Effects of Smoking on the Gut Microbiota in Individuals with Type 2 Diabetes Mellitus

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Abstract: Smoking affects eating habits; however, few studies on smoking and the gut microbiota have reported the effects of diet in detail. This cross-sectional study aimed to determine the association between smoking and the gut microbiota, considering the impact of smoking on dietary intake. Dietary habits and the composition of the gut microbiota were assessed in 195 men with type 2 diabetes (164 non-current smokers and 31 current smokers) using a brief self-administered diet history questionnaire and 16S ribosomal RNA gene sequencing of fecal samples. The data were compared according to the current smoking status of the participants. Current smokers had high alcohol and sugar/sweetener intake and low fruit intake. The proportion of the Coprococcus genus was higher among current smokers. Multiple regression analysis adjusted for current smoking, age, exercise habits, alcohol intake, sugar and sweetener intake, and fruit intake showed that smoking was associated with the proportion of the Coprococcus genus. Current smoking was associated with both dietary intake and composition of the gut microbiota. Although dietary intake should be considered when investigating the association between smoking and the gut microbiota, the results suggest that the direct effect of smoking is more significant.

Keywords: smoking; dietary intake; gut microbiota; type 2 diabetes mellitus

1. Introduction

Cigarette smoking increases the risk of incident diabetes mellitus and exacerbates the microvascular and macrovascular complications of diabetes mellitus [1]. Smoking has also been shown to increase insulin resistance [2]. Moreover, smoking causes systemic inflammation and oxidative stress [3], which not only reduces vascular endothelial function [4] but also directly damages pancreatic β-cell function [5].

Smoking has also been shown to affect the gut microbiota [6]. Gut microbiota affects the regulation of nutritional metabolism [7] and of the immunological and defense mechanisms in the body [8]. Gut dysbiosis participates in the development and progression of many diseases, including type 2 diabetes mellitus [9] and inflammatory bowel disease [10]. Many factors influence the gut microbiota, including diet, lifestyle, smoking, medications, and the genetic background of the host. Weight gain often occurs after smoking cessation [11], and changes in the gut microbiota have been suggested to play a role in this
phenomenon [12,13]. Additionally, smoking has different effects on inflammatory bowel disease, a risk factor for Crohn’s disease, and a protective factor against ulcerative colitis, which may also be linked to the gut microbiota [14].

Furthermore, smokers tend to have unhealthy diets [15], including high intake of junk food [16] and energy-dense foods [17], and excessive alcohol consumption [18,19]. This has been attributed partly to changes in taste [20] and smell [21] caused by smoking. Studies have also been conducted on the neuronal and behavioral overlap between food addiction and nicotine in the brain reward system [22,23], as well as on the association between smoking and appetite-related hormones, such as leptin, glucagon-like peptide-1, and ghrelin [24,25].

Smoking, the gut microbiota, and dietary intake are all involved in the pathogenesis of type 2 diabetes; however, no studies have assessed the association between smoking and gut microbiota in people with type 2 diabetes. In addition, few studies on smoking and the gut microbiota have examined the effects of diet in detail. Recent studies have also revealed that current smoking plays a more important role than past smoking in affecting the gut microbiota [6]. Therefore, this study investigated the relationship between current smoking and the gut microbiota in people with type 2 diabetes mellitus, taking into account the impact of current smoking on dietary intake.

2. Materials and Methods
2.1. Study Population

This study was designed to determine the association between diabetes mellitus, the gut microbiota, and various background factors [26,27]. The participants were outpatients at Kyoto Prefectural University of Medicine (KPUM) Hospital and Kameoka Municipal Hospital, Japan. Fecal samples were collected from 522 individuals (17 patients with type 1 diabetes, 383 patients with type 2 diabetes, 8 patients with other types of diabetes, and 114 individuals without diabetes) between November 2016 and October 2018, who had not received antibiotics within the prior 3 months. Individuals without diabetes and those with diabetes other than type 2 diabetes were excluded from the study.

2.2. Data Collection

Subject data were collected from medical records on sex, age, blood pressure, height, body weight, body mass index (BMI), duration of diabetes, and available medications for dyslipidemia, hypertension, diabetes, and proton pump inhibitor use. In the questionnaire, participants who reported they were currently smoking even one cigarette per day were categorized as current smokers, whereas those who reported having smoked in the past and who reported never having smoked before were categorized as non-current smokers. The daily number of cigarettes smoked and the years of smoking history were also assessed. Participants who performed some type of sporting activity at least once a week were defined as regular exercisers [28].

Fasting venous blood samples were collected to measure plasma glucose, hemoglobin A1c, triglyceride, high-density lipoprotein cholesterol, uric acid, and creatinine levels. The estimated GFR (eGFR) was defined as $194 \times \text{creatinine}^{-1.094} \times \text{age}^{-0.287} \times (\text{mL}/\text{min}/1.73 \text{m}^2) \times 0.739$ (women) according to the equation presented by the Japanese Society of Nephrology [29].

Habitual dietary intake data were assessed using a brief self-administered diet history questionnaire (BDHQ). The BDHQ can be used to estimate the dietary intake of 58 food and beverage items in the previous month. The BDHQ has been validated previously [30,31]. In the present study, we excluded patients who did not respond to the BDHQ and those who reported extremely high (>4000 kcal) or low (<600 kcal) energy intake, because of poor reliability [32]. When evaluating energy, protein, fat, and carbohydrate intake, the intake per kilogram of ideal body weight (IBW) was calculated to consider the differences in body size. IBW was calculated as $22 \times$ the square of the participant height (m$^2$) [33]. Habitual alcohol intake was defined as alcohol consumption $>20$ g/day in the BDHQ analysis [34].
2.3. Gut Microbiota Data Sampling, DNA Extraction, Sequencing, and Data Analysis

Fecal samples were collected and analyzed for gut microbiota composition using previously described methods [35–37]. Preservation of the collected fecal samples was performed using a guanidine thiocyanate solution (Feces Collection kit; Techno Suruga Lab, Shizuoka, Japan). Genomic DNA was isolated and purified using a NucleoSpin Microbial DNA kit (Macherey-Nagel, Düren, Germany) and Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA).

