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# ANALYSIS OF SHORT-CHAIN FATTY ACIDS AND ASSOCIATION WITH GLUCAGON-LIKE PEPTIDE-1 IN HEALTHY, OBESE, PREDIABETES AND TYPE 2 DIABETES INDIVIDUALS

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## Abstract

**Context.** Short-chain fatty acids (SCFAs) play a critical role in host metabolism, both directly and through specific G protein-coupled receptors, and may provide a link between glucose and lipid metabolism. This study aimed to compare fecal SCFA concentrations and to evaluate associations between SCFAs and glucagon-like peptide 1 (GLP-1) in individuals with type 2 diabetes (T2DM), prediabetes, obesity, and health.

**Subjects and Methods.** The study included 60 volunteers in four groups: T2DM (n= 15), prediabetes (n= 15), obesity (n= 15), and healthy individuals (n= 15). We analyzed fecal SCFA and GLP-1 concentrations by HPLC-UV and ELISA method, respectively.

**Results.** In comparison to healthy individuals, obese, prediabetic and T2DM exhibited notably elevated total SCFA levels respectively (p=0.019, p=0.017, p=0.024). Acetic acid concentration showed an increase in both obese and prediabetic groups compared to the healthy group (p=0.02, p=0.017). Butyric acid concentration was elevated in T2DM and prediabetic groups in comparison to both healthy and obese groups (p=0.024, p=0.017, p=0.07, p=0.56). The GLP-1 levels significantly decreased in the obese and prediabetic groups compared to the healthy and T2DM groups (p=0.00, p=0.021, p=0.000, p=0.005). GLP-1 was correlated with acetic acid, butyric acid, and the total SCFA concentrations in the T2DM and obese groups (r= 0.479, p= 0.098; r= 0.441, p= 0.099; r= 0.654, p= 0.015, respectively) while there was no correlation in the prediabetic group.

**Conclusion.** Our results extend the knowledge on the alteration of SCFA levels in the states of obesity, prediabetes, and T2DM and enrich the understanding of the relationship between SCFAs and GLP-1.

**Keywords:** fecal short-chain fatty acids, glucagon-like peptide 1, type 2 diabetes, prediabetes, obesity, microbiota.

## INTRODUCTION

Gut microbiota and their metabolites are increasingly acknowledged as crucial factors influencing health and disease status, including obesity and type 2 diabetes mellitus (T2DM) (1, 2). Short-chain fatty acids (SCFAs) are an important class of compounds produced by the gut microbiota through the fermentation of indigestible carbohydrates, such as dietary fiber in the colon (3). SCFAs, classified as organic linear carboxylic acids containing less than six carbons, primarily consist of acetic acid (C2), propionic acid (C3), and butyric acid (C4), collectively constituting 90% to 95% of the total SCFAs (4,5). Other fermentation byproducts like caproate and valerate are found in smaller quantities (6).

SCFAs are involved in various important molecular biological processes (7). Butyrate notably serves as the primary energy source for colonocytes by undergoing β-oxidation in the mitochondria, accounting for approximately 70% of their energy needs, supporting metabolic homeostasis, and preserving the integrity of the colonic epithelium (8). Furthermore, acetate, propionate, and butyrate have been implicated in the regulation of immunity, apoptosis, inflammation, and the control of glucose and lipid metabolism (9, 4).

Besides these effects, SCFAs are associated with two major signaling pathways, G-protein-coupled receptors (GPCRs) and histone deacetylases (HDACs) (6). SCFAs act as signaling molecules for and activate GPCRs known as GPR41 and GPR43, which are expressed in intestinal enteroendocrine L-cells (8, 5). The release of SCFA-related GLP-1 has several effects closely related to obesity and type 2 diabetes mellitus,

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including increasing energy expenditure, improving glucose metabolism, and insulin secretion, slowing gastric emptying and gut transit, decreasing food intake, and reducing weight gain (1-3). These beneficial effects of GLP-1 and the evidence that hormone analogs are effective in the treatment of T2DM and obesity (10) make studies particularly attractive to understand the pathways affecting the natural release of GLP-1.

T2DM, obesity, and prediabetes are closely linked, and understanding the relationships may lead to effective preventive treatments. In this study, we compared the profile of SCFAs and also investigated the relations between fecal SCFAs and GLP-1 in individuals with T2DM, prediabetes, obesity, and healthy controls. Many human clinical trials have investigated analyses of SCFAs, and most of them have focused only on obesity or T2DM separately, and in relation to various metabolic risk factors (11-15). In particular, studies measuring GLP-1 are very limited (16,17). It may be useful to understand the relationships of changes in SCFAs on GLP-1 concentrations in different health states. Our study is the first to investigate changes in SCFA levels and their relationship with GLP-1 in four groups.

## MATERIALS AND METHODS

### *Materials*

GLP-1 ELISA kit was obtained from Cloud-Clone Corporation, Houston, Texas, USA. Acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, diethyl ether, and acetonitrile were obtained from Sigma-Aldrich, St. Louis, Missouri, USA.

### *Study participants*

The study included 60 volunteers in four groups: with T2DM (n= 15), prediabetes (n= 15), obesity (n= 15), and healthy individuals (n= 15), who came to the endocrinology clinic. Participants in the study were included according to the following criteria: individuals of both genders, aged between 18 and 65 years, with a body mass index (BMI) ranging from 18.5 to 24.9 kg/m$^2$ for those in good health and ≥30 kg/m$^2$ for those classified as obese. Participants with T2DM were required to be newly diagnosed. Individuals who had inflammatory bowel disease (such as Crohn's disease, ulcerative colitis), colorectal carcinoma, and other chronic diseases other than obesity and diabetes (hypertension, chronic kidney failure, chronic liver disease, hypo/hyperthyroidism, coronary artery disease, polycystic ovarian syndrome causing insulin resistance, acanthosis nigricans, lipoatrophy/ lipodystrophy syndromes, etc.) were not included. Those who had undergone medical treatments, including antibiotics and oral contraceptives, within the last 3 months, as well as those who consumed alcohol or had gastrointestinal diseases and bowel-related operations in the same period, were also excluded. Furthermore, individuals who were pregnant or breastfeeding were not included in the study.

