SHORT REPORT

# Exenatide and Metformin Improve Serum Indices and Intestinal Flora in patients with Type 2 Diabetes Mellitus and Non-Alcoholic Fatty Liver Disease

Xiaojie Jiang[1], Tingting Shi[2], Dan Han[3], Juan Chen[4]

## Abstract

The aim of the study was to investigate the influence of Exenatide combined with Metformin on fasting blood glucose, postprandial glucose, triglycerides, total cholesterol, alanine aminotransferase, aspartate aminotransferase, and intestinal flora in type 2 diabetes mellitus cases with non-alcoholic fatty liver disease. A total of 128 type 2 diabetes mellitus patients with non-alcoholic fatty liver disease, diagnosed from January 2019 to January 2022, were included and randomly assigned to either Group A (n=64) or Group B (n=64). Group A received Metformin, while Group B received Exenatide injection and Metformin. After 24 weeks of treatment, blood glucose indices (fasting blood glucose and postprandial glucose), blood lipid indices (triglycerides and total cholesterol), liver function indices (alanine aminotransferase and aspartate aminotransferase) were all lower in Group B than in Group A ($p$<0.001 for all). Counts of *Escherichia coli* and *Enterococcus faecalis* were lower in Group B than in Group A (both $p$<0.05), counts of *Bifidobacteria* and *Lactobacillus* were higher in Group B than in Group A (both $p$<0.05). Combination of Exenatide and Metformin may have synergistic effects in improving metabolic and hepatic parameters, as well as regulating intestinal flora, which could provide a promising therapeutic option for the management of these patients.

**Keywords:** Type 2 diabetes mellitus; Non-alcoholic fatty liver disease; Metformin; Exenatide; Intestinal flora.

**DOI: https://doi.org/10.47391/JPMA.8295**

## Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that is characterised by elevated blood glucose levels over a prolonged period of time. T2DM may lead to multi-system damage. Non-alcoholic fatty liver disease (NAFLD) is a clinical condition in which there is an accumulation of fat in the liver cells, mainly in the lobule of the liver. It is not associated with a history of excessive alcohol consumption. T2DM is associated with NAFLD bidirectionally.[1] T2DM patients have a high prevalence of NAFLD. The combined occurrence of T2DM and NAFLD makes it difficult to control blood glucose and increases the risk of complications.[2]

The management of NAFLD should include treating metabolic complications, such as T2DM, in addition to liver disease.[3] The basic treatment of NAFLD is change of lifestyle, but it has little effect on reducing body mass. Therefore, drug therapy is a common treatment of NAFLD. Metformin has been observed to be effective in treating T2DM with NAFLD. However, its efficacy as a standalone treatment is limited.[4]

This important role between incretin and the pathological mechanism of T2DM has been widely recognised. Exenatide is an important antidiabetic drug of incretin analogues. It is a synthetic peptide product, which is homologous to glucagon like peptide-1 (GLP-1).[5] GLP-1 is a bioactive substance secreted by the gastrointestinal tract to improve blood glucose level when eating.

Under normal circumstances, intestinal flora maintains a balanced state with human body. It has been observed that disorders of intestinal flora can lead to metabolic diseases such as T2DM.[6] *Escherichia coli, Enterococcus faecalis, Bifidobacteria, Lactobacillus,* and other intestinal microbiota are closely related to lipid metabolism.[7] Lipid is the substrate of bacterial metabolism.[8] The abnormal lipid metabolism indices, such as total cholesterol (TC) and triacylglycerol (TG), directly affects the number and distribution of intestinal flora. Gastrointestinal micro-ecological changes caused by the disorders of the intestinal flora are also one of the adverse consequences of NAFLD.[9]

Some studies have shown that Exenatide combined with Metformin has a certain effect on patients with T2DM complicated with NAFLD.[10] However, only a few studies have reported the impact of Exenatide and Metformin combination therapy on intestinal flora in these patients.