Sequencing libraries were generated by a two-step polymerase chain reaction (PCR) of the purified DNA samples and the prepared libraries of 250 paired-end sequences were sequenced using the MiSeq Reagent v3 kit and MiSeq (Illumina, San Diego, CA, USA), at Takara Bio’s Biomedical Center, as described in a previous study [37].

The DADA2 plugin of Quantitative Insights into Microbial Ecology 2 version 2019.4 was used for table generation for amplicon sequence variants (ASVs) [26] by setting and performing noise removal by DADA2 with the trimming length from the left set at 17 and from the right at 19. Both reads were truncated to <250 base pairs. Each ASV was classified using the sklearn classifier algorithm against Greengenes database version 13_8. Singletons and ASVs assigned to chloroplasts and mitochondria were deleted in this study. Phylogenetic trees were created using SATé-compatible phylogenetic arrangements [38]. Consequently, 6902 ASVs were obtained. Functional profiles from the 16S rRNA dataset were predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States version 2.1.4 [35], as previously described [37].

2.4. Statistical Analysis

Clinical characteristics, nutrient intake, food group intake, and the proportions of phyla and genera in the gut microbiota were compared between the non-current and current smoker groups using the Mann–Whitney U test. Multiplicity was adjusted by obtaining q values using the Benjamini-Hochberg method to compare the proportions of the gut microbiota.

Furthermore, to assess the effects of smoking on the composition of the gut microbiota, a multiple regression analysis was performed using smoking, smoking-related background factors, and dietary intake as covariates. To investigate multicollinearity, the variance inflation factor (VIF) was checked and all VIFs were confirmed to be less than 2.

Statistical significance was set at \( p < 0.05 \) and \( q < 0.1 \). JMP version 14.0 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analyses.

2.5. Ethics

This study was approved by the Ethics Committee of KPUM (no. ERB-C-534 and no. RBMR-E-466-5) and was performed in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants at the time of enrollment in the study.

3. Results

3.1. Clinical Characteristics

This study analyzed the data of 195 men with type 2 diabetes mellitus (Figure 1). Women were excluded from the analysis because the number of current smokers was very low (31/195 men and 8/160 women) and to follow the methods of previous studies [6,39].
Figure 1. Study flow diagram of the registration of patients. BDHQ, brief self-administered diet history questionnaire.

Table 1 shows the clinical characteristics of participants according to their current smoking status. Of the 195 men, 164 were non-current smokers (84.1%) and 31 were current smokers (15.9%). Comparing the two groups, the current smoker group was younger, had smoked over a longer time period, had fewer exercise habits, and had less sulfonylurea use. There were no differences in BMI, hemoglobin A1c, high-density lipoprotein cholesterol, triglycerides, uric acid, creatinine, and eGFR between the two groups.

Table 1. Clinical characteristics of the subjects.

|                                  | Non-Current Smokers | Current Smokers | p Value |
|----------------------------------|---------------------|-----------------|---------|
| Age (years)                      | 67.8 (10.9)         | 63.9 (9.5)      | 0.023   |
| Duration of diabetes (years)     | 15.5 (9.7)          | 12.7 (8.5)      | 0.155   |
| Body mass index (kg/m²)          | 23.9 (3.4)          | 23.5 (3.5)      | 0.652   |
| Smoking amount (cigarettes/day)  | 18.2 (17.7)         | 20.3 (10.4)     | 0.134   |
| Smoking duration (years)         | 18.9 (17.3)         | 43.1 (9.6)      | <0.001  |
| Exercise (−/+)                   | 73/91               | 20/11           | 0.041   |
| Habitual alcohol intake (−/+)    | 86/78               | 11/20           | 0.083   |
| Systolic blood pressure (mmHg)   | 134.3 (17.5)        | 129.4 (21.0)    | 0.070   |
| Diastolic blood pressure (mmHg)  | 78.9 (10.5)         | 80.2 (12.7)     | 0.799   |
| Insulin (−/+)                    | 127/37              | 21/10           | 0.247   |
| Sulfonylureas (−/+)              | 116/48              | 29/2            | 0.008   |
| Glinides (−/+)                   | 153/11              | 30/1            | 0.460   |
| Thiazolidines (−/+)              | 165/9               | 31/0            | 0.182   |
| Biguanides (−/+)                 | 100/64              | 20/11           | 0.710   |
| Glucagon-like peptide-1 receptor agonist (−/+) | 142/22 | 26/5 | 0.688 |
| Dipeptidyl peptidase-4 inhibitors (−/+) | 65/99 | 18/13 | 0.057 |
| Sodium-glucose cotransporter inhibitors (−/+) | 135/29 | 26/5 | 0.834 |
| α-glucosidase inhibitors (−/+)   | 144/20              | 28/3            | 0.690   |
| Proton pump inhibitors (−/+)     | 130/34              | 24/7            | 0.817   |
| Hemoglobin A1c (mmol/mol)        | 56.4 (12.4)         | 59.8 (18.7)     | 0.834   |
Table 1. Cont.

|                          | Non-Current Smokers n = 164 | Current Smokers n = 31 | p Value |
|--------------------------|-----------------------------|------------------------|---------|
| Hemoglobin A1c (%)       | 7.3 (1.1)                   | 7.6 (1.7)              | 0.834   |
| HDL cholesterol (mmol/L) | 1.5 (0.4)                   | 1.5 (0.5)              | 0.942   |
| Triglycerides (mmol/L)   | 1.5 (0.9)                   | 1.7 (1.1)              | 0.482   |
| Uric acid (mmol/L)       | 324.9 (76.1)                | 303.5 (82.0)           | 0.159   |
| Creatinine (µmol/L)      | 83.6 (37.2)                 | 80.0 (28.3)            | 0.474   |
| eGFR (mL/min/1.73m²)     | 68.6 (19.7)                 | 73.1 (22.7)            | 0.372   |

Values are presented as the mean (standard deviation) or number. HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate. Differences between the groups were analyzed using the chi-square test for categorical variables or the Mann–Whitney U test for continuous variables.

