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Basic Study

Characterization of gut microbial and metabolite alterations in faeces of Goto Kakizaki rats using metagenomic and untargeted metabolomic approach

Zhao JD *et al.* Gut microbial and metabolite characterization of GK

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Abstract

BACKGROUND

⁹ In recent years, the incidence of type 2 diabetes (T2DM) has shown a rapid growth trend. Goto Kakizaki (GK) rats are a valuable model for the study of T2DM and share common glucose metabolism features with human T2DM patients. A series of studies indicate that ⁸ T2DM is associated with the gut microbiota composition and gut metabolites. We aimed to systematically characterize the faecal gut microbes and metabolites of GK rats and analyse the relationship between glucose and insulin resistance.

AIM

To evaluate the gut microbial and metabolite alterations in GK rat faeces based on metagenomics and untargeted metabolomics.

METHODS

Ten GK rats (model group) and Wistar rats (control group) (all 5-6 wk old) were observed for 10 wk. Indexes related to glucose, mainly including weight, ²⁴ fasting blood glucose

(FBG) and insulin levels, homeostasis model assessment (HOMA) of insulin resistance (IR), and HOMA- β , were assessed. The faecal gut microbiota was sequenced by metagenomics, and faecal metabolites were analysed by untargeted metabolomics. Multiple metabolic pathways were evaluated based on the differential metabolites identified, and the correlations between blood glucose and the gut microbiota and metabolites were analysed.

RESULTS

The model group displayed significant differences in weight, FBG, insulin, HOMA-IR and HOMA- β ($P < 0.05$, $P < 0.01$) and a shift in the gut microbiota structure compared with the control group. The results demonstrated significantly decreased abundances of *Prevotella* sp. CAG:604 and *Lactobacillus murinus* ($P < 0.05$) and a significantly increased abundance of *Allobaculum stercoricanis* ($P < 0.01$) in the model group. A correlation analysis indicated that FBG and HOMA-IR were positively correlated with *Allobaculum stercoricanis* and negatively correlated with *Lactobacillus murinus*. orthogonal partial least squares discriminant analysis suggested that the faecal metabolic profiles differed between the model and control groups. Fourteen potential metabolic biomarkers, including glycochenodeoxycholic acid, uric acid, 13(S)-hydroxyoctadecadienoic acid (HODE), N-acetylaspartate, β -sitosterone, sphinganine, 4-pyridoxic acid, and linoleic acid, were identified. Moreover, FBG and HOMA-IR were positively correlated with glutathione, 13(S)-HODE, uric acid, 4-pyridoxic acid and allantoic acid and negatively correlated with 3- α , 7- α , chenodeoxycholic acid glycine conjugate and 26-trihydroxy-5- β -cholestane ($P < 0.05$, $P < 0.01$). *Allobaculum stercoricanis* was positively correlated with linoleic acid and sphinganine ($P < 0.01$), and 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylate was negatively associated with *Prevotella* sp. CAG:604 ($P < 0.01$). The metabolic pathways showing the largest differences were arginine biosynthesis; primary bile acid biosynthesis; purine metabolism; linoleic acid metabolism; alanine, aspartate and glutamate metabolism; and nitrogen metabolism.

CONCLUSION

Metagenomics and untargeted metabolomics indicated that disordered compositions of gut microbes and metabolites may be common defects in GK rats.

Key Words: Type 2 diabetes mellitus; Gut microbial; Metabolites; Goto-Kakizaki rats; Metagenomic; Untargeted metabolomic

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Core Tip: Studies suggested that the gut microbial and metabolites has an essential role in Goto Kakizaki rats. ³ The results revealed evidence of a decrease in *Prevotella* sp. CAG:604 and *Lactobacillus_murinus*. *Allobaculum_stercoricanis* is increase. Fourteen potential metabolism biomarkers were glycochenodeoxycholic acid, uric acid, N-acetylaspartate, β -sitosterone, sphinganine, 4-pyridoxic acid, 13(S)-hydroxyoctadecadienoic acid, linoleic acid, *et al.*

