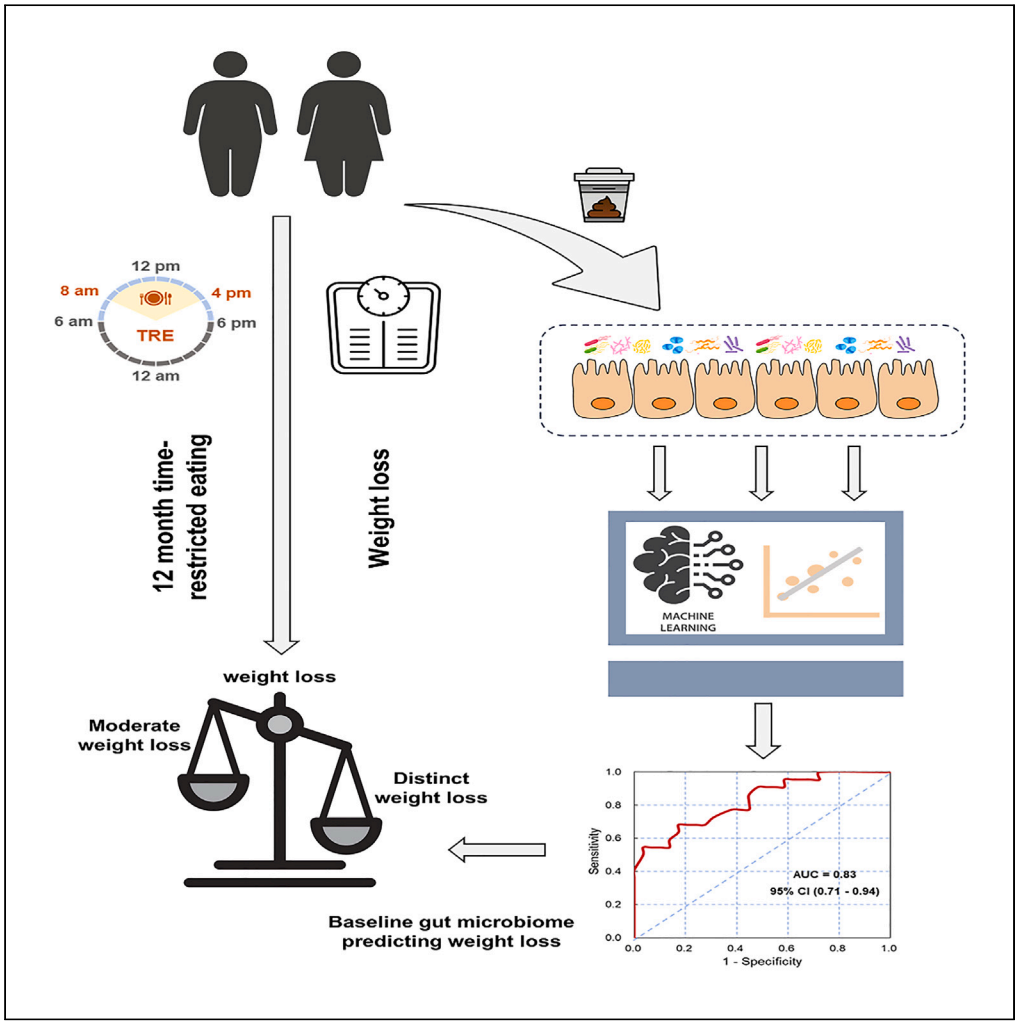
iScience

CellPress
OPEN ACCESS

Article

Effect of time-restricted eating regimen on weight loss is mediated by gut microbiome



Chensihan Huang, Deying Liu, Shunyu Yang, ..., Yafeng Li, Jin Li, Huijie Zhang

dr.yafengli@gmail.com (Y.L.)
jinli807@126.com (J.L.)
huijiezhang2005@126.com (H.Z.)

Highlights

Weight change varied significantly among individuals in the same TRE intervention

Gut microbiota contributes to the heterogeneity of responsiveness to TRE

Baseline gut microbiota signatures can be a strong predictor of individualized weight-loss efficacy

Huang et al., iScience 27, 110202
July 19, 2024
https://doi.org/10.1016/j.isci.2024.110202

iScience

CellPress
OPEN ACCESS

Article

Effect of time-restricted eating regimen on weight loss is mediated by gut microbiome

Chensihan Huang,[1,7] Deying Liu,[1,7] Shunyu Yang,[2] Yan Huang,[1] Xueyun Wei,[1] Peizhen Zhang,[1] Jiayang Lin,[1] Bingyan Xu,[1] Yating Liu,[1] Dan Guo,[1] Yafeng Li,[3,4,5,*] Jin Li,[6,*] and Huijie Zhang[1,7,8,*]