3.2. Nutritional Intake and Food Group Intake

Table 2 shows the differences in nutritional intake between current and non-current smokers. Energy, protein, fat, and carbohydrate intakes did not differ between the two groups in either total intake or intake per IBW. There were also no differences in the intake of dietary fiber, sucrose, salt, and various vitamins according to the current smoking status. In contrast, current smokers consumed significantly more alcohol than did non-current smokers.

Table 2. Nutritional intake by current smoking status.

|                          | Non-Current Smokers n = 164 | Current Smokers n = 31 | p Value |
|--------------------------|-----------------------------|------------------------|---------|
| Total energy (kcal/day)  | 1874.6 (539.0)              | 2016.3 (645.6)         | 0.191   |
| Energy (kcal/IBW/day)    | 30.6 (8.9)                  | 32.6 (10.6)            | 0.323   |
| Total fat (g/day)        | 57.8 (20.2)                 | 58.5 (22.2)            | 0.986   |
| Fat (g/IBW/day)          | 0.9 (0.3)                   | 0.9 (0.4)              | 0.873   |
| Fat per energy (%)       | 27.8 (6.4)                  | 26.3 (5.9)             | 0.255   |
| Total protein (g/day)    | 75.1 (25.5)                 | 76.2 (31.0)            | 0.972   |
| Protein (g/IBW/day)      | 1.2 (0.4)                   | 1.2 (0.5)              | 0.827   |
| Protein per energy (%)   | 16.1 (3.1)                  | 15.1 (3.2)             | 0.092   |
| Total carbohydrate (g/day)| 237.9 (80.4)                | 248.1 (92.2)           | 0.795   |
| Carbohydrate (g/IBW/day) | 3.9 (1.3)                   | 4.0 (1.5)              | 0.970   |
| Carbohydrate per energy (%) | 50.9 (9.4)                | 49.3 (8.3)             | 0.209   |
| Dietary fiber (g/day)    | 12.4 (5.0)                  | 12.4 (5.6)             | 0.747   |
| Sucrose (g/day)          | 11.8 (8.1)                  | 13.8 (9.0)             | 0.242   |
| Salt (g/day)             | 11.4 (3.4)                  | 11.9 (4.0)             | 0.677   |
| Alcohol (g/day)          | 11.4 (23.1)                 | 23.4 (30.0)            | 0.040   |
| Vitamin A (µgRAE/day)    | 774.7 (542.2)               | 941.7 (945.0)          | 0.525   |
| Vitamin B1 (mg/day)      | 0.8 (0.3)                   | 0.8 (0.3)              | 0.989   |
| Vitamin B2 (mg/day)      | 1.4 (0.5)                   | 1.5 (0.6)              | 0.928   |
| Vitamin B6 (mg/day)      | 1.3 (0.5)                   | 1.4 (0.7)              | 0.757   |
| Vitamin B12 (µg/day)     | 11.5 (6.5)                  | 11.4 (7.1)             | 0.635   |
| Vitamin C (mg/day)       | 120.5 (60.4)                | 120.6 (76.8)           | 0.644   |
| Vitamin D (µg/day)       | 17.4 (10.5)                 | 14.9 (9.4)             | 0.144   |
| Vitamin E (mg/day)       | 8.0 (2.9)                   | 7.8 (3.1)              | 0.593   |

Values are presented as the mean (standard deviation). IBW, ideal body weight. Differences between the groups were analyzed using the Mann–Whitney U test.

Table 3 shows food group intake compared by current smoking status. Of the 15 food groups, two food groups (“sugar/sweeteners” and “fruits”) showed differences in intake between current smokers and non-current smokers. Sugar and sweetener intake for coffee or tea and for cooking were significantly higher among the current smokers, whereas the current smokers had lower fruit intake.
Table 3. Food group intake by current smoking status.

|                      | Non-Current Smokers | Current Smokers | p Value |
|----------------------|---------------------|-----------------|---------|
|                      | n = 164             | n = 31          |         |
| Cereals (g/day)      | 390.8 (172.5)       | 419.7 (191.5)   | 0.631   |
| Potatoes (g/day)     | 35.3 (34.0)         | 38.2 (38.4)     | 0.736   |
| Sugar and sweeteners (g/day) | 4.1 (4.0)    | 6.5 (6.4)       | 0.034   |
| Pulses (g/day)       | 64.0 (52.7)         | 57.7 (38.3)     | 0.816   |
| Green and yellow vegetables (g/day) | 118.7 (83.3) | 113.6 (83.4)   | 0.649   |
| Other vegetables (g/day) | 181.0 (119.1) | 198.8 (139.2)  | 0.670   |
| Fruits (g/day)       | 124.7 (111.6)       | 84.6 (81.2)     | 0.044   |
| Fish and shellfish (g/day) | 93.6 (57.6)   | 85.5 (53.0)     | 0.457   |
| Meat (g/day)         | 72.0 (45.1)         | 86.8 (61.7)     | 0.346   |
| Eggs (g/day)         | 47.5 (30.9)         | 41.8 (26.2)     | 0.415   |
| Milk (g/day)         | 166.0 (121.9)       | 181.1 (160.4)   | 0.879   |
| Fat and oil (g/day)  | 11.3 (6.7)          | 12.1 (5.9)      | 0.267   |
| Snacks (g/day)       | 40.4 (37.7)         | 40.2 (41.3)     | 0.765   |
| Alcoholic and non-alcoholic beverages (g/day) | 809.5 (465.0) | 835.3 (504.1)  | 0.704   |
| Seasonings (g/day)   | 224.9 (121.9)       | 253.4 (148.0)   | 0.287   |

Values are presented as the mean (standard deviation). Differences between groups were analyzed using the Mann–Whitney U test.

3.3. Composition of Gut Microbiota

Figure 2 shows the proportions of phyla according to the current smoking status. There was no difference between the two groups in the proportions of phyla (Table S1).

![Figure 2](image-url)

**Figure 2.** The phylum proportions by current smoking status. Differences between groups were analyzed using the Mann–Whitney U test adjusted with the Benjamini-Hochberg method.

Of the highest 30 gut microbiota proportions at the genus level, the proportion of *Coprococcus* was significantly higher in current smokers than in non-current smokers (0.039 versus 0.025, \( q = 0.030 \)) (Table S2). Figure 3 shows the difference in the proportion of *Coprococcus* according to the current smoking status.
Of the highest 30 gut microbiota proportions at the genus level, the proportion of *Coprococcus* was significantly higher in current smokers than in non-current smokers (0.039 versus 0.025, \( q = 0.030 \)) (Table S2). Figure 3 shows the difference in the proportion of *Coprococcus* according to the current smoking status.