INTRODUCTION

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In recent years, the incidence of type 2 diabetes (T2DM) has shown a rapid growth trend in China, where its current prevalence is 12.8%^[1]. T2DM is a metabolic disorder whose major pathological hallmark is insulin resistance^[2]. Goto-Kakizaki (GK) noninsulin-dependent rats are a valuable animal model for studies of T2DM^[3]. At 4 wk of age, GK rats exhibit insulin resistance, basal hyperglycaemia development, increased hepatic glucose production, and impaired insulin secretion^[4,5]. GK rats share common features with human T2DM. Therefore, the results obtained using GK rats as the research subjects will be more similar to those of patients with T2DM than the results obtained in other systems and will thus provide more useful evidence for analysing the pathogenesis and treatment of T2DM.

The gut microbiota of the host constitutes a massive, complex microecosystem. Exploring the composition of the gut microbiota will improve our understanding of the relationship with the host as one of the key factors in host health or disease. Recent studies suggest that both humans and animal models of T2DM and its complications, including GK rats, exhibit dysbiosis of the gut microbiota and further indicate that this characteristic microbiota imbalance, which may involve decreased bacterial diversity, contributes to the development of T2DM^[6-8]. In addition, studies have shown that undesirable shifts in metagenomic- and microbiota-associated metabolite production may negatively impact glucose tolerance and insulin resistance^[9].

Because the human dietary structure is complex and diverse, the changes in gut microbes and metabolites observed in different studies vary, even when the type and amount of diets administered to patients with T2DM (*e.g.*, dietary fibre contents or low-carbohydrate diets) and nutritional advice are controlled^[10-12]. In this study, we selected a fixed diet with balanced nutrient proportions for rats to avoid deviations in the results due to dietary differences. In animal models of diabetes, streptozotocin or alloxan is usually selected as an agent for damaging pancreatic function. This type of model is more similar to type 1 diabetes. In the classification of diabetes, T2DM accounts for approximately 90% of cases. Therefore, the further exploration of gut microbes and

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metabolites in a T2DM model is desired. Moreover, a joint analysis of the intestinal microbiota and metabolite structure has not previously been conducted^[13]. Therefore, the faeces of GK rats were collected as the study object, and this study aimed to systematically characterize the faecal gut microbes and metabolites of GK rats using metagenomic and untargeted metabolomic approaches and to analyse the relationship between gut microbes and metabolites with glucose and insulin resistance.

MATERIALS AND METHODS

Experimental design

Ten male GK rats (aged 5-6 wk) were procured from Changzhou Cavens Model Animal Co., Ltd. (Changzhou, China; certificate No. 202145537), and ten male Wistar rats were purchased from (Beijing) Biotechnology Co., Ltd. (Beijing, China; certificate No. 110324210106676238). The animals were acclimated in a controlled laboratory (25 ± 2 °C temperature, 60% ± 5% humidity) with free access to breeding feed and water. The Animal Committee of Anhui University of Chinese Medicine (Hefei, China; Approval AHUCM-rats-2021133) approved the experiments.

The animals were fed growth and reproduction feed during the 1-wk acclimatization period and were observed for 9 wk. The fasting blood glucose (FBG) level was measured, and nine rats in the model group showed levels exceeding 11.1 mmol/L, whereas the FBG level in the other rat was not as high. Additionally, one rat in the control group escaped due to poor management, and the analysis was thus performed using nine rats from each group. Body weight, food intake and FBG were measured every two weeks. FBG levels in blood sampled from the tail vein were measured with a glucose metre after food deprivation for 12 h overnight.

Sample collection

Eighteen rats were anaesthetized *via* the intraperitoneal administration of pentobarbital sodium (30 mg/kg, Merck, United States). Blood samples were collected in

nonheparinized tubes and centrifuged to obtain serum. The contents of the caecum were placed in Eppendorf tubes. The serum and caecal contents were stored at -80 °C^[14].