### *Fecal and blood sample collection*

The fecal and blood samples from the participants were collected following a fasting period of at least 12 hours. Fecal samples were collected in tubes at the hospital and stored at -20˚C for a maximum of three days. The samples were transported by cold chain to arrival and stored at -80˚C until analysis at the university.

Blood samples were taken into serum separation tubes and kept at room temperature for two hours. After that, the blood samples were centrifuged at 2000 g for 20 min, and serum samples were stored at -80˚C until analysis.

### *Analysis of short-chain fatty acids*

SCFAs (acetic acid, propionic acid, butyric acid, and valeric acid) in fecal samples were detected and measured by a previously described method, with some modifications (18). Briefly, 150 mg of fecal samples were weighed in a tube and suspended in 1 mL diethyl ether. The samples were homogenized using a vortex (Dlab, USA) and an ultrasonic water bath (P-Selecta, Spain) for approximately 10 minutes. The samples were centrifuged at 4˚C and 13.200 g for 15 minutes. The supernatant was then transferred into a tube and evaporated at 50˚C using a nitrogen evaporator (Teknosem, Turkey) to remove diethyl ether. Acetonitrile solution (2 mL, 25% v/v) was added to the dried sample, and the mixture was placed in an ultrasonic water bath for 1–5 minutes. The solution was filtrated using a 0.45 µm syringe filter and then stored in a vial. SCFAs were measured by high-performance liquid chromatography (HPLC) using a Shimadzu Nexera-i LC 2040C 3D pump with a Shimadzu 5PD-20A ultraviolet detector (Shimadzu Corporation, Kyoto, Japan) at a wavelength of 210 nm. A 5% (v/v) acetonitrile solution, prepared with distilled water, was used as the mobile phase. The separation was performed using a Gemini C18 analytical column (5 µm, NX-C18 110A, 4.6 × 250 mm; LC Column, USA), and the flow rate was 1 mL/min. The column oven temperature was maintained at 30˚C; the injection volume was 20 µL, and the analysis time was 33 min.

**Table 1.** Clinical characteristics of healthy, obese, prediabetic, and T2DM groups

| Characteristics | Healthy (n = 15) | Obese (n = 15) | Prediabetic (n = 15) | T2DM (n = 15) | $p_1$- value | $p_2$- value | $p_3$- value | $p_4$- value | $p_5$- value | $p_6$- value |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sex (male; female)** | 8; 7 | 8; 7 | 8; 7 | 8; 7 | | | | | | |
| **Age (years)** | 45.00 (35.00-51.00)/ 41.93 ± 11.3 61.00 | 41.00 (39.50- 49.00)/ 41.86 ± 8.53 88.00 | 48.00 (44.00- 57.00)/ 49.6 ± 8.91 93.00 | 56.00 (47.00- 62.00)/ 54 ± 7.76 85.00 | | [b]0.049 | [b]0.002 | [a]0.001* | [a]0.079*** | [b]0.137 |
| **Weight (kg)** | (57.00-65.50)/ 61.57 ± 6.83 22.80 | (79.50-101.25)/ 91.63 ± 14.03 34.40 | (80.00- 98.00)/ 90.39 ± 16.97 35.60 | (75.00- 93.00)/ 83.93 ± 13.73 31.150 | [b]0.000 | [b]0.000 | [b]0.000 | [b]0.212 | [b]0.990 | [b]0.262 |
| **BMI (kg/m$^2$)** | (21.60-24.10)/ 22.85 ± 1.58 5.30 | (31.60-37.77)/ 35.86 ± 4.72 5.250 | (29.30- 36.90)/ 34.94 ± 7.73 6.00 | (27.15- 32.27)/ 30.27 ± 5.74 7.200 | [b]0.000 | [b]0.000 | [b]0.000 | [b]0.025 | [b]0.896 | [b]0.108 |
| **HbA1c (%)** | (5.00- 5.45)/ 5.24 ± 0.24 85.00 | (4.57- 5.37)/ 5.21 ± 0.38 93.00 | (5.80- 6.20)/ 6.01 ± 0.24 106.00 | (6.65- 8.95)/ 7.79 ± 1.42 133.00 | [b]0.445 | [b]0.000 | [a]0.001* | [a]0.000* | [b]0.000 | [a]0.000* |
| **FBG (mg/dL)** | (82.00-92.00)/ 86.2 ± 5.72 60.00 | (85.00- 96.00)/ 92 ± 9.32 116.00 | (96.00- 122.00)/ 109.67 ± 15.25 118.00 | (125.50- 153.00)/ 139.92 ± 17.46 191.00 | [a]0.051*** | [b]0.000 | [b]0.000 | [a]0.000* | [a]0.002* | [b]0.000 |
| **Triglyceride (mg/dL)** | (52.25-73.00)/ 61.75 ± 10.9 | (72.50- 155.00)/ 125.89 ± 60.53 | (81.00- 204.00)/ 155.7 ± 116.19 | (110.00- 289.25)/ 227.2 ± 147.63 | [b]0.064 | [a]0.009* | [a]0.007* | [a]0.054*** | | |

Data are median (25- 75% percentiles)/ mean ± standard deviation; a, results of Mann-Whitney U; b, results of T test; BMI, body mass index; HbA1c, hemoglobin A1c; FBG, fasting blood glucose. p1, obese vs healthy; p2, prediabetic vs healthy; p3, T2DM vs healthy; p4, obese vs T2DM; p5, obese vs prediabetic; p6, T2DM vs prediabetic; *, 99% level of significance; **, 95% level of significance; ***, 90% level of significance.