**Figure 3.** The difference in the proportion of genus *Coprococcus* by current smoking status. The difference between the groups was analyzed by the Mann–Whitney U test adjusted with the Benjamini-Hochberg method; * \( q < 0.1 \).

Multiple regression analysis using current smoking status, age, exercise habits, alcohol intake, sugar and sweetener intake, and fruit intake as covariates showed that smoking was associated with the proportion of the *Coprococcus* genus (Table 4).

**Table 4.** Multiple regression analysis of the proportion of genus *Coprococcus*.

|                          | Standardized Coefficient | \( p \) Value |
|--------------------------|--------------------------|---------------|
| Current smoking          | 0.253                    | <0.001        |
| Age (years)              | −0.068                   | 0.353         |
| Exercise                 | −0.050                   | 0.495         |
| Alcohol intake (g/day)   | −0.039                   | 0.593         |
| Sugar and sweeteners intake (g/day) | −0.055 | 0.443         |
| Fruits intake (g/day)    | 0.060                    | 0.404         |

Smoking status was defined as non-current smoker (=0) or current smoker (=1), and exercise status was defined as non-regular exercise (=0) or regular exercise (=1).

4. Discussion

This study of individuals with type 2 diabetes mellitus examined the association between smoking and dietary habits and between smoking and the gut microbiota. Current smokers consumed more alcohol, sugar, and sweeteners, and less fruit. We also reported that the gut microbiota of current smokers has a higher proportion of *Coprococcus* at the genus level. Current smoking was associated with a higher proportion of the *Coprococcus* genus even after adjusting for background and dietary factors related to current smoking.

Recent studies have suggested that smoking may alter the gut microbiota. Although clinical studies have been reported in healthy people [6,40], patients with inflammatory bowel disease [41,42], and patients with coronary artery disease [39], this is the first study to evaluate the association between smoking and the gut microbiota in a population of people with type 2 diabetes mellitus. Furthermore, despite many studies reporting that smoking affects eating habits [43,44], previous studies on smoking and the gut microbiota found no difference in dietary intake according to smoking status or did not assess dietary intake. A significant finding of this study is the association between current smoking and dietary intake. After considering the effects of these factors, we identified the relationship between current smoking and the gut microbiota.
A previous large cross-sectional study found no significant differences in the composition of the gut microbiota between those who had never smoked and former smokers, indicating that recovery of the gut microbiota composition to its pre-smoking status is likely to occur if smokers quit smoking [6]. Another longitudinal study on changes in the gut microbiota before and after smoking cessation showed that the effects of smoking cessation on the gut microbiota occurred quickly [12]. Similarly, the change in eating habits due to smoking cessation is also expected to occur relatively rapidly, as taste recovery was observed after two weeks [45] and weight gain appeared within three months [46] after smoking cessation. Therefore, in this study, the gut microbiota and dietary intake were compared between two groups depending on current smoking status.

The dietary habits of smokers are characterized by a low intake of vegetables and fruits, high intake of meat and alcoholic beverages, and low intake of antioxidant β-carotene and vitamin C [43,44]. The current smokers in this study also had high alcohol and low fruit intake, similar to previous findings. In contrast, the intake of vegetables and antioxidant nutrients did not differ between current and non-current smokers. This may be because of the influence of daily dietary advice and encouragement of vegetable consumption as part of the treatment of diabetes mellitus. It was also noted that the validity of the BDHQ used in this study may have been lower if only men responded without the advice from their wives [30]. Remarkably, the amounts of sugar and sweeteners used in coffee, tea, and cooking were higher among smokers in this study. Excessive intake of sucrose has been suggested to cause dysbiosis [47], and it is important to evaluate the intake of sucrose. However, the BDHQ is unreliable for measuring sucrose consumption and it is difficult to estimate the amount of sucrose consumed.

The relationship between smoking and the gut microbiota has been investigated in terms of diversity, composition, and function. Many studies have reported a reduction in the diversity of bacterial species in fecal samples from smokers [48]. Several studies have shown that smokers have a higher proportion of the Bacteroidetes phylum than nonsmokers [6,41,42]. Furthermore, there have been several reports of higher proportions of Prevotella in smokers [49–51]. The present study did not find results similar to those previously reported; however, new findings were obtained for the genus Coprococcus. The genus Coprococcus plays an important role as a protective factor against Clostridium difficile infection, and its proportion has been reported to decrease with strong inhibition of gastric acid secretion [52]. Smoking stimulates gastric acid secretion [53], which may increase the proportion of Coprococcus.

The mechanisms by which smoking affects the gut microbiota include immunosuppression, increased oxidative stress, altered gut barrier function, and changes in acid-base equilibrium [54]. Cigarette smoke contains nicotine, aldehydes, polycyclic aromatic hydrocarbons, heavy metals, toxic gases, and volatile organic compounds. The effects of each of these substances on the gut microbiota have been studied; however, the co-impact of multiple toxic substances in cigarette smoke on the gut microbiota remains unknown [55]. In addition, alcohol consumption, which was positively correlated with current smoking in this study, was reported to be associated with gut dysbiosis [56]. High fat, high sugar, and low fiber diets are also thought to cause gut dysbiosis in smokers [57]. Therefore, smoking could affect the gut microbiota not only directly through toxic substances but also indirectly through changes in dietary intake. Moreover, animal studies have indicated that a fiber-free diet further contributes to the reduction in antioxidant capacity caused by smoking [58], and smoking is expected to have a synergistic direct and indirect negative effect.

This study had several limitations. The study population of individuals with type 2 diabetes mellitus was originally prone to dysbiosis, and changes in the gut microbiota due to smoking may not have been adequately assessed. Moreover, if the direct effects of smoking on the gut microbiota are offset by indirect effects via dietary changes, they may not be detected as differences in the gut microbiota of current and non-current smokers. The quantitative impact of the number of cigarettes smoked or years of smoking was not
evaluated in this study. Additionally, since this was a cross-sectional study, the causal relationship between changes in the gut microbiota, smoking, and diet is unknown.