Faecal sample collection and metagenomic analysis

Total DNA was extracted from 1 g of faeces using a kit (Omega Bio-Tek, Norcross, GA, United States). We determined the concentration, purity and quality of the DNA^[15].

The extracted DNA (average length of 400 bp) was fragmented using a Covaris M220 ultrasonicator (Gene Company Limited, China). A paired-end library was constructed using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, United States). Sequencing was performed with an Illumina NovaSeq system (Illumina Inc., San Diego, CA, United States) using NovaSeq Reagent Kits according to the manufacturer's instructions^[16]. The paired-end Illumina reads were trimmed of adaptors, and reads with a low quality (length < 50 bp, quality value < 20, containing N bases) were removed by fastp^[17].

Metagenomic data were collected using MEGAHIT. Contigs with a length ≥ 300 bp were selected as the final assembly output and used for further gene annotation. Amino acid sequences from the predicted open reading frames with a length ≥ 100 bp were retrieved and translated from the NCBI database^[18]. The nonredundant gene catalogue was constructed using CD-HIT and aligned to high-quality reads using SOAP aligner.

Faecal metabolomic analysis

The supernatant was extracted from 200 mg of faeces and transferred to sample vials^[19]. Two microlitres of a sample was separated with an HSS T3 column and used for LC-MS/MS analysis. Mass spectrometric data were collected using a UHPLC-Q Exactive system (Thermo Fisher Scientific, Waltham, MA, United States) with an electrospray ionization source operating in positive- and negative-ion modes. Data acquisition was performed in data-dependent acquisition mode.

The raw LC-MS/MS data were preprocessed using Progenesis QI (Waters Corporation, Milford, MA, United States) software. Internal standard peaks and false-

positive peaks were removed from the data matrix, redundant signals were removed, and the peaks were pooled. In addition, the metabolites were searched and identified in the HMDB and Metlin databases.

Metabolites detected in at least 80% of any set of samples were retained^[20]. After filtering, the metabolite response intensity of the mass spectrum peaks was normalized using the sum-normalization method. Moreover, variables with a relative standard deviation > 30% relative to the quality control samples were removed, and log₁₀ Logarithmization was performed to obtain the final data matrix for subsequent analysis.

Statistical analysis

SPSS 23.0 (International Business Machines Corporation, NY, United States) and GraphPad Prism 9.0 (San Diego, CA, United States) software were used for statistical and graphical analyses with Student's *t* tests and fold difference analysis. The heatmap data were normalized via the z score method [$z = (x - \mu) / \sigma$] and graphically visualized using the pheatmap package.

Variance analysis was performed with the matrix file after data preprocessing. The R package ropls (Version 1.6.2) was used to perform orthogonal partial least squares discriminant analysis (OPLS-DA). The stability of the model was assessed by 7-cycle interactive validation. The significantly different metabolites were selected based on the variable importance in projection score (> 1) of OPLS-DA and the *P* value < 0.05 obtained by Student's *t* test^[21].

Differential metabolites were screened and mapped to their biochemical pathways through metabolic enrichment and pathway analysis based on the Kyoto Encyclopedia of Genes and Genomes (<http://www.genome.jp/kegg/>). These metabolites were classified according to their functions. Significantly enriched pathways were identified with MetaboAnalyst using degree centrality and Fisher's exact test (<https://www.metaboanalyst.ca/>). The molecular weights of potential biomarkers were obtained from biochemical databases (KEGG Compound, <https://www.genome.jp/kegg/compound/>). The data were analysed on the Majorbio

Cloud Platform (<https://www.majorbio.com>) and Personalbio Cloud Platform (<https://www.genescloud.cn/>).

RESULTS

Body weight

The initial body weights did not significantly differ between the groups ($P > 0.05$). In the final weeks of the experimental period, the model group had a significantly lower weight than the control group ($P < 0.05$; Figure 1).

