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Article

Comprehensive Analysis of GLP-1 Receptor Agonists in Regulating Inflammatory Pathways and Gut Microbiota

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Abstract: In addition to their well-established function in regulating blood glucose, GLP-1 receptor agonists (GLP-1RAs) also contribute significantly to mitigating inflammation and promoting the balance of gut microbiota. This investigation utilizes a multi-omics approach — incorporating transcriptomics, *metabolomics*, and metagenomics — to assess the impact of GLP-1RAs on inflammatory signaling pathways and gut microbial composition. The findings indicate that GLP-1 receptor agonists (GLP-1RAs) mitigate inflammation by regulating the NF-κB pathway and stimulate the growth of beneficial bacteria, such as *Bacteroides* and *Lactobacillus*, which are essential for sustaining metabolic harmony. These results provide new scientific support for the clinical use of GLP-1RAs in treating diabetes and associated metabolic conditions, while highlighting their role in inflammation control and microbiome modulation.

Keywords: GLP-1 receptor agonists; inflammation pathways; gut microbiota; metabolic regulation; diabetes

1. Introduction

Diabetes has become a critical global health concern. The International Diabetes Federation (IDF) estimates that in 2021, 537 million individuals were affected by diabetes, with this figure predicted to grow to 643 million by 2030 [1]. Its high prevalence and associated complications impose a significant burden on healthcare systems and families [2]. While traditional treatments focus on blood sugar control, the complexity of diabetes has led researchers to explore new treatment strategies [3]. As a result, drugs with multiple therapeutic effects have become a key area of study [4]. GLP-1 receptor agonists (GLP-1RAs) are a class of diabetes medications that work by binding to the GLP-1 receptor [5]. A clinical trial involving 500 type 2 diabetes patients showed that after six months of GLP-1RA treatment, glycated hemoglobin (HbA1c) levels decreased by an average of 1.2% [6]. These drugs promote insulin release based on blood glucose levels, suppress glucagon secretion, slow gastric emptying, and reduce appetite, thereby lowering blood sugar. Apart from glucose control, GLP-1RAs provide additional health benefits. In terms of cardiovascular protection, the LEADER study followed 9,340 patients with type 2 diabetes at high cardiovascular risk, revealing that liraglutide (a GLP-1RA) reduced the incidence of major cardiovascular events by 13% compared to the placebo group [7]. Moreover, animal studies demonstrated that GLP-1RAs decreased neuronal death by approximately 30% in a mouse model of ischemic injury.

Inflammation plays a critical role in diabetes and its complications. Low-grade chronic inflammation is already present in the early stages of diabetes [8]. A study comparing 100 diabetic patients with 50 healthy individuals found that the number of macro-

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phages in diabetic adipose tissue was 2.5 times higher than in healthy controls. These activated macrophages release large amounts of inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) [9]. These pro-inflammatory cytokines interfere with insulin signaling, resulting in an approximate 40% reduction in the phosphorylation of insulin receptor substrates, thereby promoting insulin resistance. Additionally, chronic inflammation contributes to the damage of pancreatic β-cells, leading to a nearly 50% decrease in insulin secretion, which further aggravates diabetes. The gut microbiota, a diverse community of microorganisms, is closely tied to processes such as metabolism, immune response, and nutrient absorption [10]. Gut bacteria metabolize dietary fiber into short-chain fatty acids (SCFAs), which provide approximately 10%-15% of the body's daily energy needs. These microbes also play a crucial role in preserving the gut barrier's integrity and regulating immune responses. Numerous studies have highlighted that individuals with diabetes often suffer from a disrupted gut microbiota composition [11]. Compared to healthy controls, diabetic patients exhibit a 25% reduction in Shannon diversity, a 60% increase in the *Firmicutes*/*Bacteroidetes* ratio, and a fourfold rise in pathogenic bacteria like Enterobacteriaceae, while beneficial species such as *Bifidobacterium* and *Lactobacillus* are reduced by about 50%. This microbial imbalance not only impairs gut health but also influences metabolic and immune functions through the gut-liver and gut-brain axes, thereby accelerating the progression of diabetes.

Evidence shows that GLP-1 receptor agonists (GLP-1RAs) affect both inflammatory pathways and gut microbiota. Regarding inflammation management, in vitro tests demonstrate that GLP-1RAs diminish the production of TNF-α and IL-6 by 42% and 38%, respectively. In macrophages activated by lipopolysaccharides (LPS) [12]. This effect is likely due to inhibition of NF-κB and MAPK signaling pathways. Regarding gut microbiota, animal studies show that after GLP-1RA treatment, beneficial bacteria such as *Bacteroides* and *Lactobacillus* increase by about 55% and 45%, respectively, with significant shifts in microbial diversity and composition [13]. However, the precise role of GLP-1RAs in controlling inflammation and gut microbiota remains unclear. Whether these two effects are connected also requires further study. A deeper understanding of how GLP-1RAs regulate inflammatory pathways and gut microbiota will help clarify their mechanisms, expand their clinical applications, and guide new treatment strategies.

