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A ketogenic diet reduces body weight gain and alters insulin sensitivity and gut microbiota in a mouse model of diet-induced obesity

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ABSTRACT

Purpose: Ketogenic diets (KDs) have anti-obesity effects that may be related to glucose control and the gut microbiota. This paper hypothesizes that KD reduces body weight and changes the insulin sensitivity and gut microbiota composition in a mouse model of diet-induced obesity.
Methods: In this study, C57BL/6 male mice were assigned randomly to 3 groups. The assigned diets were provided to the control and high-fat (HF) diet groups for 14 weeks. The KD group was given a HF diet for 8 weeks to induce obesity, followed by feeding the KD for the next 6 weeks.
Results: After the treatment period, the KD group exhibited a 35.82% decrease in body weight gain compared to the HF group. In addition, the KD group demonstrated enhanced glucose control, as shown by the lower levels of serum fasting glucose, serum fasting insulin, and the homeostatic model assessment of insulin resistance, compared to the HF group. An analysis of the gut microbiota using 16S ribosomal RNA sequencing revealed a significant decrease in the proportion of *Firmicutes* when the KD was administered. In addition, feeding the KD reduced the overall alpha-diversity measures and caused a notable separation of microbial composition compared to the HF diet group. The KD also led to a decrease in the relative abundance of specific species, such as *Acetatifactor_muris, Ligilactobacillus_apodemi,* and *Muribaculum_intestinale*, compared with the HF group. These species were positively correlated with the body weight, whereas the abundant species in the KD group (*Kineothrix_alysoides* and *Saccharofermentans_acetigenes*) showed a negative correlation with body weight.
Conclusion: The current study presents supporting evidence that KD reduced the body weight and altered the insulin sensitivity and gut microbiota composition in a mouse model of diet-induced obesity.

Keywords: body weight; gut microbiota; insulin sensitivity; ketogenic diet; obesity

INTRODUCTION

Obesity is a complex medical condition involving excess fat accumulation in the body. The causes of obesity include overnutrition, insufficient physical activity, and several endocrine imbalances [1]. Obesity is linked to various chronic conditions, including type 2 diabetes, cardiovascular diseases, and cancers [1]. Approaches for treating obesity include lifestyle

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Conflict of Interest

There are no financial or other issues that might lead to conflict of interest.

interventions such as diet and exercise, medicines, and bariatric surgery [2]. A conventional dietary strategy for obesity is the reduction of energy intake, and recently, alterations in macronutrient composition have been tried for weight management [2].

A ketogenic diet (KD) is composed of high fats (typically 80–90% energy from fat) and low carbohydrates, which induce nutritional ketosis [3]. A KD has been applied as a treatment option for epilepsy [4], and recently, its application has been extended to weight management, cognitive impairment, type 2 diabetes, and cancers [5-8]. The anti-obesity effects of a KD may be due to reduced appetite and increased energy expenditure [9,10], and are often superior to other low-calorie diets, even over the long-term [11,12].

In addition to the aspects of appetite or energy expenditure, gut microbiota may be involved in the effects of a KD. A KD raised seizure thresholds and changed the alpha-diversity of the gut microbiota and microbial composition, including *Akkermansia muciniphila* in a mouse model of the seizure [13]. The anti-seizure effect of KD was mediated by altered gut microbiota, and supplementation of 2 KD-associated gut bacteria to KD feeding synergistically increased seizure thresholds [13]. In contrast to the anti-seizure effects of KD, there is limited evidence of the involvement of gut microbiota in the anti-obesity effects of KD. Several studies have investigated the effects of a KD on blood lipid levels or fat accumulation in specific tissues, but have reported conflicting results [14,15]. Mice fed 2 types of KDs showed distinct changes in microbial composition and metabolite profiles, and these changes were associated with different effects on glucose control and lipid accumulation in the liver and fat pads [14]. In addition, improvements in glucose tolerance and insulin sensitivity were observed in a mouse model of type 2 diabetes fed a KD [15]. So far, current evidence has shown inconsistent findings, and the anti-obesity effects of KD relating to insulin sensitivity and gut microbiota have not been reported.

Therefore, we hypothesize that a KD reduces body weight and alters insulin sensitivity and gut microbiota composition in a mouse model of diet-induced obesity. To test the hypothesis, we investigated the effects of a KD on body weight control and glucose homeostasis in a mouse model of diet-induced obesity. Furthermore, we analyzed the gut microbiota profiles of the mice to investigate the impact of the KD on the composition of gut microbiota.