The aim of this research was to explore the impact of

[1,2]Department of Pharmaceutical Preparation, The Hangzhou Xixi Hospital Affiliated to Zhejiang University School of Medicine, China; [3]Department of Pharmacy, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, China; [4]Department of Endocrine, The Hangzhou Xixi Hospital Affiliated to Zhejiang University School of Medicine, China.
**Correspondence:** Xiaojie Jiang. e-mail: 61432169@qq.com
ORCID ID. 0009-0006-9570-9561

**Submission complete:** 27-03-2023 **Review began:** 16-05-2023
**Acceptance:** 18-10-2023 **Review end:** 06-09-2023

Exenatide and Metformin combination therapy on various factors, such as fasting blood glucose (FBG), postprandial glucose (PPG), triglycerides (TG), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and intestinal flora, in patients diagnosed with both T2DM and NAFLD.

## Patients/Methods and Results

This is a prospective, randomised, double-blind trial study, and was approved by the medical ethics committee of the Hangzhou Xixi Hospital Affiliated to Zhejiang University School of Medicine, China. The G Power software[11] was utilised to determine the appropriate sample size, based on a significance level of 0.05 and a power of 80%. The total sample size was calculated as 128 cases, and the subjects were divided into two groups, with 64 cases in each group. So, 128 patients diagnosed with both T2DM and NAFLD between January 2019 and January 2022 were included.

Inclusion criteria were: cases who were diagnosed with T2DM and with glycosylated haemoglobin (GHbAlc) <8% according to the diagnostic criteria of diabetes mellitus of WHO in 1999,[12] patients who met the diagnostic criteria of NAFLD,[13] no alcohol history, or alcohol equivalent to ethanol <20g/d for men and less than 10g/d for women, no drugs affecting the research process like liver protection drugs or lipid-lowering drugs been used for nearly half a month. Patients and their families were fully informed about the research protocol and they provided written consent.

Exclusion criteria were: cases with chronic viral hepatitis and autoimmune liver disease; cases with severe cardiopulmonary dysfunction, hepatic dysfunction, or renal dysfunction; patients with contraindications or allergic reactions and severe adverse reactions to the drugs used in this study; cases with type 1 diabetes, and other types of diabetes; cases with tumours and severe infectious diseases; cases with a past history of mental illness or cognitive impairment; and cases with a history of heavy alcohol consumption.

The 128 patients with T2DM complicated with NAFLD were randomly assigned to either Group A (n=64) or Group B (n=64). The two groups received routine low-salt and low-fat diet restriction and exercise intervention.

Group A received Metformin as an initial dose of 500mg twice a day. Group B received additional 5μg Exenatide injection 60 minutes before breakfast and dinner via subcutaneous route based on Group A. Both groups received the treatment continuously for 24 weeks.

Before starting the treatment and after 24 weeks of the treatment, FBG, PPG, TG, TC, ALT and AST were measured and compared between the two groups. The 0.5g faecal samples were collected from the two groups to measure and compare the changes of intestinal flora indices on *Escherichia coli, Enterococcus faecalis, Bifidobacteria* and *Lactobacillus*.

The blood glucose indices of FBG and PPG were determined by glucose oxidase method. The blood lipid indices TG and TC as well as the liver function indices ALT and AST were measured by automatic biochemical analyser. *Escherichia coli, Enterococcus faecalis, Bifidobacteria* and *Lactobacillus* were identified and counted by plate count method.

SPSS 26.0 was used for data processing. Normal distribution of measurement data was assessed using Kolmogorov-Smirnov test. Normally distributed data were presented as mean±standard deviation, and was conducted via independent sample *t*-tests. Categorical data were presented as n (%), and used the chi-square test. Statistical significance was defined as a $p<0.05$.

Group A had 37 (57.81%) males and 27 (42.19%) females, aged 55.56±6.03 years, body mass index (BMI) was 20.39-34.07 kg/m$^2$ with an average of 25.41±2.32 kg/m$^2$.

