobacteria have also been found to rise in obese people or animals, and that giving *Enterobacter*, a *phylum Proteobacteria*, to germ-free mice causes the animals to become obese and insulin resistant. One possibility is that the intestinal lumen produces less butyrate when the butyrate-producing bacteria in fat people decline and are gradually replaced by other bacteria from the same phylum [7,8,11]. For instance, it has been observed that obese individuals have higher levels of the *phylum Firmicutes bacteria Staphylococcus spp.* and *Lactobacillus reuteri*, which are positively connected with calorie intake and plasma C-reactive protein (CRP), respectively. Conversely, the degree of low-grade inflammation in obese individuals and patients with type 2 diabetes was adversely connected with the reduced abundance of the butyrate-producing *Faecalibacterium prausnitzii (Firmicutes phylum). A. muciniphila (Verrucomicrobia phylum),* a mucin-degrading bacterium that helps stabilize the function of the intestinal barrier, secrete antimicrobial peptides, and regulate inflammation, was similarly shown to be less abundant in obese individuals [2,6,9,10].

Why There Are Differences in Research on the Gut Microbiota and Obesity. The Methodological Differences Among Research. Differences in sample processing and data analysis, such as the DNA extraction technique, the primer selection for the amplified 16S rRNA region, the sequencing technique, and the bioinformatic analysis (taxonomy database and taxonomy assignment algorithm used), may also account for the previously reported inconsistent results. Therefore, it is difficult to remove the bias caused by PCR amplification artifacts, primer design, library preparation, and DNA separation techniques, which can lead to the over- or under-representation of specific species within complex communities. Additionally, the identification of bacterial communities may be impacted by sample storage [7-11]. The platform used to sequence the 16S rRNA amplicons is another crucial element. There are currently a number of platforms (Ion Torrent PGM, Illumina MiSeq, Illumina HiSeq, and Roche GS FLX+) that employ various sequencing chemistries and have the potential to introduce internal biases, such as affecting the detection and abundance of microorganisms with high or low genomic GC content. Furthermore, it is impossible to completely rule out the possibility that the adapters and barcodes added to the sequencing primers will eventually have an influence that is unique to each platform. Therefore, when identifying bacterial taxa, technological factors might have a significant impact and obscure biological differences in the samples, particularly when the sample size is small. The findings of a meta-analysis employing amplicons from high-throughput sequencing studies in obese subjects demonstrate this issue by demonstrating that the composition of the gut microbiota clustered by study rather than by subject's BMI, indicating that the per-study effect was larger than the biological effect [1-6].

The Variability of the Populations' Gut Microbiomes. Authors used information on the microbiota composition from nine published studies conducted in seven countries (USA, UK, India, Pakistan, Chile, Argentina, and Colombia) with 728 healthy subjects in order to evaluate the significance of the

Firmicutes/Bacteroidetes ratio as a taxonomic signature of obesity. High-throughput 16S rRNA gene sequence data produced by the Illumina MiSeq Platform and corresponding to V3–V4 or V4 hypervariable areas were gathered from earlier investigations. All the reads were filtered using the DADA2 pipeline, aligned and trimmed to the same length (80 bp) using Mothur, and then taxonomically identified using the DADA2 pipeline based on the identification of exact sequence variants. This allowed for direct comparisons between sequences from various studies [11-15]. Since less than 0.02% of the reads were not assigned, we believed that an 80 bp sequence length was sufficient for examining the microbial communities at the Phylum level. As previously mentioned, we anticipated removing all bias produced by sequencing and bioinformatic tools by using a special pipeline using reads produced by the same sequencing platform. We then examined the ratio and relative abundance of Bacteroidetes and Firmicutes. Overall, the findings show that the abundance of Bacteroidetes in the gut microbiota of healthy people ranges from 0.6% to 86.6%, while that of Firmicutes varies from 11% to 95% [16-20]. Accordingly, we recently found that the fecal microbiota of young, healthy Chilean volunteers had a high variability of both Firmicutes and Bacteroidetes, ranging from 25% to 67% and 4% to 64%, respectively. This was in spite of strict inclusion criteria that included dietary intake, biomarkers of colonic and systemic inflammation (plasma IL-6 and high sensitivity C-reactive protein, respectively), and anthropometrical and biochemical markers. Once more, the primary cause of these differences in the healthy population is most likely dietary heterogeneity, which may ultimately make it challenging to identify certain microbial fingerprints [5-12].