METHODS

Animal experiment

The experimental protocol received approval from the Institutional Animal Care and Use Committee at Seoul Women's University (protocol No. SWU IACUC 2021A-11). Male C57BL/6J mice, aged 6 weeks, were acquired from Raon Bio (Yongin, Republic of Korea). They were housed in a facility with a 12-hour light and 12-hour dark cycle, maintained at a constant temperature of 24 ± 1°C, and controlled humidity of 40 ± 10%. Following a one-week acclimatization period, the mice were randomly divided into 3 groups; 1) the lean control (CON, n = 7) group, 2) the high-fat diet-fed obese control group (HF, n = 7), and 3) the KD group (KD, n = 6). Mice in the CON group were fed a standard diet (10% calories from fat; D12450J, Research Diets, New Brunswick, NJ, USA). The HF diet (60% calories from fat; D12492, Research Diets) was fed for 8 weeks to induce obesity, and then, the HF diet or the KD (90% calories from fat; D16062902, Research Diets) was provided to the mice according to the assigned diet for the next 6 weeks. Diets were fed *ad libitum*, and energy density

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values were 3.85, 5.24, and 6.7 kcal/g for the standard low-fat diet, the HF diet, and the KD, respectively.

The total experimental period was 14 weeks, following a one-week period of acclimatization. The consumption of food and the weight of the body were constantly monitored. After a 12-hour period of fasting, the animals were humanely euthanized using carbon dioxide at the end of the experiment. Blood samples were collected by cardiac puncture. Fat pads were collected from fat depots of subcutaneous fat, epididymal fat, mesenteric fat, and retroperitoneal fat. Intestinal contents were collected from the large intestine, and stored at -80°C until use.

Homeostatic model assessment of insulin resistance (HOMA-IR)

The measurement of fasting serum insulin was conducted utilizing an Ultra Sensitive Mouse Insulin enzyme-linked immunosorbent assay kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan), and the analysis of fasting serum glucose concentrations was performed using a glucose assay kit from Sigma-Aldrich (St. Louis, MO, USA). HOMA-IR was determined through the following calculation: [fasting glucose concentrations (mmol/L) × fasting insulin concentrations (μU/mL)]/22.5 [16]. HOMA-beta, an equation to express beta-cell function, was calculated as HOMA-beta = [20 × fasting insulin concentrations (μU/mL)]/[fasting glucose concentrations (mmol/L) − 3.5] [16]. The quantitative insulin sensitivity check index (QUICKI) was calculated as QUICKI = 1/{log [fasting insulin concentrations (μU/mL)] + log [fasting glucose concentrations (mmol/L)]} [17].

Beta-hydroxybutyrate (BHB) measurement

BHB concentrations in serum were analyzed using a BHB colorimetric assay kit (BioVision, Waltham, MA, USA), following the guidelines provided by the manufacturer. In brief, specimens were subjected to incubation with BHB dehydrogenase, and the generated products were reacted with the colorimetric probe. The measurement of absorbance was taken at a wavelength of 450 nm, and the determination of BHB concentrations was derived from a reference curve with different amounts of BHB.

16S ribosomal RNA (rRNA)-amplicon sequencing of gut microbiota

The analysis of the gut microbiota was conducted at Macrogen (Seoul, Republic of Korea). The DNeasyPowerSoil Kit (Qiagen, Hilden, Germany) was utilized to extract DNA, and DNA concentrations were measured using the Quant-IT PicoGreen assay kit (Invitrogen, Carlsbad, CA, USA). The Illumina MiSeq platform (San Diego, CA, USA) was used to amplify the V3 and V4 hypervariable regions of the gene encoding 16S rRNA. The sequencing data underwent processing using QIIME (version 1.9). Following quality filtering and sample assignment, the sequences were subjected to denoising and clustering to generate amplicon sequence variants, and taxonomic classification was assigned using the NCBI BLAST+ (V 2.9.0) [18].

Statistical analysis

The sample size was estimated to detect a 8 g difference in body weight between mean values of HF and KD groups with a 5% significance level, 90% power, and a standard deviation of 4 g, which was based on findings in a previous study [19]. The sample size was calculated using the epicalc package (V 2.15.1.0) in R software (V 4.2.1). Sufficient power was achieved if 5 mice were included in each group. Alpha-diversity indices, including Chao1, Shannon, and Inverse-Simpson, were calculated using R software and expressed as boxplots. Beta-diversity was assessed in R through principal coordinate analysis (PCoA) utilizing UniFrac distance

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metrics, including unweighted and weighted UniFrac. To evaluate the separation of groups in the PCoA, a permutational multivariate analysis of variance was conducted.