FBG and insulin resistance

The FBG levels significantly differed from weeks 0 to 11, and over this time period, the significant difference became increasingly pronounced (Figure 2). The mean FBG of the model group exceeded 11.1 mmol/L at week 5 and was close to 16.7 mmol/L at week 11.

Starting from week 11, the fasting insulin level of the model group was significantly higher than that of the control group (Figure 3). Additionally, the HOMA-IR index of the model group was markedly higher than that of the control group from week 11 onward (Figure 4). Moreover, the HOMA- β index of the model group was significantly lower than that of the control group (Figure 5).

Community structure of the gut microbiota

In total, 4.15×10^{10} raw bases (bp) and 4.33×10^8 raw reads were obtained. Subsequently, 1.59×10^8 nonredundant genes were predicted from the reads after quality control and the removal of host sequences. In total, five domains, 14 kingdoms, 181 phylum, 316 classes, 551 orders, 940 families, 2764 genus and 12302 species were identified. At the domain level, the gut microbiota comprised bacteria, archaea, eukaryotes, viruses and unclassified. Bacteria accounted for more than 99.67% of the organisms in both groups.

A principal coordinate analysis of the distance matrix showed that the first and second principal components (PC1 and PC2, respectively) accounted for 12.19% and 9.19% of the observed variation, respectively (Figure 6). The boxplots illustrate the

distribution of different groups on the PC1 axis and reveal that the greatest difference existed between the control and model groups (Figure 7).

At the phylum level, Firmicutes, Bacteroidetes, Actinobacteria, Spirochaetes, Proteobacteria and Candidatus Saccharibacteria were identified as the main phylum (Figure 8). The relative abundances of Firmicutes and Bacteroidetes were 54.85% and 27.16% in the control group and 57.75% and 23.82% in the model group, respectively. Although no significant differences were detected, a greater abundance of Firmicutes and a lower abundance of Bacteroidetes were found in the model group than in the control group.

In addition to *Allobaculum*, which was the only genus showing a significant difference, *Prevotella*, *Bacteroides*, *Dorea*, *Phascolarctobacterium*, *Faecalibacterium* and *Ruminococcus* were enriched in the control group, and *Collinsella*, *Clostridium*, *Blautia* and *Lactobacillus* were the main components of the model group (Figure 9). Among the 30 species with the highest abundance, 3 species showed significant differences between the control and model groups ($P < 0.05$). A higher abundance of *Allobaculum stercoricanis* and lower abundances of *Prevotella* sp. CAG:604 and *Lactobacillus murinus* were found in the model group compared with the control group (Figure 10).

Based on the heatmap, we found that FBG and HOMA-IR were moderately positively correlated with *Allobaculum stercoricanis* and moderately negatively correlated with *Lactobacillus murinus* ($P < 0.05$, $P < 0.01$; Figure 11).

Metabolomic analysis

The faecal metabolic profile data of the control and model groups were separated by an OPLS-DA model, which indicated that the positive- and negative-ion metabolic profiles of the samples differed between the two groups. The evaluation parameters of the OPLS-DA models from the positive- and negative-ion profiles showed values of $R_2 = 0.941$ and $Q_2 = -0.199$ and $R_2 = 0.944$ and $Q_2 = -0.068$, respectively, demonstrating good explanation and prediction with $200 \times$ permutation testing. For the positive-ion profiles, the first principal component showed a value 14.00%, and the second principal component

showed a value of 30.70%. For the negative-ion profiles, the first principal component showed a value of 13.60%, and the second principal component showed a value of 32.10% (Figures 12-15).

We identified 815 positive-ion and 678 negative-ion metabolites, and 596 metabolites were found in the KEGG compound database. The differential metabolites were filtered in volcano plots and included 145 positive- and 106 negative-ion metabolites and 180 upregulated and 71 downregulated metabolites (Figure 16). The most reasonable molecular formula was obtained from a search of the KEGG Compound database. According to the abovementioned principle, 14 potential metabolic biomarkers were identified in GK rats (Table 1).