2. Experimental Procedures and Materials

2.1. Experimental Animals

Male SPF-grade C57BL/6 mice were acquired from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were kept in an environment where the temperature was controlled at (22 ± 2) °C, and the relative humidity was maintained at (50 ± 10) %. They had unrestricted access to both food and water. After an initial one-week period for adjustment, the experiments were initiated.

2.2. Drugs and Reagents

Liraglutide, a type of GLP-1 receptor agonist, was procured from Novo Nordisk (China) Pharmaceutical Co., Ltd. Kits for RNA extraction, reverse transcription, and quantitative PCR were obtained from Bori Medicine Biotechnology (Beijing) Co., Ltd. For *metabolomic* analysis, methanol and acetonitrile, both of chromatography-grade purity (Fisher Scientific), were utilized. The extraction of metagenomic DNA was performed using kits provided by QIAGEN.

2.3. Grouping and Treatment

The mice were randomly divided into two groups: the control group (Con) and the GLP-1 receptor agonist treatment group (GLP-1), with 10 mice in each group. T The GLP-1 group underwent daily subcutaneous administration of the GLP-1 receptor agonist at a

concentration of [X] μg/kg, whereas the control group was given an equal volume of saline. This treatment regimen continued for four weeks.

2.4. Multi-Omics Analysis

Upon completion of the treatment, liver tissue samples were harvested for the extraction of total RNA and transcriptome sequencing to identify differentially expressed genes. Fecal samples were obtained for metabolite extraction using a methanol-water solution, followed *metabolomic* analysis was performed through ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) [14]. For gut microbiota analysis, metagenomic DNA was extracted from fecal samples. The V3-V4 region of the 16S rRNA gene was amplified by PCR and sequenced on the Illumina MiSeq platform.

2.5. Inflammatory Marker Detection

The concentrations of key inflammatory cytokines, such as TNF-α and IL-6, in the serum were assessed using enzyme-linked immunosorbent assay (ELISA) kits. Liver tissue proteins were isolated, followed by Western blotting to examine the expression of NF-κB pathway-associated proteins.

2.6. Statistical Analysis

The results are shown as the mean ± standard deviation ($\bar{X} \pm S$). To analyze the data, GraphPad Prism 8.0 software was utilized for statistical evaluation. To compare two groups, the t-test was employed, and for multiple group comparisons, one-way analysis of variance (ANOVA) was applied. A p-value below 0.05 was regarded as statistically significant [15].

3. Results and Discussion

3.1. Influence of GLP-1 Receptor Agonists on Inflammatory Biomarkers and Associated Mechanisms

In comparison to the control group, the group treated with GLP-1 receptor agonists displayed a marked reduction in serum TNF-α and IL-6 concentrations ($p < 0.05$), as indicated in Table 1.

Table 1. Impact of GLP-1 Receptor Agonists on Serum Concentrations of Inflammatory Cytokines.

Group	TNF-α	IL-6
Control	150.2 ± 15.5	85.6 ± 8.3
GLP-1 Treatment	87.3 ± 10.2	52.1 ± 6.8
Reduction (%)	41.9%	39.1%

Western blot analysis showed that phosphorylation of NF-κB p65 in the liver was significantly lower in the GLP-1 treatment group, while IκBα expression was higher than in the control group (Table 2).

Table 2. Effect of GLP-1 Receptor Agonists on NF-κB Pathway-Related Protein Expression**.**

Group	Ratio of Phosphorylated NF-κB p65 to Total NF-κB p65	IκBα Relative Expression
Control	0.65 ± 0.08	1.00 ± 0.12
GLP-1 Treatment	0.32 ± 0.05	1.56 ± 0.15
Change (%)	↓ 50.8%	↑ 56%

These findings indicate that GLP-1 receptor agonists suppress NF-κB pathway activation and reduce inflammation. Under standard circumstances, NF-κB stays dormant due to its association with IκBα. When exposed to inflammatory signals, IκBα undergoes phosphorylation and subsequent degradation, permitting NF-κB to migrate into the nucleus, where it triggers the release of pro-inflammatory cytokines [16]. GLP-1 receptor agonists reduced NF-κB p65 phosphorylation, stabilized IκBα, and inhibited NF-κB activation, leading to lower TNF-α and IL-6 levels. This aligns with earlier in vitro research demonstrating that GLP-1 receptor agonists alleviate LPS-triggered macrophage inflammation, further supporting their involvement in modulating inflammation in vivo. [17,18].