Phenotypic variables and the abundance of each phylum or species were presented as means ± standard error of the mean. Mann-Whitney U test or 1-way analysis of variance followed by Bonferroni post hoc analysis was performed to compare the differences between the groups. The correlations between the mouse phenotypic variables and the gut microbial species were analyzed with Spearman's correlation analysis and expressed as Spearman's correlation coefficients. The statistical analysis was conducted using IBM SPSS Statistics 26 software (IBM Corp., Armonk, NY, USA), with a significance level of $p < 0.05$.

RESULTS

A KD reduced body weight gain

During the dietary treatment period, the CON group exhibited the lowest increase in body weight, while the KD group demonstrated significantly less body weight gain compared to the HF group (**Table 1**). After the dietary intervention, the average body weights of the HF and KD groups were 42.97 ± 1.99 and 34.23 ± 1.33 g, respectively (**Table 1**). The KD led to a reduction in body weight gain of 8.37 g, representing a significant decrease of 35.82% when compared to the HF group ($p = 0.002$; **Table 1**). Food intake (g/day) was not different among the groups. However, the KD group exhibited the highest energy intake (kcal/day), while the CON group demonstrated the lowest energy intake (**Table 1**). According to the findings presented in **Table 1**, the HF group exhibited the highest weights of the total fat pads, shown as a percentage of body weight, which decreased in the KD group ($p = 0.005$).

A KD enhanced blood glucose homeostasis

Impaired glucose homeostasis was observed in the mice fed the HF diet, as shown by higher fasting serum glucose and insulin concentrations, as well as the increased HOMA-IR, but a lower HOMA-beta and QUICKI (**Fig. 1**). The KD treatment significantly improved fasting serum glucose and insulin concentrations, as well as the calculated insulin sensitivity and beta-cell function indices (**Fig. 1**).

Effect of a KD on BHB

As shown in **Table 1**, feeding the HF reduced serum BHB concentrations compared with those in the CON group, which was reversed to similar levels of those in the CON group by feeding the KD.

Table 1. BW changes, food intake, total fat pads, and serum BHB concentrations in the KD-fed mice

	CON (n = 7)	HF (n = 7)	KD (n = 6)
Initial BW (g)	19.71 ± 0.53	19.60 ± 0.26	19.23 ± 0.47
Final BW (g)	29.97 ± 0.84[a]	42.97 ± 1.99[b]	34.23 ± 1.33[a]
Weight gain (g/day)	0.11 ± 0.01[a]	0.22 ± 0.02[b]	0.14 ± 0.01[a]
Food intake (g/day)	2.59 ± 0.07	2.50 ± 0.03	2.61 ± 0.14
Food intake (kcal/day)	9.99 ± 0.32[a]	13.14 ± 0.37[b]	15.34 ± 0.63[c]
Total fat pads (% BW)	5.54 ± 0.62[a]	16.71 ± 1.95[b]	9.67 ± 0.90[a]
Serum BHB (mmol/L)	2.82 ± 0.29[a]	2.13 ± 0.14[b]	3.00 ± 0.18[a]

Data are expressed as means ± standard error of the mean. One-way analysis of variance with Bonferroni post hoc analysis was used to compare the differences between groups. Different letters within a variable are significantly different at $p < 0.05$. Total fat pads included subcutaneous fat, epididymal fat, mesenteric fat, and retroperitoneal fat, and were demonstrated as a percentage of BW.
BW, body weight; BHB, beta-hydroxybutyrate; KD, ketogenic diet; CON, control; HF, high-fat diet.

