Different alterations in gut microbiota caused by combining with metformin liraglutide or pioglitazone in overweight individuals diagnosed with diabetes

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Abstract.
This study aimed to evaluate the effect of liraglutide, a GLP1-RA, in combination with metformin on the gut microbiota of individuals with type 2 diabetes. The study found that after 6 months of treatment, of individuals with type 2 diabetes specifically, the combination of liraglutide and metformin led to a decrease in the levels of Bacteroidetes and an increase in the levels of Firmicutes and Actinobacteria. This resulted in a reduction in the Firmicutes/Bacteroidetes ratio and an increase in the Bacteroides fragilis group/Faecalibacterium prausnitzii ratio. These findings suggest that liraglutide, combined with metformin, can significantly impact the composition of the gut microbiota in individuals with type 2 diabetes. Further research is needed to fully understand the metabolic implications of these changes and their potential consequences for treating diabetes.

Keywords:
liraglutide
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gut microbiota
Introduction. Diabetes mellitus (DM) is a medical condition that significantly contributes to the occurrence of disabilities, impairments, and fatalities among the population. According to the Ministry of Health of Ukraine's Center for Medical Statistics, there were officially registered 1,237,270 diabetes patients in Ukraine as of January 1, 2017, excluding certain regions under different authorities [1]. In recent times, there has been an increasing interest among scientists in studying the gut microbiota. However, there is still much to be explored in this field. While the effects of metformin on the gut microbiota have already been extensively studied [1], the influence of GLP1-RA, such as liraglutide, remains relatively uncharted. Given that liraglutide has known impacts on the intestine and stomach, we were motivated to investigate the potential changes in the gut microbiota accompanying this drug.

The primary objective of our study was to evaluate the levels of critical microbial species, including Firmicutes, Bacteroidetes, and Actinobacteria, along with the ratios of Firmicutes/Bacteroidetes and Bacteroides fragilis group/Faecalibacterium prausnitzii, in individuals with type 2 diabetes who were previously untreated and had not received any medication. Subsequently, we aimed to assess any alterations in these microbial profiles after a 6-month treatment period with liraglutide in combination with metformin.

By conducting this study, we aimed to contribute to the existing scientific knowledge regarding the effects of liraglutide on the gut microbiota, particularly in individuals with type 2 diabetes. This research would not only fill the existing gaps in our understanding of the relationship between liraglutide and the gut microbiota but also provide valuable insights into the potential impact of this medication on overall health and metabolic processes.

Materials and methods: We conducted a study involving 68 newly diagnosed diabetics. The study group consisted of 37 women and 31 men, all with an average age of 54±7.2. After an initial examination, the participants were divided into two groups and assigned different treatment plans.

The first group, comprising 28 individuals, was prescribed a combination of Liraglutide at a dose of 1.8 mg
and metformin at a daily dosage of 2000 mg. The second group, consisting of 29 patients, received a daily dose of pioglitazone 30 mg in combination with metformin 2000 mg.

Following a six-month stable treatment regimen, the patients underwent a follow-up examination. Several factors were analyzed during this assessment, including body mass index (BMI), fasting glucose levels, fasting insulin levels, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), and HbA1c levels. Additionally, stool samples were collected and analyzed using amplicon sequencing to study the gut microbiota composition.

Stool samples were collected from the participants and analyzed using amplicon sequencing. This method allowed us to examine and determine the gut microbiota composition in each individual. By analyzing the genetic material obtained from the stool samples, we identified and characterized the various microbial species present in the gut. This information provided valuable insights into how different treatment plans may influence the gut microbiota and their potential impact on diabetes management.

Stool samples were collected from each participant to assess the microbial profiles. The DNA from these samples was extracted, and specific genetic markers were targeted to quantify Firmicutes, Bacteroidetes, and Actinobacteria levels among the major gut bacteria species.

Additionally, we calculated the ratios of Firmicutes/Bacteroidetes and Bacteroides fragilis group/Faecalibacterium prausnitzii, as these ratios have been suggested to play a role in metabolic disorders, including diabetes.

Using advanced sequencing and bioinformatic techniques, we analyzed the microbial composition and ratios in each group, compared the results between the different treatment plans, and assessed whether any specific treatment significantly impacted the gut microbial profiles.

Results: The results suggest that the different treatment plans positively affected glycemic control, as evidenced by the significant decrease in HbA1c levels from 8.45±0.27 % to 6.91±0.21 %, P<0.05. HbA1c is a long-term marker of blood sugar control, and decreased levels indicate improved glycemic control.
Additionally, the study found that HOMA-IR, a measure of insulin resistance, improved significantly after treatment. After treatment, HOMA-IR changed from 6.76±0.48 to 3.53±0.21 (P<0.05). Insulin resistance is a major underlying factor in the development and progression of diabetes, and improving insulin sensitivity can help manage the condition better.

These findings highlight the effectiveness of the treatment plans in achieving normoglycemia and improving insulin resistance in individuals with diabetes. By targeting both glycemic control and insulin resistance, these treatment options can positively influence the long-term management of diabetes and its associated complications.