### *Biochemical analyses*

The GLP-1 analysis was carried out using the GLP-1 ELISA kit (Cloud-Clone Corporation, USA) according to the manufacturer's instructions. The concentrations of serum lipids were determined using a commercially available reagent kit based on an enzymatic colorimetric technique. An automated spectrophotometer was used to measure the colorimetric reaction (ABX Pentra 400 autoanalyzer, Horiba ABX Pentra). Also, FBG was measured by the commercial reagent kit based on the enzymatic colorimetric method of glucose-oxidase using an automated spectrophotometer (ABX Pentra 400 autoanalyzer, Horiba ABX Pentra). The Friedwald formula was used to calculate LDL-cholesterol concentration. Biochemical analyses except for GLP-1 were made in the hospital and the results were obtained.

### *Statistical analyses*

Statistical analyses of the findings obtained in the study were carried out with the IBM SPSS Statistics Version 22.00 software. The sample size was calculated at the beginning of the study. In the case of using the Anova test from the F test family, alpha 0.05, beta 0.80, effect size 0.45, and for the number of 4 groups sample size was determined as 60. The sample size was calculated using G Power 3.1.9.7 (Supplementary File, Figure S1, Table S4). Normality tests were conducted by considering the z-scores of Skewness and Kurtosis values (Supplementary File, Table S2 A, B, C, D). The equality of variances was tested using the Levene test. These tests were carried out to determine whether the assumptions of the analysis of variance were met. Accordingly, when the normality assumption was satisfied and the variances were equal, the ANOVA test was employed. In cases where the normality assumption was met but the variances were unequal, the robust alternative to the Anova test, the Welch test was used. When the normality assumption was not met, the nonparametric alternative to variance analysis Kruskal-Wallis H test was used (Supplementary File, Table S1). T-test was employed for variables that followed

**Table 2.** Fecal SCFA concentrations and GLP-1 levels in healthy, obese, prediabetic, and T2DM groups

| SCFA (mg/g) | Healthy | Obese | Prediabetic | T2DM | $p_1$-value | $p_2$-value | $p_3$-value | $p_4$-value | $p_5$-value | $p_6$-value |
|---|---|---|---|---|---|---|---|---|---|---|
| AA | 0.017 (0.000- 0.136)/ 0.217± 0.487 | 0.105 (0.039- 0.208)/ 0.209± 0.301 | 0.170 (0.050- 0.249)/ 0.461± 0.837 | 0.071 (0.016- 1.083)/ 0.571± 0.712 | [a] 0.020** | [a] 0.017** | | | | |
| BA | 0.053 (0.003- 0.139)/ 0.086± 0.080 | 0.109 (0.057- 0.369)/ 0.350± 0.613 | 0.268 (0.162- 1.023)/ 0.505± 0.488 | 0.259 (0.03- 0.635)/ 0.395± 0.397 | | [b] 0.024 | [b] 0.017 | | [a] 0.072*** | [b] 0.561 |
| Total SCFA | 0.393 (0.010- 1.164)/ 1.101± 2.275 | 0.616 (0.301- 1.567)/ 1.730± 2.960 | 1.675 (0.493- 2.428)/ 1.771± 1.529 | 1.479 (0.815- 7.755)/ 3.308± 4.016 | [a] 0.019** | [a] 0.017** | [a] 0.024** | | | |
| GLP-1 (pg/mL) | 32.66 (22.45- 53.66)/ 38.27± 20.80 | 17.53 (11.74- 27.88)/ 20.01± 9.29 | 14.69 (12.32- 28.85)/ 23.91± 19.91 | 52.38 (38.38- 62.28)/ 48.24± 19.28 | [b] 0.006 | [a] 0.021** | [b] 0.184 | [b] 0.000 | | [a] 0.005* |

Data are median (25- 75% percentiles)/ mean ± standard deviation; a, results of Mann-Whitney U; b, results of T test; AA, acetic acid; PA, propionic acid; BA, butyric acid; GLP-1, glucagon-like peptide 1. p1, obese vs healthy; p2, prediabetic vs healthy; p3, T2DM vs healthy; p4, obese vs T2DM; p5, obese vs prediabetic; p6, T2DM vs prediabetic; *, 99% level of significance; **, 95% level of significance; ***, 90% level of significance.

a normal distribution, otherwise while the Mann–Whitney U test was used. Spearman's correlation test was performed to explore the correlation between SCFAs and the biochemical parameters.

## RESULTS

### *Clinical characteristics of participants*

Fasting blood glucose (FBG) demonstrated a progressive and statistically significant increase in the obese, prediabetic, and T2DM groups compared to the healthy group (Table 1). The prediabetic and T2DM groups showed FBG levels exceeding the reference range of 70–100 mg/dL, whereas the obese group's levels were within the reference range. Both obese and healthy individuals exhibited normal hemoglobin 1Ac (Hb1Ac) levels falling within the reference range of 3.5–5.6%. Notably, HbA1c levels were elevated in the prediabetic and T2DM groups, with a significant pairwise increase observed among the groups. Additionally, body mass index (BMI) values were markedly higher in the other three groups when compared to the healthy group. The levels of triglyceride were within reference ranges in obese, prediabetic, and healthy groups, but were higher in the T2DM group (triglyceride, <150 mg/dL) and significantly elevated in all groups compared to the healthy group (Table 1).