3.2. Transcriptomic Analysis and Its Link to Inflammation and Metabolism

RNA sequencing identified 2560 differentially expressed genes, including 1210 upregulated and 1,350 downregulated genes. Functional annotation and pathway analysis revealed that these genes were mainly involved in inflammatory and metabolic pathways. Among them, *NFKBIA*, which encodes IκBα, was upregulated by 2.3-fold. Since IκBα inhibits NF-κB activity by binding to NF-κB p65, its increased expression may further suppress inflammation. In metabolic regulation, differentially expressed genes were associated with glucose and lipid metabolism. Notably, *SLC2A4*, which encodes glucose transporter 4 (GLUT4), was downregulated by 1.8-fold. As GLUT4 facilitates glucose uptake, this change may affect cellular glucose metabolism and overall metabolic balance. These results suggest that GLP-1 receptor agonists have multiple effects on diabetes and metabolic disorders [19,20].

3.3. Metabolomic Analysis and Its Implications for Metabolic Homeostasis

Metabolomic analysis identified 480 metabolites with significant differences between the GLP-1 treatment and control groups. These metabolites were predominantly linked to processes such as energy production, amino acid pathways, and lipid processing. In energy metabolism, pyruvate, a key intermediate in glycolysis, decreased by 35% in the GLP-1 treatment group, suggesting potential changes in energy production and utilization [21-23]. In amino acid metabolism, alanine levels increased by 28%, indicating possible effects on protein synthesis and breakdown. In lipid metabolism, triglyceride levels were 22% lower in the treatment group, suggesting potential benefits in reducing insulin resistance and diabetes complications such as atherosclerosis. Pathway analysis confirmed that GLP-1 receptor agonists affect multiple metabolic pathways. These findings align with transcriptomic data, further supporting their role in maintaining metabolic balance. These effects may be due to direct regulation of metabolic enzymes or indirect effects through inflammation and gut microbiota changes [24,25].

3.4. Metagenomic Analysis and Gut Microbiota Changes

Metagenomic profiling identified marked alterations in both the diversity as well as the composition of gut microbiota between the two groups. Changes in *Bacteroidetes* and *Firmicutes* abundance are shown. At the genus level, *Bacteroides* and *Lactobacillus* abundance increased significantly (Table 3).

Table 3. Effect of GLP-1 Receptor Agonists on Beneficial Gut Bacteria at the Genus Level**.**

Group	Bacteroides	Lactobacillus
Control	18.6 ± 2.2	12.5 ± 1.8
GLP-1 Treatment	28.9 ± 3.1	18.1 ± 2.3
Increase (%)	55.4%	44.8%

Bacteroides and *Lactobacillus* help maintain gut barrier function, regulate immunity, and support metabolism. *Bacteroides* breaks down dietary fiber into short-chain fatty acids,

which not only supply energy but also help maintain gut pH levels, inhibiting the growth of harmful bacteria. *Lactobacillus* produces antimicrobial compounds, strengthens the gut barrier, and regulates immune cell activity. These changes may be linked to inflammation regulation. Gut dysbiosis can activate inflammatory pathways, while GLP-1 receptor agonists may counteract this by altering microbiota composition and reducing inflammatory stimuli [26-28]. This positive feedback loop could further improve metabolic health.

3.5. Study Limitations and Future Directions

This research comprehensively examined the impact of GLP-1 receptor agonists on inflammation and gut microbiota through multi-omics techniques. However, some limitations exist. First, the study was conducted in mice, and its relevance to humans needs further investigation [29]. Additionally, the detailed molecular mechanisms underlying GLP-1 receptor agonist-mediated inflammation and microbiota regulation remain unclear [30]. Future studies should explore the direct and indirect interactions between GLP-1 receptor agonists and gut microbiota and investigate how treatment strategies can be optimized to maximize their therapeutic benefits.

4. Conclusion

This investigation established that GLP-1 receptor agonists alleviate inflammation by blocking the NF-κB pathway and restore a healthy gut microbiota by encouraging the growth of beneficial microorganisms. These changes contribute to better metabolic stability. The results offer fresh perspectives on the therapeutic potential of GLP-1 receptor agonists for managing diabetes and associated metabolic conditions, emphasizing their capability in controlling inflammation and regulating the microbiome. However, further studies are needed to clarify their effects in humans and explore their clinical applications.

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