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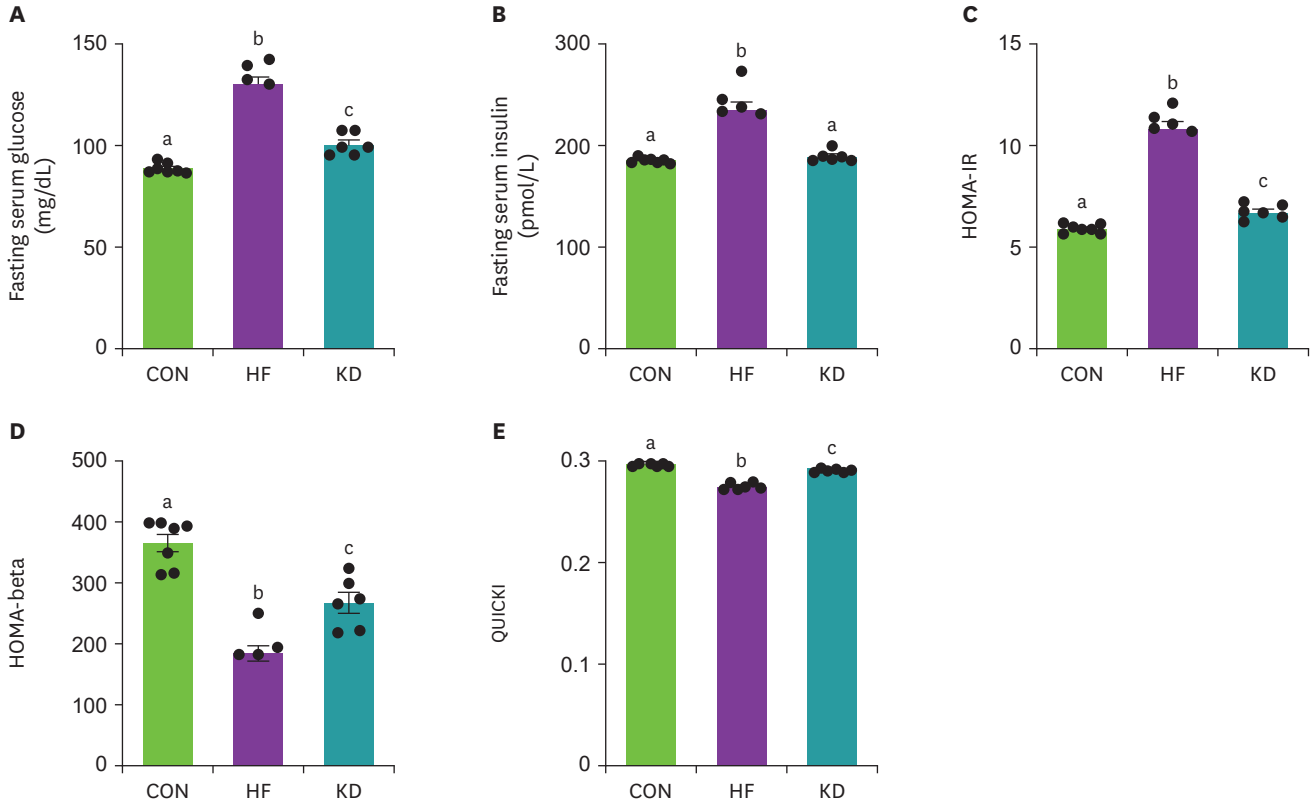

Fig. 1. The effects of a KD on fasting serum glucose, serum insulin, HOMA-IR, HOMA beta, and QUICKI in a mouse model of diet-induced obesity.
KD feeding improved glucose control and insulin sensitivity in mice. Data were expressed as means ± standard error of the mean (n = 6–7 per group). One-way analysis of variance with Bonferroni post hoc analysis was used to compare the differences between groups. Different letters within a variable are significantly different at $p < 0.05$.
CON, control; HF, high-fat diet; KD, ketogenic diet; HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index.

A KD modulated gut microbial community

The 7 intestinal content samples yielded a total of 2,158,416 sequencing reads, averaging 308,345 reads per sample. The gut microbiota of the KD group exhibited reduced alpha-diversity, as indicated by lower values of the Chao1, Shannon, and Inverse Simpson indices, compared to the HF group (**Fig. 2**). A UniFrac-based PCoA demonstrated notable