Smoking cessation is important for people with diabetes because smoking has a significant negative influence on the pathogenesis of diabetes and its complications. Unhealthy diets consumed by patients with diabetes may have been influenced by smoking. However, many people are reluctant to quit smoking because of weight gain concerns due to smoking cessation. Weight gain associated with smoking cessation has been shown to not reduce the long-term benefit of smoking cessation in reducing cardiovascular and all-cause mortality [11,59]. More research is needed to clarify the relationship between smoking cessation and the gut microbiota, diet, and body weight because if means can be found to control weight gain with smoking cessation, more people are likely to quit smoking, and the benefits of smoking cessation would increase.

5. Conclusions

This study revealed that both dietary intake and the gut microbiota are associated with current smoking status in men with type 2 diabetes mellitus. However, even after adjusting for the effects of smoking on dietary intake, associations between current smoking and the gut microbiota were still observed. The results of this study suggest that although dietary intake should be considered when examining gut microbiota associations, the non-dietary effects of smoking are more significant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14224800/s1, Table S1: The proportions of phyla by current smoking status; Table S2: The proportions of genera by current smoking status.

Author Contributions: Conceptualization, M.H.; methodology, Y.K. and Y.H.; software, M.F.; validation, Y.K. and Y.H.; formal analysis, Y.K. and Y.H.; investigation, Y.K., Y.H., M.H., S.K., K.M., K.U., T.T. and Y.N.; resources, M.F.; data curation, Y.K., Y.H., A.K. and R.S.; writing—original draft preparation, Y.K. and Y.H.; writing—review and editing, Y.K., Y.H., M.H., A.K., R.S., R.I., S.K., K.M., K.U., T.T., Y.N. and M.F.; supervision, M.F.; funding acquisition, Y.N. and M.F.; visualization, Y.K. and Y.H.; project administration, M.H., T.T., Y.N. and M.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partly supported by MAFF Commissioned project study (Grant Number JPJ009842) on “Project for the realization of foods and dietary habits to extend healthy life expectancy” to Y.N. No funding bodies played a role in this study.

Institutional Review Board Statement: The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Research Ethics Committee of KPUM (no. ERB-C-534 and no. RBMR-E-466-5).

Informed Consent Statement: Informed consent was obtained from all the participants involved in the study.

Data Availability Statement: The sequence data used in this study were submitted to the Sequence Read Archive (SRA) with the accession number PRJNA766337 (available on 1 November 2021).

Conflicts of Interest: Y.H. received personal fees from Ono Pharmaceutical Co., Ltd., Sanoﬁ K.K., Novo Nordisk Pharma Ltd., Takeda Pharmaceutical Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Mitsubishi Tanabe Pharma Corp., Daiichi Sankyo Co. Ltd., and Kowa Company, Ltd., separate from the submitted work. M.H. received grants from Nippon Boehringer Ingelheim Co. Ltd., Yamada Bee Farm, Oishi Kenko Inc., AstraZeneca K.K., and Ono Pharma Co. Ltd., and personal fees from Mitsubishi Tanabe Pharma Corp., Ono Pharma Co. Ltd., Sanofi K.K., Lilly Japan, AstraZeneca K.K., Daiichi Sankyo Co. Ltd., Sumitomo Dainippon Pharma Co., Ltd., and Kowa Pharma Co. Ltd., separate from the submitted work. T.T. received a collaborative research fund from FUJIFILM Corp. separate from the submitted work. Y.N. received a collaboration research fund from Fujifilm Medical Co., Ltd., a scholarship from EA Pharma Co., Ltd., and lecture fees from EA Pharma Co., Ltd., Mochida Pharma Co., Ltd., Janssen Pharma K.K., Otsuka Pharma Co., Ltd., Astellas Pharma Co., Ltd., Mylan EPD Co., Takeda Pharma Co., Ltd., and Miyarisan Pharma Co., Ltd. This study was partially supported by these funds. Neither the funding agency nor any outside organization participated
in the study design nor had any competing interests. All the pharmaceutical companies approved the final version of the manuscript. Moreover, M.I. received grants from Sanofi K.K., Kyowa Kirin Co., Ltd., Takeda Pharma Co. Ltd., Nippon Boehringer Ingelheim Co. Ltd., Sumitomo Dainippon Pharma Co., Ltd., Ono Pharma Co. Ltd., Oishi Kenko inc., Kissei Pharma Co. Ltd., Daiichi Sanky Co. Ltd., Nippon Chemiphar Co., Ltd., Teijin Pharma Co., Ltd., Terumo Corp., Abbott Japan Co. Ltd., Astellas Pharma Inc., MSD K.K., Kowa Pharma Co. Ltd., Mitsubishi Tanabe Pharma Corp., Novo Nordisk Pharma Ltd., Sanwa Kagaku Kenkyusho CO., Ltd., Eli Lilly, Japan, K.K., Yamada Bee Farm, TERUMO CORPORATION, and Johnson & Johnson K.K. Medical Co., and received personal fees from Takeda Pharma Co. Ltd., Bayer Yakuhin, Ltd., Novo Nordisk Pharma Ltd., Taisho Pharma Co., Ltd., Mochida Pharma Co. Ltd., AstraZeneca K.K., Sanofi K.K., Daiichi Sanky Co. Ltd., Nippon Boehringer Ingelheim Co., Ltd., Arkray Inc., Kissei Pharma Co., Ltd., Mitsubishi Tanabe Pharma Corp., Astellas Pharma Inc., MSD K.K., Teijin Pharma Ltd., Medtronic Japan Co. Ltd., Kowa Kirin Co. Ltd., Sumitomo Dainippon Pharma Co. Ltd., Kowa Pharma Co. Ltd., Ono Pharma Co. Ltd., Sanwa Kagaku Kenkyusho Co. Ltd., Eli Lilly Japan K.K., Abbott Japan Co. Ltd., TERUMO CORPORATION, and Nipro Corp., separate from the submitted work. outside of the submitted work. The other authors have no conflict of interest to declare.