### *Fecal short-chain fatty acids and GLP-1 levels*

SCFAs and GLP-1 levels, along with statistical group comparisons, are outlined in Table 2. As anticipated, acetic, propionic, and butyric acids were identified as the fecal SCFAs across all groups. Valeric acid was not detected in certain stool samples. The total SCFA concentration exhibited a noteworthy increase in the obese, prediabetic, and T2DM groups when compared to the healthy group. Acetic acid levels displayed a significant rise in both the obese and prediabetic groups as opposed to the healthy group. Butyric acid levels were significantly elevated in the prediabetic and T2DM groups relative to the healthy group. Within the prediabetic group, the levels of butyric acid demonstrated a significant increase in comparison to both the obese and T2DM groups. The levels of propionic and valeric acid, however, did not exhibit significant differences among the groups (Supplementary File, Table S2).

GLP-1 levels showed a statistically significant decrease in the obese and prediabetic groups compared to the healthy group. Contrary to expectations, the GLP-

1 levels of the T2DM group were higher than all other groups, while statistical significance was observed in the pairwise comparison with the obese and prediabetic groups (Table 2).

***Relationships among the fecal short-chain fatty acids and GLP-1***

The relationships between SCFAs and GLP-1 for the obese, prediabetic, and T2DM groups are shown in Table 3. In the T2DM group, the total SCFA and acetic acid levels correlated positively with GLP-1 (r= 0.479, p= 0.098; r= 0.441, p= 0.099). The butyric acid concentration in the obese group correlated positively with GLP-1 concentrations (r= 0.654, p= 0.015). In the prediabetic group, the concentrations of SCFAs were not statistically correlated with GLP-1 concentrations (Table 3).

## DISCUSSION

In recent years, the effects of microbiota and its synthesis products on health have been extensively recognized. In the current study, it was observed that propionic acid formed the dominant SCFA in the colon, followed by increasing levels of butyric and acetic acid in the T2DM and prediabetic groups. Compared to the healthy individuals, both the T2DM and prediabetic groups exhibited significantly higher levels of butyric acid and total SCFAs. In a study of Indonesian women with type 2 diabetes, no significant differences in SCFA levels were observed between those with and without T2DM (11). Contrary to our results, a study conducted by Sato *et al.* revealed that the total SCFA concentrations, as well as fecal concentrations of acetic and propionic acids, were lower in the T2DM group than in the healthy control group (13). Zhao *et al.* found that in T2DM patients, fecal SCFA results indicated significantly reduced levels of acetate and butyrate compared to the healthy group (15). A study of individuals with T2DM showed higher acetic acid and propionic acid (19). Furthermore, in our study, although fecal propionic acid levels showed a distinct increase in both the prediabetic and T2DM groups compared to the healthy and obese groups, this increase did not have statistical significance. Nevertheless, this increase seems to support the findings of a study conducted by Sanna *et al.* Their statistical analysis, based on information gathered from 952 normoglycemic individuals, indicated a relationship between elevated fecal propionate levels and the risk of developing T2DM (20). An increase in fecal propionate levels might prove useful in assessing the risk of T2DM. However, further research is needed to investigate the underlying mechanisms that explain the connection between fecal propionate levels and T2DM risk.

In the obese group, the concentrations of total SCFAs and acetic acid have shown a statistically significant increase compared to the healthy group. Similarly, Fernandes *et al.* have reported that obese individuals have higher levels of acetic, propionic, and butyric acids, as well as total SCFA concentrations, compared to lean individuals (21). In Teixeira's study, it has been indicated that obese individuals exhibit a greater increase in fecal concentrations of acetic, propionic, and butyric acids compared to lean individuals (12). Schwiertz *et al.* have reported that there is an increment in total SCFAs and propionic acid concentrations from lean individuals to overweight and obese individuals (14). Although the site and method of administration or different SCFAs may have different effects on metabolic outcomes, most studies demonstrate the beneficial effects of SCFAs in obesity studies (16, 30, 7). Therefore, it is thought that increased SCFA levels have a positive effect on individuals through antiobesity and antidiabetic effects. However, no consistent conclusions have been reached about the effect of intestinal SCFA production in obese individuals (22, 20). The most important feature of SCFAs is that most are used as an energy source. It has been stated that absorbed and diffused SCFAs meet

**Table 3.** Correlations among SCFAs and GLP-1 in obese, prediabetic, and T2DM groups

| Parameter | AA | | PA | | BA | | Total SCFA | |
|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p |
| **Obese Group** | | | | | | | | |
| **GLP-1 (pg/mL)** | 0.071 | 0.800 | 0.033 | 0.915 | **0.654*** | **0.015** | 0.268 | 0.334 |
| **Prediabetic Group** | | | | | | | | |
| **GLP-1 (pg/mL)** | 0.327 | 0.253 | -0.13 | 0.709 | -0.455 | 0.187 | -0.068 | 0.810 |
| **T2DM Group** | | | | | | | | |
| **GLP-1 (pg/mL)** | **0.479***** | **0.098** | 0.378 | 0.225 | -0.190 | 0.535 | **0.441***** | **0.099** |

GLP-1, glucagon-like peptide 1; AA, acetic acid; PA, propionic acid; BA, butyric acid; *, 99% level of significance; **, 95% level of significance; ***, 90% level of significance.

approximately 5–15% of daily needed energy in human metabolism (5). There is a general belief that these excess SCFAs produced in obese individuals provide additional energy increases and cause weight gain (20). However, the increasing fecal levels of SCFAs may also reflect a generally higher substrate presence, decreased absorption capacity, or a condition of SCFA resistance (23).