Group B had 39 (60.94%) males and 25 (39.06%) females, aged 25.86±6.29 years, and BMI 20.33-35.05 kg/m$^2$ with an average of 25.44±2.47kg/m2. Sex, age and BMI between the two groups ($p$=0.719, 0.786, 0.954, respectively) had no difference, so they were comparable.

Before the treatment, no differences were observed between the two groups with regard to blood glucose indices (FBG and PPG), blood lipid indices (TG and TC), and liver function indices (ALT and AST) ($p$=0.886, 0.676, 0.130, 0.525, 0.632, 0.901, respectively, Table 1).

**Table-1:** Comparison of serum indexes.

| Study parameters | Time | Group A (n=64) Mean ±SD | Group B (n=64) Mean ±SD | *p*-value |
|---|---|---|---|---|
| FBG (mmol/L) | Before treatment | 142.74±12.96 | 143.10±13.86 | 0.886 |
| | After 24 weeks of treatment | 118.08±10.80 | 104.4±10.08 | <0.001 |
| PPG (mmol/L) | Before treatment | 223.56±20.34 | 225.18±21.78 | 0.676 |
| | After 24 weeks of treatment | 168.48±15.30 | 149.04±14.40 | <0.001 |
| TG (mmol/L) | Before treatment | 248.69±23.01 | 255.77±25.67 | 0.130 |
| | After 24 weeks of treatment | 203.55±18.59 | 154.88±15.93 | <0.001 |
| TC (mmol/L) | Before treatment | 226.64±19.69 | 229.73±23.17 | 0.525 |
| | After 24 weeks of treatment | 189.96±17.76 | 163.32±16.60 | <0.001 |
| ALT (U/L) | Before treatment | 59.36±5.41 | 59.84±5.89 | 0.632 |
| | After 24 weeks of treatment | 47.04±4.29 | 40.61±4.00 | <0.001 |
| AST (U/L) | Before treatment | 60.93±5.56 | 61.05±6.01 | 0.901 |
| | After 24 weeks of treatment | 50.27±4.58 | 42.84±4.16 | <0.001 |

FBG: fasting blood glucose, PPG: postprandial glucose, TG: triacylglycerol, TC: total cholesterol, ALT: alanine aminotransferase, AST: aspartate aminotransferase, SD: standard deviation.

**Table-2:** Comparison of serum indexes.

| Study parameters | Time | Group A (n=64) Mean ±SD | Group B (n=64) Mean ±SD | *p*-value |
|---|---|---|---|---|
| *Escherichia coli* | Before treatment | 11.73±1.07 | 11.81±1.14 | 0.662 |
| (lg CFU/g) | After 24 weeks of treatment | 9.82±0.89 | 8.95±0.87 | <0.001 |
| *Enterococcus faecalis* | Before treatment | 11.06±1.01 | 11.10±1.08 | 0.806 |
| (lg CFU/g) | After 24 weeks of treatment | 9.38±0.94 | 8.83±1.09 | 0.003 |
| *Bifidobacteria* | Before treatment | 7.23±0.80 | 7.17±0.86 | 0.679 |
| (lg CFU/g) | After 24 weeks of treatment | 8.50±0.95 | 9.14±1.10 | 0.001 |
| *Lactobacillus* | Before treatment | 7.36±0.82 | 7.31±0.84 | 0.762 |
| (lg CFU/g) | After 24 weeks of treatment | 8.75±0.97 | 9.14±1.07 | 0.033 |

SD: standard deviation.

After 24 weeks of treatment, the blood glucose indices (FBG and PPG), blood lipid indices (TG and TC), as well as liver function indices (ALT and AST) were all lower in Group B than in Group A ($p<0.001$ for all, (Table 1)

Before the treatment, there were no differences in counts of *Escherichia coli, Enterococcus faecalis, Bifidobacteria* and *Lactobacillus* between the two groups ($p=0.662$, 0.806, 0.679, 0.762, respectively, Table 2). After 24 weeks of treatment, counts of *Escherichia coli* and *Enterococcus faecalis* were lower in Group B than in Group A ($p<0.001$, $p=0.003$, respectively, Table 2), and counts of *Bifidobacteria* and *Lactobacillus* were higher in Group B than in Group A ($p=0.001$, $p=0.033$, respectively, Table 2).