Based on the heatmap, we found that FBG and HOMA-IR were positively correlated with 13(S)-HODE, glutathione, uric acid, 4-pyridoxic acid and allantoinic acid and negatively associated with 3- α , 7- α , 26-trihydroxy-5- β -cholestane and chenodeoxycholic acid glycine conjugate ($P < 0.05$, $P < 0.01$; Figure 17).

Based on the heatmap, we found that sphinganine and linoleic acid were positively correlated with *Allobaculum stercoricanis* ($P < 0.01$) and that 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylate was negatively associated with *Prevotella*_sp._CAG:604 ($P < 0.01$; Figure 18).

The KEGG compound IDs of endogenous metabolites were then introduced into the MetaboAnalyst 5.0 system for pathway and visual analyses. Based on the faecal biomarker data, the enriched pathways were found to include vitamin B6 metabolism; primary bile acid biosynthesis; arginine biosynthesis; purine metabolism; alanine, aspartate and glutamate metabolism; linoleic acid metabolism; and nitrogen metabolism. Among these pathways, six pathways were found to show differences (Figure 19; Table 2). Figure 20 illustrates the key potential proteases of the metabolic pathways related to hyperglycaemia levels based on the KEGG database.

DISCUSSION

The aetiology and pathogenesis of T2DM are complex, and most experts believe that this disorder is a clinical syndrome caused by genetic and environmental factors. Because GK rats develop spontaneous diabetes and show insulin resistance, these rats constitute one of the best models for the study of T2DM. In clinical practice, we have found that some patients who suffer from T2DM do not consume a high-sugar and high-fat diet. Therefore, a high-sugar and high-fat diet was not selected in this study, which reduced the impacts of excess nutrition on glucose. Studies have shown that the glucose levels of 14- to 16-wk-old GK rats meet the diagnostic criteria for T2DM^[3]. In this study, at 14 wk, the GK rats appeared to drink and urinate more than the control rats, and their hair colour turned yellow. Before 12 wk, the GK rats were heavier than the control Wistar rats. However, at 14 wk, the body weight of the GK rats was lower than that of the control group, and the FBG level exceeded 11.1 mmol/L. By the 16th week, the body weight was further reduced, and the glucose level was further increased, exceeding 16 mmol/L. Furthermore, the increase in glucose was related to the increase in the HOMA-IR index and the decrease in the HOMA- β index. This finding may be related to the observed decrease in the numbers of pancreatic β -cell islets, the irregular shape of islets, and the presence of amyloid degeneration. Some researchers believe that weight loss is related to a reduction in the Firmicutes/Bacteroidetes ratio, but this phenomenon is not observed universally^[22,23] and was not found in our research. Muñoz-Garach *et al*^[24] believe that weight loss is related to a reduction in the level of bile acid, and the results of the present study are consistent with this view.

A clear separation among the communities was observed between the control and model groups, which suggested that the gut microbiota shows substantial differences between the diabetes and nondiabetes groups. At the phylum level, Bacteroidetes and Firmicutes were dominant in GK and Wistar rats, with proportions greater than 80%. Although no significant differences were detected, a higher relative abundance of Firmicutes and a lower relative abundance of Bacteroidetes were found in the model group than in the control group. These findings may be related to the fact that the rats were fed breeding feed after weaning. These results may also be related to the shorter

course of disease in the T2DM rats because the gut microbiota is seriously disturbed, and the development of characteristic hyperglycaemia lags behind.