When analyzing inter-patient groups, it was found that while SCFA levels were generally elevated in both prediabetic and T2DM groups compared to the obese group, statistical significance was observed only for butyric acid.

The incretin hormone GLP-1 reduces postprandial glycemia through a variety of mechanisms, including glucagon inhibition, insulin secretion, delayed gastric emptying, appetite suppression, reduced intestinal food absorption, improved lipid metabolism, and pancreatic cell efficiency (24). Studies have conflicting results regarding the GLP-1 levels of type-2-diabetic, prediabetic, and obese individuals. Some research studies demonstrated that GLP-1 secretion reduces and rises in people with T2DM (25, 26), while a meta-analysis states that it is unchanged (27). According to another study, individuals with prediabetes and T2DM have a 25% lower GLP-1 level than those with normal glucose tolerance. Also, independent of glucose tolerance level, obese and overweight people had a 20% lower GLP-1 response to oral glucose than normal-weight people (28). The present study examined changes in GLP-1 levels among four groups. The prediabetic and obese individuals had lower GLP-1 levels compared to the healthy group ($p= 0.006$, $p= 0.021$) while the T2DM group had higher GLP-1 levels than healthy individuals ($p= 0.184$). However, although there are different perspectives in the literature, one of the assumptions of GLP-1-based therapies is that the GLP-1 level is generally low in diabetics (10,29). Healthy individuals, those with impaired glucose tolerance (often considered prediabetic), and diabetic individuals were shown in this study as groups with decreased GLP-1 levels, respectively. Acetate and propionate preferentially activate GPR43, while propionate and butyrate activate GPR41 (22). Acetate and butyrate have been shown to improve glucose homeostasis by inducing intestinal production of GLP-1 and PYY, which in turn stimulates insulin secretion (9). Considering these data, the increased fecal butyric acid concentration in the T2DM group may have an effect that causes an increase in plasma GLP-1.

Oral, intravenous, and colonic infusion of SCFAs, including microbial-derived SCFAs, beneficially affects metabolism, improving insulin sensitivity, substrate metabolism, and body weight regulation (30-32). Rahat- Rozenbloom *et al.* examined the effects of SCFAs on carbohydrate metabolism and showed that increased propionic acid in the colon stimulates GLP-1 activity and insulin secretion in beta cells (33). In another study, intra-colonic injection of propionate stimulated the simultaneous release of GLP-1 in rats (34). Examining the effect of fecal SCFAs on GLP-1 release constitutes the main basis of the present study. In our study, the results showed no general correlations between fecal SCFAs and GLP-1, except for acetic acid, butyric acid, and total SCFA in the obese and T2DM groups (Table 3). Müller *et al.* investigated the relationship between fecal and plasma SCFAs and GLP-1 in obese individuals with normal and impaired glucose tolerance. In contrast to our study, they found no association with fecal SCFAs but did observe a positive correlation between plasma SCFAs and GLP-1 (17). Similarly, Solar *et al*. found a correlation between increased GLP-1 levels and total plasma SFCA levels in various obese groups (16). Although fecal SFCAs are widely used as an indicator of microbial fermentation, this may not fully reflect the intestinal absorption of SCFAs because approximately 95% of SCFAs are effectively absorbed in the intestine, while 5% remain in the feces (32, 21). The present study has some limitations, including, for example, the number of participating individuals and the lack of eating habits of participants.

**In conclusion,** our study investigated the fecal SCFA profiles of four closely related study groups - obese, prediabetic, T2DM, and healthy individuals - and the relationship between SCFAs and GLP-1. The results showed a significant difference in SCFA levels of the four groups, in terms of total SCFA, acetic acid, and butyric acid levels. Acetic acid, butyric acid, and the total SCFA concentrations were associated with the GLP-1 in the T2DM and obese groups. Metabolic diseases and homeostasis can be managed through the regulation of SCFA levels. Further research in this area could offer promising avenues for the development of interventions aimed at managing T2DM and its related diseases and improving overall health outcomes.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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# SUPPLEMENTARY FILE

Analysis of Short-Chain Fatty Acids and Association with Glucagon-Like Peptide-1 in Individuals with Healthy, Obese, Prediabetes and Type 2 Diabetes

**Table S1.** Tests of the feasibility of variance analysis assumptions

| Parameters | Gruops that deviate from normality | Condition of equality of variances | Analysis technique | Pairwise comparison |
|---|---|---|---|---|
| **AA** | H,P,O | Not equal | Kruskal- Wallis H | Man- Whitney U |
| **PA** | H,P,O | Not equal | Kruskal- Wallis H | Man- Whitney U |
| **BA** | O | Not equal | Kruskal- Wallis H | Man- Whitney U/ T test |
| **VA** | H,O | Equal | Kruskal- Wallis H | Man- Whitney U/ T test |
| **Total SCFA** | H, P,O,T | Not equal | Kruskal- Wallis H | Man- Whitney U |
| **GLP-1** | P | Not equal | Kruskal- Wallis H | Man- Whitney U/ T test |
| **Age** | O | Equal | Kruskal- Wallis H | Man- Whitney U/ T test |
| **Weight** | | Equal | Anova | T test |
| **Hb1Ac** | T | Not equal | Kruskal- Wallis H | Man- Whitney U/T test |
| **FBG** | O | Not equal | Kruskal- Wallis H | Man- Whitney U/T test |
| **Triglicerides** | P, T | Equal | Kruskal- Wallis H | Man- Whitney U/ T test |
| **BMI** | | Not equal | Welch | T test |

H, healthy group; P, prediabetic group; O,obese group; T, T2DM group; BMI, body mass index; HbA1c, hemoglobin A1c; FBG, fasting blood glucose; AA, acetic acid; PA, propionic acid; BA, butyric acid.