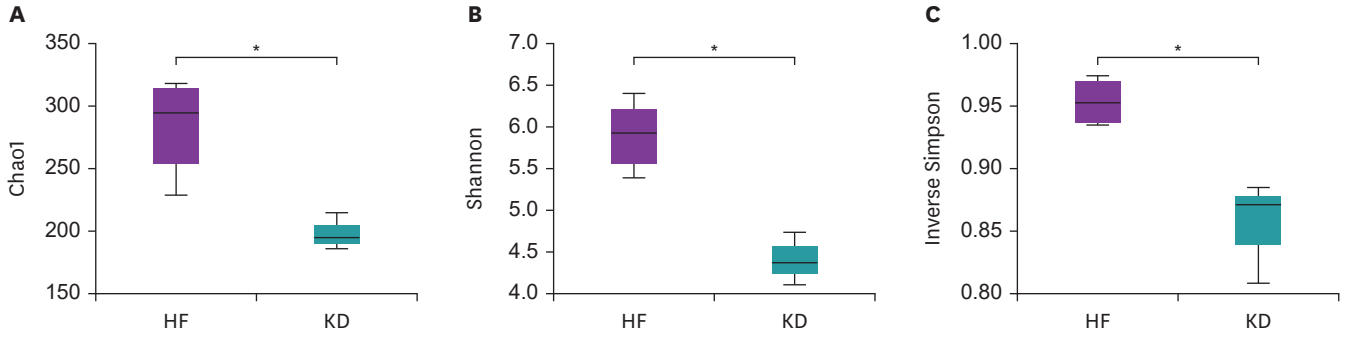

Fig. 2. Boxplots of alpha-diversity indices in mice of the HF (n = 4) and the KD (n = 3) groups.
KD feeding reduced indices of alpha-diversity (the species diversity of microbiomes) in mice. Mann-Whitney U test was used to compare the differences between groups.
HF, high-fat diet; KD, ketogenic diet.
*$p < 0.05$.

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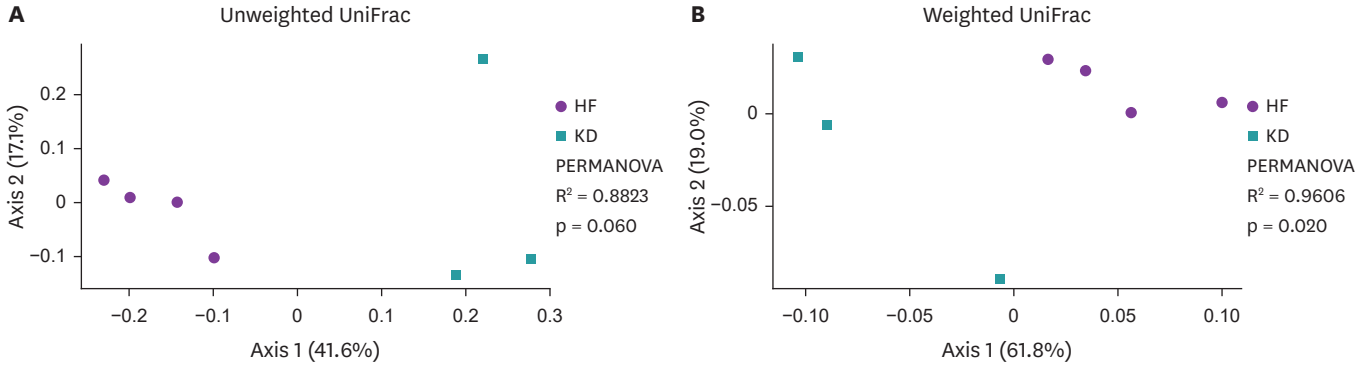

Fig. 3. (A) Unweighted and (B) weighted UniFrac-based PCoA plots with PERMANOVA test of gut microbiota in mice of the HF (n = 4) and KD (n = 3) groups.
The unweighted and (B) weighted UniFrac-based PCoA plots showed significant separation between the HF and KD groups.
HF, high-fat diet; KD, ketogenic diet; PERMANOVA, permutational multivariate analysis of variance; PCoA, principal coordinate analysis.

differentiation between the 2 groups, as evidenced by significant separation observed on both the unweighted (R^2 = 0.8823, p = 0.060) and weighted (R^2 = 0.9606, p = 0.020) plots (**Fig. 3**). The initial 2 components of the unweighted UniFrac analysis accounted for 58.7% of the overall variance, with PC1 contributing 41.6% and PC2 contributing 17.1%. Similarly, the first 2 components of weighted UniFrac analysis explained 80.8% of the total variance, with PC1 contributing 61.8% and PC2 contributing 19.0%.

In the HF group, the phyla *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* collectively represented 94.74% of the total composition, while in the KD group, these phyla accounted for 73.21% of the total composition (**Fig. 4**). KD group revealed a lower relative abundance of *Firmicutes* (24.31 ± 3.13%) than in the HF group (49.21 ± 8.81%) (**Table 2**). The *Firmicutes* to *Bacteroidetes* (F/B) ratio declined from 1.47 in the HF-fed mice to 0.82 in the KD-fed mice. Taxonomic composition in gut microbiota at the species level between the HF and KD groups is shown in **Table 3**, and only species with statistical differences were included. *Acetatifactor_muris*, *Ligilactobacillus_apodemi*, *Muribaculum_intestinale*, and *Ruminococcus_gnavus* were differentially abundant taxa in the HF-fed mice, whereas *Kineothrix_alysoides* and *Saccharofermentans_acetigenes* were at a higher abundance in the KD-fed mice.