At the species level, a higher abundance of *Allobaculum stercoricanis* and lower abundances of *Prevotella* sp. CAG:604 and *Lactobacillus murinus* were found in the model group compared with the control group. *Allobaculum stercoricanis* belongs to the *Allobaculum* genus (Firmicutes), for which a significant difference was found at the genus level. A previous study showed that *Allobaculum* presented a 10.95-fold increase in HF diabetic mice compared with ND-fed mice^[25], and the corresponding increase found in this study was 2.75-fold. In a previous study, *Allobaculum* was identified as an important biomarker of metabolic disorders, particularly diabetes^[26,27]. This study also showed that *Allobaculum stercoricanis* was closely related to FBG, the HOMA-IR index and a decrease in the HOMA- β index. Different individual animals, even within the same species, show different feed conversion efficiencies. Animals with a high feed conversion efficiency show higher mRNA expression levels of insulin-like growth factor^[28], which can further regulate glucose. The abundance of *Prevotella* sp. CAG:604 is related to the feed conversion efficiency^[29]. This study revealed a decrease in the abundance of *Prevotella* sp. CAG:604, which may be one of the reasons for the diabetic phenotype of GK rats, and a direct negative correlation was found between these factors. *Prevotella* sp. CAG:604 could be considered a potential biomarker of diabetes. *Lactobacillus murinus* was decreased in GK rats, and this finding is consistent with the results reported by Cui *et al.*^[30] and Yue *et al.*^[31]. With increases in the abundance of *Lactobacillus murinus*, the glucose level and the HOMA-IR index showed a downwards trend. *Lactobacillus murinus* is considered a beneficial gastrointestinal microbe^[32]. In a previous study, a probiotic group whose members drank *Lactobacillus casei* strain milk showed a significant increase in the faecal count of *Lactobacillus*. The condition of patients with T2DM has also been controlled^[33]. We speculate that drinking milk containing *Lactobacillus murinus* may also control glucose levels.

The OPLS-DA model suggested that the positive- and negative-ion metabolic profiles differed between GK and Wistar rat faeces. Fourteen potential metabolic

biomarkers were assigned to bile acids, benzenoids, amino acids, steroids, amines, pyridine carboxylic acids and derivatives, and fatty acids. The topological analysis of the metabolic pathways showed that the difference between GK and Wistar rats was mainly due to arginine biosynthesis; ²⁸ primary bile acid biosynthesis; alanine, aspartate and glutamate metabolism; purine metabolism; linoleic acid metabolism; and ⁴⁵ nitrogen metabolism. ¹ These results were consistent with those of previous studies ^[34,35]. ²⁷ Metabolic pathway analysis showed that the differences were mainly due to amino acid metabolism, lipid metabolism, nucleotide metabolism, and energy metabolism. These pathways provide ideas for future research, and the results show that in addition to abnormal carbohydrate pathways, fat and protein are also metabolized in a disorderly manner leading to dysfunctions of the eyes, kidneys, blood vessels, and nerves, among other organs.

We observed marked decreases in the excretion of glycochenodeoxycholic acid and glycocholic acid in rat faeces, as reported by Sun *et al* ^[36]. The levels of glycochenodeoxycholic acid are increased in the liver, blood and ileum ^[36-38]. Lu *et al* ^[39] showed that increased plasma glycochenodeoxycholic acid levels are ⁹ associated with an increased risk of T2DM. The relationship among 3- α , 7- α , 26-trihydroxy-5- β -cholestane and T2DM has been less well studied; we found that 3- α , 7- α , 26-trihydroxy-5- β -cholestane was positively related to FBG and HOMA-IR, which may be a research topic that will be addressed in the future. Hyperuricaemia is particularly common in patients with T2DM ^[40]. Some clinical studies have observed that ursodeoxycholic acid, as the representative drug regulating bile acid metabolism, exerts a certain effect on reducing glucose, glycosylated haemoglobin A1c, and weight and increasing GLP-1 secretion ^[41,42]. Although ursodeoxycholic acid is not widely used in clinical practice, it may be used as a potential drug for the treatment of T2DM. In addition to excretion in the kidneys, uric acid can also be secreted into the intestine and metabolized by microorganisms ^[43]. This interaction could potentially modulate serum uric acid levels ^[44]. This study revealed an increase in the intestinal excretion of uric acid, indicating that uric acid in the body is

likely to be a risk factor for T2DM and to aggravate this condition. In ¹our study, we also found that uric acid was positively related to FBG and HOMA-IR.