Before treatment, we examined all patients, and there were noticeable changes in the microbial community. The levels of Firmicutes were 33.2 ±1.37 %, suggesting a potentially altered metabolic function of the gut microbiota. On the other hand, the levels of Bacteroidetes are 45.16±1.11 %, indicating a possible shift in the diversity of the microbial community. This shift may have implications for the overall gut health and digestion. Additionally, the levels of Actinobacteria are 4.27±0.32 %, and other groups 17.37±2.41 %, suggesting a potential proliferation of bacterial groups, which may have specific functions within the gut ecosystem. The indexes of Firmicutes/Bacteroidetes ratio 0.73±0.06 and Bacteroides fragilis group/Faecalibacterium prausnitzii ratio 72±4.11 further support the notion of altered microbial composition and may have implications for metabolic processes within the gut.

After 6 months of administering pioglitazone in combination with metformin, we observed the following changes in bacterial populations: a decrease in Firmicutes to 24.42 ±1.1%, an increase in Bacteroidetes growth to 56.41±1.54%, no significant change in Actinobacteria levels at 4.02±0.3%, and a decrease in other groups to 14.81±1.18%. The Firmicutes/Bacteroidetes ratio was also found to be 0.43±0.03, much lower, while the Bacteroides fragilis group/Faecalibacterium prausnitzii ratio was 11±1.14.

The administration of pioglitazone in combination with metformin has several advantages based on the observed changes in bacterial populations. Decrease in Firmicutes: Firmicutes are a species of bacteria associated with obesity and
metabolic disorders. The reduction in Firmicutes levels suggests the treatment may help reduce these risks. Increase in Bacteroidetes: Bacteroidetes are a species of bacteria known to be associated with a healthy gut microbiota. Increased Bacteroidetes levels indicate a potential improvement in gut health and overall well-being. Maintained levels of Actinobacteria: Actinobacteria are a group of bacteria known for their beneficial effects on the gut. The fact that their levels remained relatively stable suggests that the treatment did not disrupt their presence, which is essential for a balanced gut microbiota [2,3]. Decrease in other groups: The decrease in other bacterial groups might suggest a reduction in potentially harmful or pathogenic bacteria, further promoting gut health. Lower Firmicutes/Bacteroidetes ratio: The Firmicutes/Bacteroidetes ratio is a valuable indicator of an individual's gut health. A lower ratio is often associated with a healthier gut microbiota. In this case, the decrease in Firmicutes and increase in Bacteroidetes resulted in a significantly lower ratio, indicating improved gut health. Higher Bacteroides fragilis group/Faecalibacterium prausnitzii ratio: The ratio between specific bacterial groups can provide insights into the gut ecosystem. In this case, the increase in the Bacteroides fragilis group/Faecalibacterium prausnitzii ratio suggests a shift towards a more favorable microbial balance [4,5,6].

After 6 months of administering liraglutide in combination with metformin, we observed significant shifts in the gut microbiota composition. Specifically, there was a notable increase in the abundance of Firmicutes, constituting approximately 47.35±4.1% of the total microbial population. In contrast, the levels of Bacteroidetes decreased to 39.13±2.54%. Another bacterial group, Actinobacteria, showed a significant increase to 10.07±2.1%. The abundance of other bacterial groups decreased to 3.12±0.89%. Significantly, the Firmicutes/Bacteroidetes ratio also changed, measuring 1.02±0.04 after the treatment period. Moreover, the ratio of the Bacteroides fragilis group to Faecalibacterium prausnitzii substantially increased, measuring 120±4.17.

The treatment in this group of patients resulted in changes that led to a more equitable distribution of the gut
microbiota, aligning with findings from previous research.

These findings align with previous research on the impact of liraglutide and metformin on gut microbiota composition. Studies have demonstrated that these medications can influence the relative abundance of different bacterial groups [7,8,9], with increases in Firmicutes and decreases in Bacteroidetes being commonly observed [2,3]. Overall, these changes in microbial composition after treatment suggest that the therapy impacted the gut microbiota and its ecological balance.

**Conclusion:** Based on the data obtained, it can be concluded that the combination treatment of liraglutide and metformin leads to a significant shift in the composition of the gut microbiota. These changes are consistent with previous research indicating that this treatment can influence the relative abundance of different bacterial groups, specifically an increase in Firmicutes and a decrease in Bacteroidetes [1,9]. These changes in the gut microbiota have been associated with metabolic and inflammatory processes. Such changes in microbial composition have been associated with alterations in metabolic and inflammatory processes [4,5].

Our study provides novel evidence that the combination of GLP1-RA Liraglutide and metformin can directly influence gut microbiota composition. It is hypothesized that the decrease in intestinal transit time associated with this treatment leads to a significant increase in the abundance of the Bacteroides fragilis group, leading to a substantial shift in the Bacteroides fragilis group/Faecalibacterium prausnitzii ratio. This finding highlights the potential impact of these medications on the gut microbiota and suggests potential therapeutic implications for metabolic health. Further research is needed to elucidate the underlying mechanisms and explore the clinical applications of gut microbiota targeting in managing metabolic disorders.

**References:**

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