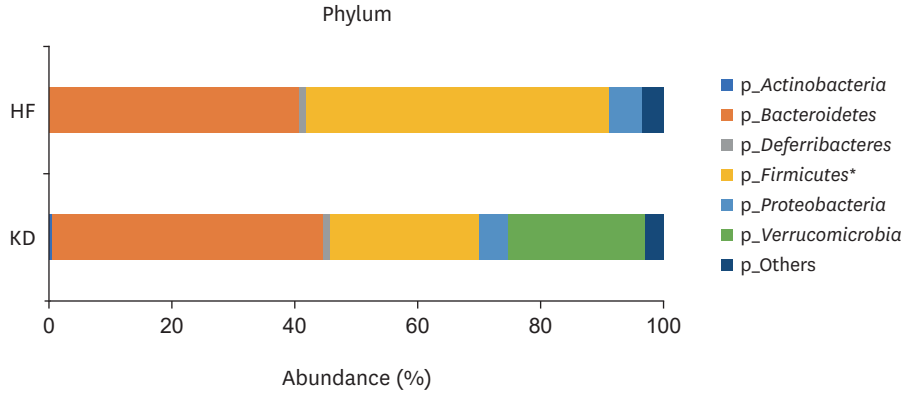

Fig. 4. The relative phylum abundance of gut microbiota in mice of the HF (n = 4) and the KD (n = 3) groups.
KD feeding reduced the abundance of *Firmicutes* in mice. Mann-Whitney U test was used to compare the differences between groups.
HF, high-fat diet; KD, ketogenic diet.
*p < 0.05.

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Table 2. The taxonomic composition at the phylum level of gut microbiota in mice of the HF and the KD groups

Phylum (%)	HF (n = 4)	KD (n = 3)	HF/KD	p-value
Actinobacteria	0.44 ± 0.16	0.64 ± 0.39	0.68	0.724
Bacteroidetes	40.33 ± 7.32	43.99 ± 17.79	0.92	1.000
Deferribacteres	1.49 ± 1.27	1.16 ± 0.92	1.28	1.000
Firmicutes	49.21 ± 8.81	24.31 ± 3.13	2.02	0.034
Proteobacteria	5.20 ± 0.83	4.92 ± 3.21	1.06	0.724
Verrucomicrobia	0.00 ± 0.00	21.83 ± 14.32	0.00	0.057
Others	3.34 ± 1.68	3.15 ± 2.23	1.06	1.000
F/B ratio	1.47 ± 0.62	0.82 ± 0.51	1.79	0.289

Data are expressed as means ± standard error of the mean. Statistical analysis was performed using the Mann-Whitney U test.
HF, high-fat diet; KD, ketogenic diet; F/B, *Firmicutes* to *Bacteroidetes*.

Table 3. The relative species abundance of gut microbiota in mice of the HF and the KD groups

Species (%)	HF (n = 4)	KD (n = 3)	HF/KD	p-value
s__Acetatifactor_muris	9.79 ± 0.16	1.22 ± 0.07	8.03	0.034
s__Kineothrix_alysoides	1.70 ± 0.36	6.45 ± 1.83	0.26	0.034
s__Ligilactobacillus_apodemi	0.81 ± 0.14	0.03 ± 0.02	24.38	0.034
s__Muribaculum_intestinale	6.37 ± 0.22	3.52 ± 0.98	1.81	0.034
s__Ruminococcus_gnavus	2.12 ± 0.45	0.45 ± 0.17	4.70	0.034
s__Saccharofermentans_acetigenes	0.00 ± 0.00	0.34 ± 0.15	-	0.019

Species with statistical significance ($p < 0.05$) were only included. Data are expressed as means ± standard error of the mean, and statistical analysis was performed using the Mann-Whitney U test.
HF, high-fat diet; KD, ketogenic diet.

The correlation analysis between gut microbiota and the host phenotype

The Spearman's correlation analysis between the gut microbial species and the mouse phenotype is shown in **Table 4**. Various correlations were detected between microbial species and specific mouse phenotypes. *Acetatifactor_muris*, *Ligilactobacillus_apodemi*, and *Muribaculum_intestinale*, abundant species in the HF group, displayed a positive correlation with body weight, whereas *Kineothrix_alysoides* and *Saccharofermentans_acetigenes*, the abundant species in the KD group, exhibited a negative correlation with body weight. Serum BHB concentrations were negatively correlated with *Muribaculum_intestinale*, but, positively correlated with *Saccharofermentans_acetigenes*.