References

1. Chang, S.A. Smoking and Type 2 Diabetes Mellitus. *Diabetes Metab. J.*, 2012, 36, 399–403. [CrossRef]
2. Bergman, B.C.; Perreault, L.; Hunerdosse, D.; Kerege, A.; Playdon, M.; Samek, A.M.; Eckel, R.H. Novel and Reversible Mechanisms of Smoking-Induced Insulin Resistance in Humans. *Diabetes 2012*, 61, 3156–3166. [CrossRef] [PubMed]
3. Garbin, U.; Fratta Pasini, A.; Stranieri, C.; Cominacinii, M.; Pasini, A.; Manfro, S.; Lugoboni, F.; Mozzini, C.; Guidi, G.; Faccini, G.; et al. Cigarette Smoking Blocks the Protective Expression of Nr2f2/ARE Pathway in Peripheral Mononuclear Cells of Youn

Heavy Smokers Favouring Inflammation. *PLoS ONE 2009*, 4, e8225. [CrossRef]
4. Zeiher, A.M.; Schächinger, V.; Minners, J. Long-Term Cigarette Smoking Impairs Endothelium-Dependent Coronary Arterial Vasodilator Function. *Circulation 1995*, 92, 1094–1100. [CrossRef] [PubMed]
5. Tong, X.; Chaudhry, Z.; Lee, C.-C.; Bone, R.N.; Kanojia, S.; Maddatu, J.; Sohn, P.; Weaver, S.A.; Robertson, M.A.; Petrocha, I.; et al. Cigarette Smoke Exposure Impairs β-Cell Function through Activation of Oxidative Stress and Ceramide Accumulation. *Mol. Metab. 2020*, 37, 100975. [CrossRef]
6. Lee, S.H.; Yun, Y.; Kim, S.J.; Lee, E.-J.; Chang, Y.; Ryu, S.; Shin, H.; Kim, H.-L.; Kim, H.-N.; Lee, J.H. Association between Cigarette Smoking Status and Composition of Gut Microbiota: Population-Based Cross-Sectional Study. *J. Clin. Med. 2018*, 7, 282. [CrossRef]
7. Nicholson, J.K.; Holmes, E.; Kinross, J.; Burcelin, R.; Gibson, G.; Jia, W.; Pettersson, S. Host-Gut Microbiota Metabolic Interactions. *Science 2012*, 336, 1262–1267. [CrossRef]
8. Hooper, L.V.; Litman, D.R.; Macpherson, A.J. Interactions between the Microbiota and the Immune System. *Science 2012*, 336, 1268–1273. [CrossRef]
9. Gurung, M.; Li, Z.; You, H.; Rodrigues, R.; Jump, D.B.; Morgun, A.; Shulzhenko, N. Role of Gut Microbiota in Type 2 Diabetes Pathophysiology. *Ebiomedicine 2020*, 51, 102590. [CrossRef]
10. Sokol, H.; Leducq, V.; Aschard, H.; Pham, H.-P.; Jegou, S.; Landman, C.; Cohen, D.; Liguori, G.; Bourrier, A.; Nion-Larmurier, I.; et al. Fungal Microbiota Dysbiosis in IBD. *Cir. 2017*, 66, 1039–1048. [CrossRef]
11. Hu, Y.; Zong, G.; Liu, G.; Wang, M.; Rosner, B.; Pan, A.; Willett, W.C.; Manson, J.E.; Hu, F.B.; Sun, Q. Smoking Cessation, Weight Change, Type 2 Diabetes, and Mortality. *N. Engl. J. Med. 2018*, 379, 623–632. [CrossRef]
12. Biedermann, L.; Zeitz, J.; Mvinyi, J.; Sutter-Minder, E.; Rehman, A.; Ott, S.J.; Steurer-Stey, C.; Frei, A.; Frei, P.; Scharl, M.; et al. Smoking Cessation Induces Profound Changes in the Composition of the Intestinal Microbiota in Humans. *PLoS ONE 2013*, 8, e9260. [PubMed]
13. Biedermann, L.; Brüllsauer, K.; Zeitz, J.; Frei, P.; Scharl, M.; Vavricka, G.; Fried, M.; Nöthniger, M.J.; Rogler, G.; Schuppler, M.; Brüllsauer, K. Smoking Cessation Alters Intestinal Microbiota: Insights from Quantitative Investigations on Human Fecal Samples Using FISH. *Mol. Cell. 2014*, 20, 1496–1501. [CrossRef] [PubMed]
14. Berkowitz, L.; Schultz, B.M.; Salazar, G.A.; Pardo-Roa, C.; Sebastián, V.P.; Álvarez-Lobos, M.M.; Bueno, S.M. Impact of Cigarette Smoking on the Gastrointestinal Tract Inflammation: Opposing Effects in Crohn’s Disease and Ulcerative Colitis. *Front. Immunol. 2018*, 9, 74. [CrossRef]
15. Alkerwi, A.; Baydarlioglu, B.; Sauvageot, N.; Stranges, S.; Lemmens, P.; Shivappa, N.; Hébert, J.R. Smoking Status Is Inversely Associated with Overall Diet Quality: Findings from the ORISCAV-LUX Study. *Clin. Nutr. 2017*, 36, 1275–1282. [CrossRef]
16. Anker, J.J.; Nakajima, M.; Raatz, S.; Allen, S.; Al’Absi, M. Tobacco Withdrawal Increases Junk Food Intake: The Role of the Endogenous Opiod System. *Drug Alcohol. Depend. 2021*, 225, 108819. [CrossRef] [PubMed]
17. MacLean, R.R.; Cowan, A.; Vernarelli, J.A. More to Gain: Dietary Energy Density Is Related to Smoking Status in US Adults. *BMC Public Health 2018*, 18, 365. [CrossRef]
26. Hashimoto, Y.; Hamaguchi, M.; Kaji, A.; Sakai, R.; Osaka, T.; Inoue, R.; Kashiwagi, S.; Mizushima, K.; Uchiyama, K.; Takagi, T.; et al. Prediction of Functional Profiles of Gut Microbiota from 16S RRNA Metagenomic Data Provides a More Robust Evaluation of Gut Dysbiosis Occurring in Japanese Type 2 Diabetic Patients. *J. Clin. Biochem. Nutr.* 2017, 61, 217–221. [CrossRef] [PubMed]