## Conclusion

Combination of Exenatide and Metformin may have synergistic effects in improving metabolic and hepatic parameters, as well as regulating intestinal flora, which could provide a promising therapeutic option for the management of these patients.

The limitation of this research is that the number of cases was small, with a short clinical observation time.

**Disclaimer:** None.

**Conflict of Interest:** None.

**Funding Sources:** The development programme of the Hangzhou science and technology committee (NO. 20181228Y24).

## References

1. Wang M, Zhao Y, He Y, Zhang L, Liu J, Zheng S, et al. The bidirectional relationship between NAFLD and type 2 diabetes: A prospective population-based cohort study. Nutr Metab Cardiovasc Dis 2023;33:1521-8. doi: 10.1016/j.numecd.2023.05.018.
2. Lv WS, Sun RX, Gao YY, Wen JP, Pan RF, Li L, et al. Nonalcoholic fatty liver disease and microvascular complications in type 2 diabetes. World J Gastroenterol 2013;19:3134-42. doi: 10.3748/wjg.v19.i20.3134.
3. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67:328-57. doi: 10.1002/hep.29367.
4. Tian F, Zheng Z, Zhang D, He S, Shen J. Efficacy of liraglutide in treating type 2 diabetes mellitus complicated with non-alcoholic fatty liver disease. Biosci Rep 2018;38:BSR20181304. doi: 10.1042/BSR20181304.
5. Goodarzi G, Tehrani SS, Fana SE, Moradi-Sardareh H, Panahi G, Maniati M, et al. Crosstalk between Alzheimer's disease and diabetes: a focus on anti-diabetic drugs. Metab Brain Dis 2023;38:1769-800. doi: 10.1007/s11011-023-01225-3.
6. Ma Q, Li Y, Li P, Wang M, Wang J, Tang Z, et al. Research progress in the relationship between type 2 diabetes mellitus and intestinal flora. Biomed Pharmacother 2019;117:109138. doi: 10.1016/j.biopha.2019.109138.
7. He YJ, You CG. The Potential Role of Gut Microbiota in the Prevention and Treatment of Lipid Metabolism Disorders. Int J Endocrinol 2020;2020:e8601796. doi: 10.1155/2020/8601796.
8. Schoeler M, Caesar R. Dietary lipids, gut microbiota and lipid metabolism. Rev Endocr Metab Disord 2019;20:461-72. doi: 10.1007/s11154-019-09512-0.
9. Li F, Sun G, Wang Z, Wu W, Guo H, Peng L, et al. Characteristics of fecal microbiota in non-alcoholic fatty liver disease patients. Sci China Life Sci 2018;61:770-8. doi: 10.1007/s11427-017-9303-9.
10. Zhao R, Li Y, Dai Q, Qian K, Li H. Clinical effects of exenatide combined with metformin on liver in type 2 diabetes mellitus patients combined with non-alcoholic fatty liver disease. Drug Evaluat Res 2016;39:623-6.
11. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods 2009;41:1149-60. doi: 10.3758/BRM.41.4.1149.
12. World Health Organization (WHO). Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus. [Online] 1999 [Cited 2023 November 16]. Available from URL: https://iris.who.int/handle/10665/66040.
13. Lin S, Huang J, Wang M, Kumar R, Liu Y, Liu S, et al. Comparison of MAFLD and NAFLD diagnostic criteria in real world. Liver Int 2020;40:2082-9. doi: 10.1111/liv.14548.

**Author Contribution:**
XJ: Analysis, drafting, revision, final approval.
TS: Revision, drafting
DH: Revision, analysis, acquisition of data
JC: Acquisition of data, revising