**Table S2A.** Descriptive statistics for the variables included in the analysis for the healthy group

| | Descriptive Statistics | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Min. | Max. | Mean | Std. Deviation | Median | Skewness | | Kurtosis | | | |
| | Statistic | Statistic | Statistic | Statistic | Statistic | Statistic | Statistic | Std. Error | Statistic | Std. Error | Skewness z score | Kurtosis z score |
| **AA** | 13 | 0.000 | 1.661 | 0.217 | 0.487 | 0.017 | 2.664 | 0.616 | 7.068 | 1.191 | 4.322 | 5.935 |
| **PA** | 14 | 0.000 | 6.257 | 0.583 | 1.639 | 0.148 | 3.697 | 0.597 | 13.758 | 1.154 | 6.189 | 11.922 |
| **BA** | 13 | 0.001 | 0.259 | 0.086 | 0.080 | 0.053 | 0.737 | 0.616 | 0.015 | 1.191 | 1.196 | 0.013 |
| **VA** | 10 | 0.000 | 1.748 | 0.442 | 0.635 | 0.100 | 1.411 | 0.687 | 0.666 | 1.334 | 2.054 | 0.499 |
| **Total SCFA** | 15 | 0.002 | 9.064 | 1.101 | 2.275 | 0.393 | 3.481 | 0.580 | 12.764 | 1.121 | 6.000 | 11.387 |
| **GLP-1** | 15 | 12.270 | 76.170 | 38.276 | 20.808 | 32.660 | 0.464 | 0.580 | -1.174 | 1.121 | 0.800 | -1.047 |
| **Age** | 15 | 18.000 | 58.000 | 41.933 | 11.304 | 45.000 | -0.622 | 0.580 | -0.241 | 1.121 | -1.073 | -0.215 |
| **Weight** | 15 | 51.000 | 76.000 | 61.567 | 6.832 | 61.000 | 0.670 | 0.580 | 0.249 | 1.121 | 1.156 | 0.222 |
| **Hb1Ac** | 15 | 4.900 | 5.500 | 5.240 | 0.241 | 5.300 | -0.601 | 0.913 | -0.945 | 2.000 | -0.659 | -0.473 |
| **FBG** | 15 | 78.000 | 97.000 | 86.200 | 5.722 | 85.000 | 0.569 | 0.580 | -0.734 | 1.121 | 0.981 | -0.655 |
| **Triglicerides** | 15 | 51.000 | 76.000 | 61.750 | 10.905 | 60.000 | 0.757 | 1.014 | -0.369 | 2.619 | 0.746 | -0.141 |
| **BMI** | 15 | 19.800 | 24.800 | 22.853 | 1.576 | 22.800 | -0.550 | 0.580 | -0.748 | 1.121 | -0.947 | -0.667 |

**Table S2B.** Descriptive statistics for the variables included in the analysis for the prediabetic group

| | Descriptive Statistics | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Min. | Max. | Mean | Std. Deviation | Median | Skewness | | Kurtosis | | | |
| | Statis-tic | Statis-tic | Statis-tic | Statis-tic | Statistic | Statistic | Statis-tic | Std. Error | Statis-tic | Std. Error | Skewness z score | Kurtosis z score |
| **AA** | 14 | 0.014 | 2.454 | 0.461 | 0.837 | 0.170 | 2.252 | 0.597 | 3.674 | 1.154 | 3.769 | 3.183 |
| **PA** | 11 | 0.024 | 5.617 | 1.069 | 1.620 | 0.458 | 2.578 | 0.661 | 7.356 | 1.279 | 3.902 | 5.749 |
| **BA** | 10 | 0.093 | 1.468 | 0.505 | 0.488 | 0.268 | 1.144 | 0.687 | -0.108 | 1.334 | 1.665 | -0.081 |
| **VA** | 8 | 0.035 | 1.339 | 0.407 | 0.449 | 0.277 | 1.417 | 0.752 | 1.980 | 1.481 | 1.884 | 1.337 |
| **Total SCFA** | 15 | 0.148 | 6.048 | 1.771 | 1.528 | 1.676 | 1.561 | 0.580 | 3.483 | 1.121 | 2.692 | 3.108 |
| **GLP-1** | 15 | 9.680 | 67.970 | 23.914 | 19.912 | 14.690 | 1.671 | 0.580 | 1.553 | 1.121 | 2.881 | 1.385 |
| **Age** | 15 | 35.000 | 64.000 | 49.600 | 8.911 | 48.000 | 0.248 | 0.580 | -0.794 | 1.121 | 0.427 | -0.708 |
| **Weight** | 15 | 51.000 | 120.000 | 90.393 | 16.972 | 93.000 | -0.401 | 0.580 | 1.148 | 1.121 | -0.692 | 1.024 |
| **Hb1Ac** | 15 | 5.700 | 6.400 | 6.013 | 0.242 | 6.000 | 0.241 | 0.580 | -1.050 | 1.121 | 0.416 | -0.937 |
| **FBG** | 15 | 91.000 | 137.000 | 109.667 | 15.253 | 106.000 | 0.544 | 0.580 | -0.579 | 1.121 | 0.937 | -0.516 |
| **Triglicerides** | 15 | 58.000 | 470.000 | 155.727 | 116.186 | 118.000 | 2.246 | 0.661 | 5.860 | 1.279 | 3.400 | 4.580 |
| **BMI** | 15 | 20.700 | 51.900 | 34.940 | 7.732 | 35.600 | 0.600 | 0.580 | 1.095 | 1.121 | 1.034 | 0.977 |