In the presence of glutathione, a series of physiological reactions result in a low antioxidative defence capacity. β cells gradually die, and progression to T2DM occurs^[45]. This study showed that the glutathione level was increased in the GK group and was positively related to the FBG level and HOMA-IR. Hyperglycaemia is a potential risk factor for the N-acetylaspartate network^[46]. The present study also identified this phenomenon. The N-acetylaspartate level was higher in GK rats. A high level of N-acetyl-L-glutamic acid may result in increased blood pressure^[47]. Uncontrolled blood pressure can significantly increase the risk of macroangiopathy in diabetes. The relationship of allantoic acid with diabetes has not been studied, but the present study revealed that allantoic acid was positively related to FBG and HOMA-IR. At baseline, a previous study showed that amino acids are upregulated in T2DM patients. The dysregulation of amino acid metabolism may occur earlier than glucose metabolism to T2DM^[48]. This finding suggests that patients with T2DM should not consume a diet with a high protein level.

We found that the concentration of sphinganine was increased in GK rats. Sphinganine is positively related to *Lachnospiraceae* bacteria, *Allobaculum stercoricanis*, and *Lactobacillus johnsonii* and is negatively related to *Prevotella* sp. CAG:604 and *Phascolarctobacterium succinatutens*. Studies have shown that when obese people lose weight, they exhibit an increase in the abundance of *Phascolarctobacterium succinatutens*^[49]. Weight loss is conducive to glucose control. Sphinganine is formed from ceramide and is a risk factor for β -cell dysfunction in T2DM^[50]. In this condition, insulin secretion was observed to be reduced, and the inhibition of ceramide metabolism was weakened. An increase in 13(S)-HODE is observed in individuals with nonalcoholic fatty liver disease^[51]. Because the common pathogenesis of nonalcoholic fatty liver and diabetes is insulin resistance, diabetes may also be accompanied by elevated levels of 13(S)-HODE, and our research confirms this hypothesis. 13(S)-HODE was found to be positively related to FBG and HOMA-IR, and *Phascolarctobacterium succinatutens* was enriched in the model group.

We observed that the linoleic acid level was elevated in GK rats. Linoleic acid levels are markedly higher among populations with a higher prevalence of T2DM^[52]. Linoleic acid is positively related to *Allobaculum stercoricanis* and is negatively related to *Prevotella* sp. CAG:604. 4-Pyridoxic acid is a catabolite of vitamin B6 metabolism, and our study showed that 4-pyridoxic acid levels were increased in the model group and positively related to FBG and HOMA-IR. A higher level of vitamin B6 is associated with a higher HOMA-IR values and T2DM^[53]. Furthermore, the risk of all-cause mortality in patients with T2DM is higher among those with 4-pyridoxic acid levels in the highest quartile^[54]. 2-Methyl-3-hydroxy-5-formylpyridine-4-carboxylate is also a catabolite of vitamin B6 metabolism, and a reduction in its content can help regulate glucose levels^[55,56]. This compound was found to be negatively related to *Prevotella* sp. CAG:604 and *Dorea* sp. CAG:317 and showed increased levels in the model group in this study. The results indicated that the glucose level was elevated in GK rats and that chenodeoxycholic acid glycine conjugate was negatively correlated with FBG and HOMA-IR, which was consistent with the results reported by Zhang *et al*^[57].

Feng *et al*^[58] found that glutamine synthetase activity was significantly increased after the induction of diabetes (streptozotocin-treated) in rats. The serum levels of ornithine carbamoyltransferase are increased in KK-Ay diabetic mice^[59]. The liver is involved in glucose metabolism. In a state of hyperglycaemia, the liver is damaged to varying degrees, resulting in the abnormal expression of some proteases. Phosphoglucomutase is a key protease in carbohydrate metabolism^[60]. In particular, this protease is closely related to obesity. Endothelial cell function damage mostly occurs in T2DM and is related to argininosuccinate synthase and argininosuccinate lyase. In particular, TNF- α downregulates argininosuccinate synthase expression^[61,62]. The activity of amidophosphoribosyltransferase is higher in the kidneys of diabetic rats^[63]. The results indicate that amidophosphoribosyltransferase is related to the accretion of nucleic acids. A low level of aspartate aminotransferase is an independent risk factor of frail T2DM patients^[64]. Glycogen is synthesized in the liver, and changes in a series of liver enzymes affect the synthesis of glycogen. For example, aspartate 4-decarboxylase