DISCUSSION

This study aimed to investigate whether KD reduces body weight gain and induces changes in glucose homeostasis and gut microbiota composition in a mouse model of diet-induced obesity. We showed that feeding a KD significantly reduced body weight gain and improved glucose control. Additionally, feeding the KD changed alpha-diversity and the taxonomic

Table 4. Spearman's correlations between gut microbial species and mouse phenotype variables of the high-fat diet and ketogenic diet groups

Species	BW (g)	Total fat pads (% BW)	Serum glucose (mg/dL)	Serum insulin (pmol/L)	HOMA-IR	Serum BHB (mmol/L)
s__Acetatifactor_muris	0.893*	0.643	0.631	0.821*	0.786*	−0.607
s__Kineothrix_alysoides	−0.821*	−0.679	−0.613	−0.857*	−0.786*	0.964*
s__Ligilactobacillus_apodemi	0.893*	0.750	0.829*	0.643	0.714	−0.750
s__Muribaculum_intestinale	0.857*	0.714	0.613	0.964*	0.964*	−0.857*
s__Ruminococcus_gnavus	0.536	0.893*	0.901*	0.643	0.643	−0.679
s__Saccharofermentans_acetigenes	−0.788*	−0.749	−0.895*	−0.749	−0.788*	0.788*

Values are Spearman's correlation coefficients.
BW, body weight; HOMA-IR, homeostatic model assessment of insulin resistance; BHB, beta-hydroxybutyrate; s_, species.
*$p < 0.05$.

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composition of the gut microbiota, which were correlated with body weight, tissue weights of the fat pads, indices of glucose control, and serum concentrations of BHB. Collectively, the present study determined the effects of a KD on body weight gain and glucose homeostasis, considering changes in gut microbes.

Previous research has demonstrated the potential benefits of a KD in terms of reducing body weight gain and enhancing insulin sensitivity [11,12,15]. The 6-week KD intervention lowered body weight gain by 35.82% without altering food intake in a mouse model of diet-induced obesity in the present study. HF diet feeding often resulted in a decrease in food intake compared with the standard rodent diet [20]. However, food intake expressed as g/day was not significantly different among the groups in the current study. The dissimilarities in diet composition (e.g., sucrose) and the environment of the facility may contribute to the different feeding pattern compared to the previous study. An analysis of energy expenditure in overweight or obese males demonstrated that KD increased energy expenditure [10]. Considering that a KD has a higher energy density than the HF diet, the body weight-lowering effects of a KD may be partly due to increased energy expenditure. A KD may change microbial profile and abundance [21], possibly affecting energy expenditure and metabolism [22,23]. Whether gut microbiota directly regulates energy expenditure or metabolism, and whether and how feeding a KD modulates gut microbial profile and energy expenditure/metabolism need to be clarified.

Dysregulation of fat mass and distribution increases the risk for obesity-associated complications [24]. Feeding a KD reduced total body fat weight, and fat percentage and distribution are closely related to insulin sensitivity [25]. In this study, glucose homeostasis and insulin sensitivity were improved in the KD group. KD treatment in diabetic animals has raised concern that hypoglycemia would occur due to the limited supply of glucose [26]; however, hypoglycemia was not observed in the current study or previous report [11,15].

Although previous reports demonstrated weight reduction and improvements in glucose tolerance and insulin sensitivity by KD feeding [11,12,15], caution is needed to interpret previous findings relating to KD. Because feeding patterns (e.g., duration and timing of feeding) and diet compositions of KD were not identical in previous studies, which might affect the results, differences in study design should be considered.

Ketone bodies (acetone, acetoacetate, and BHB) are produced from fatty acids by the liver when the glucose supply is limited. BHB is the predominant ketone body, and has renewed interest in that it has regulatory effects on inflammation, oxidative stress, learning and memory function, carcinogenesis, and glucose control [27-30]. Fasting plasma BHB concentrations were reduced in subjects with obesity and non-alcoholic fatty liver disease [31]. Also, the fasting plasma BHB concentrations were negatively correlated with hepatic fat contents, and positively correlated with insulin sensitivity assessed by hyperinsulinemic-euglycemic clamp [31]. Moreover, BHB supplementation reduced hepatic triglyceride concentrations in aged rats [32]. In the current study, HF diet-fed mice had lower BHB concentrations in fasting serum, which were increased by KD feeding. Although current evidence did not provide a direct link between increased BHB concentrations in fasting serum and reduced body weights and body fat, increased BHB concentrations similar to the extent of CON lean mice may relate to the changes in body weights and body fat.