**Table S2C.** Descriptive statistics for the variables included in the analysis for the obese group

| | Descriptive Statistics | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Min. | Max. | Mean | Std. Deviation | Median | Skewness | | Kurtosis | | | |
| | Statis-tic | Statis-tic | Statistic | Statistic | Statistic | Statistic | Statis-tic | Std. Error | Statis-tic | Std. Error | Skewness z score | Kurtosis z score |
| **AA** | 15 | 0.011 | 0.944 | 0.209 | 0.302 | 0.105 | 2.173 | 0.580 | 3.596 | 1.121 | 3.746 | 3.208 |
| **PA** | 13 | 0.000 | 8.611 | 1.154 | 2.532 | 0.097 | 2.645 | 0.616 | 6.839 | 1.191 | 4.291 | 5.743 |
| **BA** | 13 | 0.031 | 2.284 | 0.349 | 0.613 | 0.109 | 3.043 | 0.616 | 9.816 | 1.191 | 4.937 | 8.243 |
| **VA** | 8 | 0.048 | 2.030 | 0.409 | 0.662 | 0.193 | 2.707 | 0.752 | 7.483 | 1.481 | 3.600 | 5.053 |
| **Total SCFA** | 15 | 0.083 | 11.561 | 1.729 | 2.959 | 0.616 | 3.034 | 0.580 | 9.774 | 1.121 | 5.229 | 8.720 |
| **GLP-1** | 15 | 10.750 | 41.980 | 20.011 | 9.291 | 17.530 | 1.025 | 0.580 | 0.483 | 1.121 | 1.767 | 0.431 |
| **Age** | 15 | 20.000 | 54.000 | 42.000 | 8.860 | 41.000 | -1.232 | 0.616 | 2.197 | 1.191 | -1.999 | 1.845 |
| **Weight** | 15 | 71.000 | 117.000 | 90.464 | 13.779 | 88.000 | 0.466 | 0.597 | -0.811 | 1.154 | 0.780 | -0.703 |
| **Hb1Ac** | 15 | 4.500 | 5.600 | 5.075 | 0.420 | 5.250 | -0.507 | 0.752 | -1.410 | 1.481 | -0.674 | -0.952 |
| **FBG** | 15 | 82.000 | 119.000 | 92.143 | 9.654 | 93.000 | 1.592 | 0.597 | 4.003 | 1.154 | 2.664 | 3.469 |
| **Triglicerides** | 15 | 49.000 | 247.000 | 125.889 | 60.530 | 116.000 | 0.728 | 0.717 | 0.947 | 1.400 | 1.015 | 0.677 |
| **BMI** | 15 | 30.100 | 43.500 | 35.250 | 4.235 | 34.400 | 0.786 | 0.597 | -0.486 | 1.154 | 1.316 | -0.421 |

**Table S2D.** Descriptive statistics for the variables included in the analysis for the T2DM group

| | Descriptive Statistics | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Min. | Max. | Mean | Std. Deviation | Median | Skewness | | Kurtosis | | | |
| | Statis-tic | Statis-tic | Statis-tic | Statistic | Statistic | Statistic | Statis-tic | Std. Error | Statis-tic | Std. Error | Skewness z score | Kurtosis z score |
| **AA** | 13 | 0.002 | 2.182 | 0.572 | 0.712 | 0.071 | 1.115 | 0.616 | 0.456 | 1.191 | 1.810 | 0.383 |
| **PA** | 12 | 0.000 | 9.253 | 2.477 | 3.333 | 0.570 | 1.056 | 0.637 | -0.456 | 1.232 | 1.658 | -0.370 |
| **BA** | 13 | 0.001 | 1.286 | 0.395 | 0.398 | 0.259 | 1.089 | 0.616 | 0.628 | 1.191 | 1.767 | 0.527 |
| **VA** | 10 | 0.001 | 1.748 | 0.732 | 0.774 | 0.284 | 0.480 | 0.687 | -2.084 | 1.334 | 0.699 | -1.562 |
| **Total SCFA** | 15 | 0.003 | 12.070 | 3.308 | 4.016 | 1.480 | 1.234 | 0.580 | 0.025 | 1.121 | 2.127 | 0.023 |
| **GLP-1** | 15 | 12.780 | 69.810 | 48.243 | 19.283 | 52.380 | -0.946 | 0.580 | -0.322 | 1.121 | -1.631 | -0.287 |
| **Age** | 15 | 43.000 | 65.000 | 54.267 | 7.759 | 56.000 | -0.126 | 0.580 | -1.412 | 1.121 | -0.217 | -1.260 |
| **Weight** | 15 | 63.000 | 110.000 | 83.933 | 13.735 | 85.000 | 0.495 | 0.580 | 0.060 | 1.121 | 0.853 | 0.053 |
| **Hb1Ac** | 15 | 6.600 | 11.300 | 7.792 | 1.422 | 7.200 | 1.451 | 0.616 | 1.746 | 1.191 | 2.353 | 1.466 |
| **FBG** | 15 | 116.000 | 176.000 | 139.923 | 17.462 | 133.000 | 0.580 | 0.616 | -0.284 | 1.191 | 0.941 | -0.238 |
| **Triglicerides** | 15 | 90.000 | 545.000 | 227.250 | 147.631 | 191.000 | 1.599 | 0.752 | 3.000 | 1.481 | 2.126 | 2.026 |
| **BMI** | 15 | 22.300 | 44.800 | 30.729 | 5.694 | 31.150 | 1.047 | 0.597 | 1.942 | 1.154 | 1.753 | 1.683 |

**Table S3A.** The results of Anova test

| Parameters | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **Weight*** | Between Groups | 8360.052 | 3 | 2786.684 | 15.647 | 0.000 |
| | Within Groups | 9795.088 | 55 | 178.093 | | |
| | Total | 18155.140 | 58 | | | |

* There ıs a significant difference between at least two groups at 99% confidence level.