27. Kondo, Y.; Hashimoto, Y.; Hamaguchi, M.; Ando, S.; Kaji, A.; Sakai, R.; Inoue, R.; Kashiwagi, S.; Mizushima, K.; Uchiyama, K.; et al. Unique Habitual Food Intakes in the Gut Microbiota Cluster Associated with Type 2 Diabetes Mellitus. *Nutrients* 2021, 13, 3816. [CrossRef]

28. Hashimoto, Y.; Kaji, A.; Sakai, R.; Takahashi, F.; Kawano, R.; Hamaguchi, M.; Fukushima, M. Effect of Exercise Habit on Skeletal Muscle Mass Varies with Protein Intake in Elderly Patients with Type 2 Diabetes: A Retrospective Cohort Study. *Nutrients* 2020, 12, 3220. [CrossRef]

29. Matsuo, S.; Imai, E.; Horio, M.; Yasuda, Y.; Tomita, K.; Nitta, K.; Yamagata, K.; Tomino, Y.; Yokoyama, H.; Hishida, A.; et al. Revised Equations for Estimated GFR From Serum Creatinine in Japan. *Am. J. Kidney Dis.* 2009, 53, 982–992. [CrossRef]

30. Kobayashi, S.; Murakami, K.; Sasaki, S.; Okubo, H.; Hirota, N.; Notsu, A.; Fukushima, M.; Date, C. Comparison of Relative Validity of Food Group Intakes Estimated by Comprehensive and Brief-Type Self-Administered Diet History Questionnaires against 16 d Dietary Records in Japanese Adults. *Public Health Nutr.* 2011, 14, 1200–1211. [CrossRef]

31. Kobayashi, S.; Honda, S.; Murakami, K.; Sasaki, S.; Okubo, H.; Hirota, N.; Notsu, A.; Fukushima, M.; Date, C. Both Comprehensive and Brief Self-Administered Diet History Questionnaires Satisfactorily Rank Nutrient Intakes in Japanese Adults. *J. Epidemiol.* 2012, 22, 151–159. [CrossRef] [PubMed]

32. Murakami, K.; Sasaki, S.; Takahashi, Y.; Okubo, H.; Hosoi, Y.; Horiguchi, H.; Oguma, E.; Kayama, F. Dietary Glycemic Index and Load in Relation to Metabolic Risk Factors in Japanese Female Farmers with Traditional Dietary Habits. *Am. J. Clin. Nutr.* 2006, 83, 1161–1169. [CrossRef] [PubMed]

33. Lemmens, H.J.M.; Brodsky, J.B.; Bernstein, D.P. Estimating Ideal Body Weight—a New Formula. *Obes. Surg.* 2005, 15, 1082–1083. [CrossRef]

34. Kaji, A.; Hashimoto, Y.; Sakai, R.; Okada, H.; Hamaguchi, M.; Ushigome, E.; Majima, S.; Yamazaki, M.; Fukushima, M. Frequent Usage of Convenience Stores Is Associated with Low Diet Quality. *Nutrients* 2019, 11, 1212. [CrossRef]

35. Inoue, R.; Ohue-Kitano, R.; Tsukahara, T.; Tanaka, M.; Masuda, S.; Inoue, T.; Yamakage, H.; Kusakabe, T.; Hasegawa, K.; Shimatsu, A.; et al. Prediction of Functional Profiles of Gut Microbiota from 16S RNA Metagenomic Data Provides a More Robust Evaluation of Gut Dysbiosis Occurring in Japanese Type 2 Diabetic Patients. *J. Clin. Biochem. Nutr.* 2017, 61, 217–221. [CrossRef] [PubMed]

36. Nishino, K.; Nishida, A.; Inoue, R.; Kawada, Y.; Ohno, M.; Sakai, S.; Inatomi, O.; Bamba, S.; Sugimotori, M.; Kawahara, M.; et al. Analysis of Endoscopic Brush Samples Identified Mucosa-Associated Dysbiosis in Inflammatory Bowel Disease. *J. Gastroenterol.* 2018, 53, 95–106. [CrossRef]

37. Takagi, T.; Naito, Y.; Kashiwagi, S.; Uchiyama, K.; Mizushima, K.; Kamada, K.; Ishikawa, T.; Inoue, R.; Okuda, K.; Tsujimoto, Y.; et al. Changes in the Gut Microbiota Are Associated with Hypertension, Hyperlipidemia, and Type 2 Diabetes Mellitus in Japanese Subjects. *Nutrients* 2020, 12, 2996. [CrossRef] [PubMed]

38. Janssen, S.; McDonald, D.; Gonzalez, A.; Navas-Molina, J.A.; Jiang, L.; Xu, Z.Z.; Winker, K.; Kado, D.M.; Orwoll, E.; Manary, M.; et al. Phylogenetic Placement of Exact Amplicon Sequences Improves Associations with Clinical Information. *Systems* 2018, 3, e00012-18. [CrossRef]

39. Hu, X.; Fan, Y.; Li, H.; Zhou, R.; Zhao, X.; Sun, Y.; Zhang, S. Impacts of Cigarette Smoking Status on Metabolomic and Gut Microbiota Profile in Male Patients With Coronary Artery Disease: A Multi-Omics Study. *Front. Cardiovasc. Med.* 2021, 8, 766739. [CrossRef] [PubMed]

40. Yan, S.; Ma, Z.; Jiao, M.; Wang, Y.; Li, A.; Ding, S. Effects of Smoking on Inflammatory Markers in a Healthy Population as Analyzed via the Gut Microbiota. *Front. Cell Infect. Microbiol.* 2021, 11, 633242. [CrossRef]
41. Benjamin, J.L.; Hedin, C.R.H.; Koutsoumpas, A.; Ng, S.C.; McCarthy, N.E.; Prescott, N.J.; Pessoa-Lopes, P.; Mathew, C.G.; Sanderson, J.; Hart, A.L.; et al. Smokers with Active Crohn’s Disease Have a Clinically Relevant Dysbiosis of the Gastrointestinal Microbiota. *Inflamm. Bowel Dis.* 2012, 18, 1092–1100. [CrossRef] [PubMed]