The KD may exert anti-obesity effects by modulating the gut microbiota composition. In this study, the modulation in gut microbial composition by feeding the KD was analyzed through

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alpha- and beta-diversity indices. The changes, particularly at the phylum and species levels, were further examined to investigate whether the altered microbial profile was related to the anti-obesity effect observed from feeding the KD. This study demonstrated significant differences for the alpha-diversity indices of Chao1, Shannon, and Inverse Simpson, suggesting that feeding the KD decreased microbial richness and evenness. Reduced alpha-diversity of gut microbiota was often observed in unhealthy conditions such as obesity, metabolic syndrome, and depression [33,34]. However, the reduced levels of microbial richness and evenness by KD might be from high fat contents rather than health conditions. Beta-diversity measures of unweighted and weighted UniFrac suggest that feeding a KD significantly altered the microbial community, leading to differences with the HF diet group.

The increased F/B ratio was reported in leptin-deficiency (*ob/ob)* mice [35] and a mouse model of diet-induced obesity [36]. The F/B ratio has been positively associated with body weight or fat mass [37]. In this study, the decrease of *Firmicutes* and the patterns of increased *Bacteroidetes* and decreased F/B ratio, were detected in the KD-fed mice, which agrees with previous reports, although the differences in *Bacteroidetes* and the F/B ratio were not significant between the HF and KD groups.

The abundance levels of 6 species were distinctly altered by feeding the KD. Correlation analysis further revealed a relation between these key microbial species and specific phenotypes observed in the mice. Notably, species that were differentially abundant between groups exhibited correlations with mouse body weight, total fat pad weights, and the HOMA-IR, which suggests the possibility that the species might be involved with KD-mediated improvements in obesity and insulin resistance. For example, *Acetatifactor_muris*, *Ligilactobacillus_apodemi*, and *Muribaculum_intestinale* were higher in the HF group, which had positive correlations with body weights. *Acetatifactor_muris* was identified and isolated from the intestinal samples of obese mice [38]; however, its function relating to obesity and metabolism needs to be investigated. In contrast, *Kineothrix_alysoides* and *Saccharofermentans_acetigenes*, the abundant species in the KD group, were negatively correlated with body weight. Evidence regarding the link between a KD and the gut microbiota related to obesity has been so far inevident, and, previous research has focused on other conditions, including epilepsy [39], or has reported mixed results [40]. Considering these findings, it is meaningful that the current study reported a distinct profile of gut microbiota in KD-fed mice, and identified the specific phylum and species correlated with obesity and insulin resistance.

Although the current study provided significant findings demonstrating that KD reduced body weight gain and enhanced blood glucose control, there are some limitations to consider. First, we did not provide direct evidence that increased BHB concentrations were related to the KD-mediated anti-obesity effects. Therefore, it is necessary to examine the effects of BHB supplementation on body weight and body fat to strengthen our findings. Second, we did not reveal how altered gut microbiota regulated body weights and glucose control. The microbial metabolites from the gut microbes that have been modified through KD feeding could potentially contribute to the beneficial effects of KD on body weight changes and glucose homeostasis. Further studies investigating the mechanisms by which the specific microbial species identified in this study contribute to the improvements of obesity and insulin resistance are needed in a well-controlled study design. Third, we did not investigate the optimal regimen (e.g., duration of KD feeding) of KD considering the potential adverse effects of KD. It is well known that KD may have short-term (fatigue, headache, hypoglycemia, metabolic acidosis, and so on) and long-term adverse effects, such

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as cardiovascular risks, and has relatively low tolerability and compliance [5]. Therefore, the safety and tolerability of KD should be considered for application, and the development of the appropriate regimen of KD should be followed.

SUMMARY

This study showed that the KD reduced body weight gain and improved glucose control and insulin sensitivity in a mouse model of diet-induced obesity. Additionally, the KD altered gut microbiota profile and changed the abundance levels of specific gut bacteria, such as *Acetatifactor_muris*, *Ligilactobacillus_apodemi*, *Muribaculum_intestinale*, *Kineothrix_alysoides,* and *Saccharofermentans_acetigenes*. These distinct alterations of the gut microbiota may relate to the effects of the KD on body weight gain and glucose control.

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