**Table S3B.** The result of Welch test

| Parameter | | Statistic[a] | df1 | df2 | Sig. |
|---|---|---|---|---|---|
| **BMI*** | **Welch** | 47.098 | 3 | 25.059 | 0.000 |

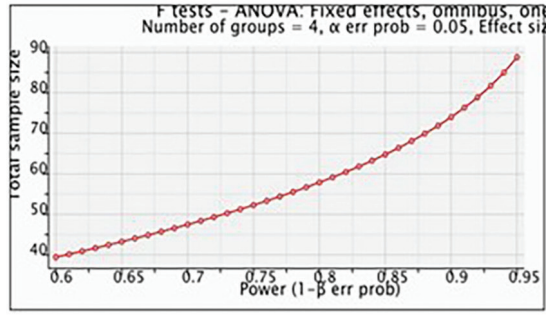
* There is a significant difference between at least two groups at 99% confidence level

**Table S3C.** The results of Kruskal Wallis H

| | AA | BA | Total SCFA | GLP-1 | Age | Hb1Ac | FBG | Trigliserid |
|---|---|---|---|---|---|---|---|---|
| **Kruskal-Wallis H** | 7.726 | 10.647 | 7.973 | 16.966 | 13.230 | 35.633 | 39.684 | 11.239 |
| **df** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Asymp. Sig.** | 0.052 | 0.014 | 0.047 | 0.001 | 0.004 | 0.000 | 0.000 | 0.011 |
| **Significance level of east two groups** | 90% | 95% | 95% | 99% | 99% | 99% | 99% | 95% |

**Table S4.** Data of sample size study

| **F tests - ANOVA** | | Fixed effects, omnibus, one-way |
|---|---|---|
| **Analysis** | A priori | Compute the required sample size |
| **Input** | Effect size f | 0.45 |
| | α err prob | 0.05 |
| | Power (1-β err prob) | 0.8 |
| | Number of groups | 4 |
| **Output** | Noncentrality parameter λ | 12.1500000 |
| | Critical F | 2.7694309 |
| | Numerator df | 3 |
| | Denominator df | 56 |
| | Total sample size | 60 |
| | Actual power | 0.8165014 |

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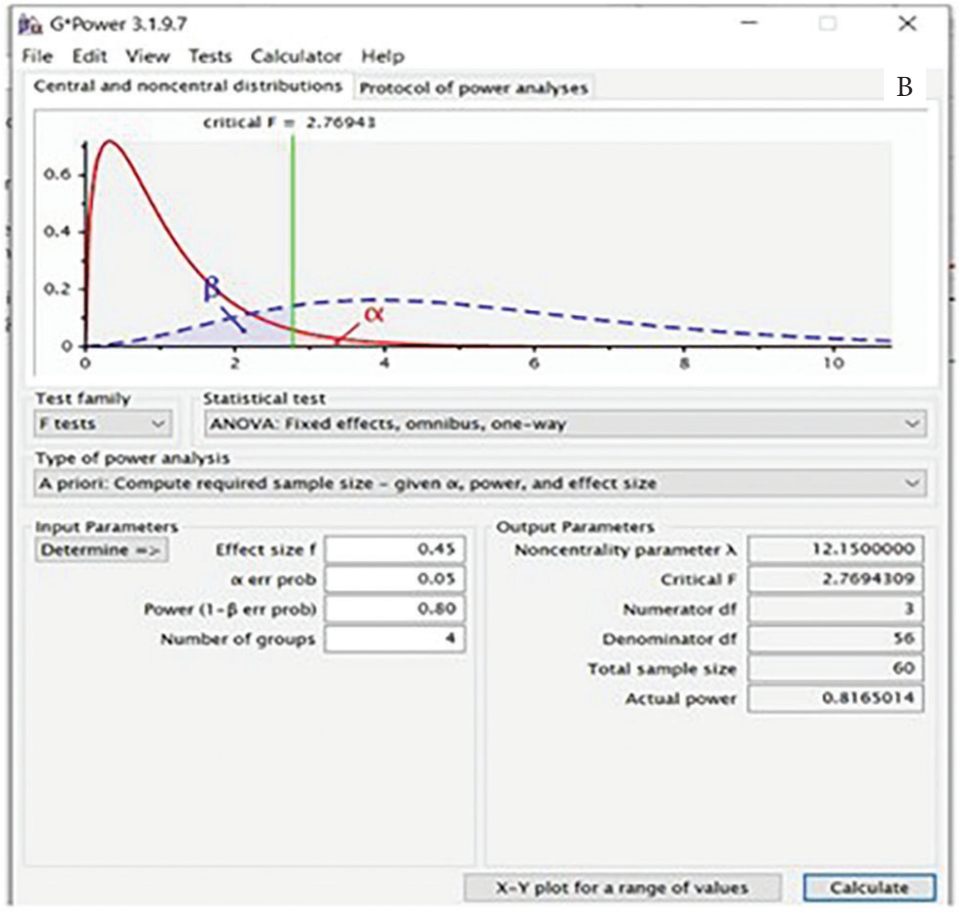


**Figure 1.** Data of sample size study (A, B).