and alanine-glyoxylate transaminase were detected in this study. The occurrence of T2DM is related to many signalling pathways. Pyruvate, which is the end product of glycolysis, is transported into mitochondria and drives ATP production^[65]. This study showed that ATP imbalance was the main reason for insulin resistance and, thus, mainly affected IRS1 and AKT2 potential in the insulin resistance signalling pathway.

We identified different gut microbes and metabolites in GK rats, and the results provide new research directions related to faecal bacteria transplantation, supplementary metabolites, and drug screening, among other topics. This study also has some limitations, such as a lack of drug interventions for verification and a lack of long-term observations of the dynamic changes in gut microbes and metabolites. In future research, we will further improve the design scheme for studying metabolic disorders through the influence of nitrogen metabolism, arginine biosynthesis, primary bile acid biosynthesis, purine metabolism, alanine, aspartate and glutamate metabolism and insulin resistance, which can lead to the emergence of T2DM. In addition to the characteristics of hyperglycaemia, disordered fat and protein levels are also observed in patients with T2DM. T2DM is a clinical syndrome with multiple causes, and we thus believe that the treatment strategy for T2DM should be comprehensive and should not focus on glucose, lipids, weight or other factors alone. This finding may provide ideas for the future management of patients with T2DM.

CONCLUSION

The present study revealed that disordered compositions of gut microbes and metabolites are putative common defects observed in GK rats by metagenomics and untargeted metabolomics. T2DM-related changes in gut microbes and metabolites may contribute to hyperglycaemia; however, further experiments are required to summarize the different stages of T2DM.

ARTICLE HIGHLIGHTS

Research background

Goto Kakizaki (GK) rats share common features with human type 2 diabetes (T2DM). Therefore, the gut microbiota and metabolite results obtained using GK rats will be more similar to those of patients with T2DM.

Research motivation

The gut microbiota and metabolites are critical in T2DM; therefore, alterations in different gut microbiota and metabolites may provide useful evidence for analysing the pathogenesis and treatment of T2DM.

Research objectives

To investigate the alterations in different gut microbiota and metabolites in the faeces of T2DM rats.

Research methods

Systematic characterization of the faecal gut microbes and metabolites of GK rats using metagenomic and untargeted metabolomic approaches and analysis of the relationship between gut microbes and metabolites under conditions of glucose and insulin resistance.

Research results

The GK rats displayed significant differences in gut microbiota structure compared with the control group. The results demonstrated that the GK rats presented significantly decreased abundances of *Prevotella* sp. CAG:604 and *Lactobacillus murinus* ($P < 0.05$) and a significantly higher abundance of *Allobaculum stercoricanis* ($P < 0.01$). orthogonal partial least squares discriminant analysis suggested that the faecal metabolic profiles differed between the GK and control groups. Fourteen potential metabolic biomarkers, including glycochenodeoxycholic acid, uric acid, 13(S)-hydroxyoctadecadienoic acid, N-acetylaspartate, β -sitosterone, sphinganine, 4-pyridoxic acid, and linoleic acid, were identified. The metabolic pathways with the main differences were arginine biosynthesis;

primary bile acid biosynthesis; purine metabolism; linoleic acid metabolism; alanine, aspartate and glutamate metabolism; and nitrogen metabolism.

Research conclusions

The present study revealed that disordered compositions of gut microbes and metabolites are putative common defects observed in GK rats by metagenomics and untargeted metabolomics.

Research perspectives

Gut microbes and metabolites play a key role in carbohydrate metabolic pathways. Therefore, the evaluation of the involvement of gut microbes and metabolite dynamic changes may be important in the future.

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