42. Opstelten, J.L.; Plassais, J.; van Mil, S.W.C.; Achouri, E.; Pichaud, M.; Siersema, P.D.; Oldenburg, B.; Cervino, A.C.L. Gut Microbial Diversity Is Reduced in Smokers with Crohn’s Disease. *Inflamm. Bowel Dis.* 2016, 22, 2070–2077. [CrossRef] [PubMed]

43. Subar, A.F.; Harlan, L.C.; Mattson, M.E. Food and Nutrient Intake Differences between Smokers and Non-Smokers in the US. *Am. J. Public Health* 1990, 80, 1323–1329. [CrossRef] [PubMed]

44. Marangon, K.; Hereth, B.; Lecomte, E.; Paul-Dauphin, A.; Grolier, P.; Chancerelle, Y.; Artur, Y.; Siest, G. Diet, Antioxidant Status, and Smoking Habits in French Men. *Am. J. Clin. Nutr.* 1998, 67, 231–239. [CrossRef] [PubMed]

45. Chéruel, F.; Jarlier, M.; Sancho-Garnier, H. Effect of Cigarette Smoke on Gustatory Sensitivity, Evaluation of the Deficit and of the Recovery Time-Course after Smoking Cessation. *Tox. Induc. Dis.* 2017, 15. [CrossRef] [PubMed]

46. Aubin, H.-J.; Farley, A.; Lyckett, D.; Lahmek, P.; Aveyard, P. Weight Gain in Smokers after Quitting Cigarettes: Meta-Analysis. *BMJ* 2012, 345, e4439. [CrossRef]

47. Sun, S.; Araki, Y.; Hanzawa, F.; Umeki, M.; Kojima, T.; Nishimura, N.; Ikeda, S.; Mochizuki, S.; Oda, H. High Sucrose Diet-Induced Dysbiosis of Gut Microbiota Promotes Fatty Liver and Hyperlipidemia in Rats. *J. Nutr. Biochem.* 2021, 93, 108621. [CrossRef]

48. Antinozzi, M.; Giffi, M.; Sini, N.; Gallè, F.; Valeriani, F.; De Vito, C.; Liguori, G.; Romano Spica, V.; Cattaruzza, M.S. Cigarette Smoking and Human Gut Microbiota in Healthy Adults: A Systematic Review. *Biomedicines* 2022, 10, 510. [CrossRef]

49. Stewart, C.J.; Auchtung, T.A.; Ajami, N.J.; Velasquez, K.; Chancerelle, Y.; Artur, Y.; Siest, G. Diet, Antioxidant Status, and Smoking Habits in French Men. *Am. J. Clin. Nutr.* 1998, 67, 231–239. [CrossRef] [PubMed]

50. Curtis, K.; Stewart, C.J.; Robinson, M.; Molfese, D.L.; Gosnell, S.N.; Kosten, T.R.; Petrosino, J.F.; de La Garza, R.; Salas, R. Insulin Resting State Functional Connectivity Is Associated with Gut Microbiota Diversity. *Eur. J. Neurosci.* 2019, 50, 2446–2452. [CrossRef]

51. Prakash, A.; Peters, B.A.; Cobbs, E.; Beggs, D.; Choi, H.; Li, H.; Hayes, R.B.; Ahn, J. Tobacco Smoking and the Fecal Microbiome in a Large, Multi-Ethnic Cohort. *Cancer Epidemiol. Biomarkers Prev.* 2021, 30, 1328–1335. [CrossRef] [PubMed]

52. Otsuka, T.; Sugimoto, M.; Inoue, R.; Ohno, M.; Ban, H.; Nishida, A.; Inatomi, O.; Takahashi, S.; Naito, Y.; Andoh, A. Influence of Potassium-Competitive Acid Blocker on the Gut Microbiome of Helicobacter Pylori-Negative Healthy Individuals. *Gut* 2017, 66, 1723–1725. [CrossRef] [PubMed]

53. Maity, P.; Biswas, K.; Roy, S.; Banerjee, R.K.; Bandyopadhyay, U. Smoking and the Pathogenesis of Gastroduodenal Ulcer–Recent Mechanistic Update. *Mol. Cell Biochem.* 2003, 253, 329–338. [CrossRef] [PubMed]

54. Huang, C.; Shi, G. Smoking and Microbiome in Oral, Airway, Gut and Some Systemic Diseases. *J. Transl. Med.* 2019, 17, 225. [CrossRef] [PubMed]

55. Gui, X.; Yang, Z.; Li, M.D. Effect of Cigarette Smoke on Gut Microbiota: State of Knowledge. *Front. Physiol.* 2021, 12, 673341. [CrossRef]

56. Qamar, N.; Castano, D.; Patt, C.; Chu, T.; Cottrell, J.; Chang, S.L. Meta-Analysis of Alcohol Induced Gut Dysbiosis and the Resulting Behavioral Impact. *Behav. Brain Res.* 2023, 376, 112196. [CrossRef] [PubMed]

57. Benitez-Páez, A.; Gómez Del Pulgar, E.M.; Kjølbaek, L.; Brahe, L.K.; Astrup, A.; Larsen, L.H.; Sanz, Y. Impact of Dietary Fiber and Fat on Gut Microbiota Re-Modeling and Metabolic Health. *Trends Food Sci. Technol.* 2016, 57, 201–212. [CrossRef]

58. Tomoda, K.; Kubo, K.; Asahara, T.; Nomoto, K.; Nishii, Y.; Yamamoto, Y.; Yoshikawa, M.; Kimura, H. Suppressed Anti-Oxidant Capacity Due to a Cellulose-Free Diet Declines Further by Cigarette Smoke in Mice. *J. Toxicol. Sci.* 2012, 37, 575–585. [CrossRef]

59. Chen, S.; Kawasaki, Y.; Hu, H.; Kuwahara, K.; Yamamoto, M.; Uehara, A.; Honda, T.; Yamamoto, S.; Nakagawa, T.; Miyamoto, T.; et al. Smoking Cessation, Weight Gain, and the Trajectory of Estimated Risk of Coronary Heart Disease: 8-Year Follow-up From a Prospective Cohort Study. *Nicotine Tob. Res.* 2021, 23, 85–91. [CrossRef]