Discussion. The primary conclusion of our research is that there is no relationship between the F/B ratio and BMI. The F/B ratio did not have any prognostic value for BMI in our study. While these findings are consistent with many other research, they are not consistent with the original Koliada et al. study, which indicated a substantial correlation between the F/B ratio and BMI. This conclusion has been replicated in multiple investigations. Additionally, when evaluated in conjunction with the F/B ratio, the multivariate analysis's findings demonstrated that greater age, male gender, and history of appendectomy were statistically significant independent predictors of excess body weight. Higher Firmicutes levels or F/B ratio values do not significantly correlate with BMI and, consequently, excess body weight, according to the data gathered for our study [1-5]. The findings imply that the F/B ratio is not a reliable indicator of excess body weight among younger, health-conscious people since it is not linked to higher BMI and obesity. The current study is constrained by the way it was designed and carried out. Since health-conscious individuals are more likely to reply and volunteer in this kind of study than those who are overweight or obese, the recruiting process was significantly skewed by a number of selection biases (volunteer bias, non-response bias, and undercoverage bias). The original study's authors advised performing small intestine sampling, but this was not possible due to technological limitations. Although the gut microbiota may play a role in the development of obesity, the evidence that suggests an association between obesity and changes in the Firmicutes/Bacteroidetes ratio is not compelling. Therefore, it is important to improve subject characterization and clearly identify co-variables that may affect microbiota composition and interfere with the interpretation of the results [6-11]. Additionally, the idea of a unique taxonomic signature associated with obesity seems compromised; instead of examining a taxonomical marker of obesity per se, studies related to the gut microbiome should focus on finding taxonomic markers for patient stratification. By directly manipulating patient microbiomes, microbiome patient stratification would improve the management of obesity by individualizing treatment decisions. In conclusion, persons from the same group exhibit significant variation in the relative abundance of the phyla *Firmicutes* and *Bacteroidetes.* The makeup of the microbiota in the gastrointestinal system is likely influenced by a variety of lifestyle factors, such as diet, physical activity, food additives and pollutants, antibiotic use, and more. This could account for the inconsistent findings between normal-weight and obese participants' microbiota, which makes it challenging to link the *Firmicutes/Bacteroidetes* ratio to a specific health condition [17-21].

Conclusions. Our review study's strength is that it is the first to examine the relationship between obesity and the F/B ratio as well as the composition of the gut microbiota in general. Our results may

support the idea that the F/B ratio is not a reliable taxonomic indicator of excess body weight and that it is not a reliable biomarker. Furthermore, it was based on an already-published observational study, the sample size of which was larger in the current investigation. By providing incentives like monetary reward, we intend to incorporate a more diverse population in future research, particularly with regard to BMI and lifestyle choices. Future studies should focus on the problems of misinformation. As previously stated, a larger sample size is necessary for high-quality results.

In conclusion, there is significant variation in the relative abundance of the phyla *Firmicutes* and *Bacteroidetes* among individuals belonging to the same group. This is most likely caused by a variety of lifestyle-related factors that affect the makeup of the microbiota in the gastrointestinal system, such as nutrition, physical activity, food additives and pollutants, antibiotic use, and more. It is challenging to link the *Firmicutes/Bacteroidetes* ratio to a specific health status, which may account for the inconsistent findings shown when comparing the microbiota of normal-weight and obese patients.

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