SUMMARY

Time-restricted eating (TRE) is a promising obesity management strategy, but weight-loss efficacy varies among participants, and the underlying mechanism is unclear. The study aimed to investigate the role of gut microbiota in weight-loss response during long-term TRE intervention. We analyzed data from 51 obese adults in a 12-month TRE program, categorizing them into distinct weight loss groups (DG) and moderate weight loss groups (MG) based on their TRE responses. Shotgun metagenomic sequencing analysis revealed a significant increase in species closely associated with weight loss effectiveness and metabolic parameter changes in the DG group. Pathways related to fatty acid biosynthesis, glycogen biosynthesis, and nucleotide metabolism were reduced in the DG group and enhanced in the MG group. Next, we identified nine specific species at baseline that contributed better responses to TRE intervention and significant weight loss. Collectively, gut microbiota contributes to responsiveness heterogeneity in TRE and can predict weight-loss effectiveness.

INTRODUCTION

Obesity is a global epidemic issue that greatly increase the risks of type 2 diabetes mellitus (T2DM), cardiovascular diseases, and certain cancers.[1] Diet is the most relevant factors associated with the development of obesity, and dietary interventions have been at the forefront of weight management.[2] Time-restricted eating (TRE) regimen, an intermittent-fasting regimen that involves a shorten period of time for eating within each 24-h period, has been proved as an effective intervention for weight-loss strategy. TRE can significantly reduce body weight, body fat, and metabolic risk factors in human individuals.[3] However, participants often respond differently to the same diet-regimen as weight changes may vary considerably between individuals.[4–6] The underlying mechanisms or potential predictors for the heterogeneity of weight-loss responsiveness remain unclear.

Diverse evidence suggests that gut microbiota is involved in the development of obesity.[7,8] Compositional and functional alterations of gut microbiota have been identified in individuals with obesity.[9] Furthermore, fecal microbiota transplantation from lean donors and specific gut microbes supplementation, such as *Akkermansia muciniphila*, could reduce body weight and obesity-related dysregulated metabolic parameters.[10–12] These findings suggest that gut microbiota may contribute to physiological inter-individual variability.

It has been proposed that dietary intervention has a regulatory effect on gut microbiota in both humans and animals.[13,14] Previous study showed that intermittent calorie restriction could ameliorate the development of obesity by modulating the composition of gut microbiota in mice.[15] Consistently, another study found that intermittent fasting significantly decreased overall gut bacterial population and increased gut bacterial diversity in healthy individuals.[16,17] There were limited clinical researches conducted regarding this subject. Some available studies indicated that TRE was associated with increased levels of *Akkermansia muciniphila* and *Bacteroides fragilis*, leading to enhanced diversity of gut microbes. While some studies revealed a higher presence of *Prevotellaceae* and *Bacteroideaceae* families, and *Butyricicoccus pullicaecorum* after TRE treatment.[18–20] In contrast, few studies reported that no substantial differences in alpha and beta diversity or gut-microbiota composition were observed between TRE and time-unrestricted intervention.[21,22] However, due to the limited sample size and short intervention duration in previous studies, the research findings have been inconsistent, and the relationship between changes in gut microbiota and long-term TRE interventions still needs to be identified.

Effects of Time-Restricted Eating on Weight loss and Cardiometabolic Risk Factors in Obese Adults (TREATY trial) was designed to compare a 12-month calorie restriction with or without TRE on weight loss in obese adults. To address the aforementioned questions, we

[1]Department of Endocrinology and Metabolism, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong, China
[2]Department of Nutrition, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong, China
[3]Department of Nephrology, Shanxi Provincial People's Hospital (Fifth Hospital) of Shanxi Medical University, Taiyuan 030000, Shanxi, China
[4]Core Laboratory, Shanxi Provincial People's Hospital (Fifth Hospital) of Shanxi Medical University; Taiyuan, China
[5]Academy of Microbial Ecology, Shanxi Medical University, Taiyuan 030000, Shanxi, China
[6]Department of Endocrinology and Metabolism, Shan Xi Medical University Second Hospital, Shan Xi Medical University, Taiyuan 030000, Shanxi, China
[7]These authors equally contributed
[8]Lead contact
*Correspondence: dr.yafengli@gmail.com (Y.L.), jinli807@126.com (J.L.), huijiezhang2005@126.com (H.Z.)
https://doi.org/10.1016/j.isci.2024.110202

analyzed the data of participants in our previously conducted TREATY trial.[3] We found reductions in body fat, visceral fat, blood pressure, glucose levels, and lipid levels in these participants over a 12-month TRE intervention. However, the weight-loss effects of TRE were observed to vary considerably in various individuals. To assess the potential role of gut microbiota in the effects of weight-loss mediated by TRE, we performed comprehensive metagenomics analysis in a subset of participants in the TREATY trial. We also analyzed baseline microbial signatures to evaluate its predictive effects for the heterogeneity of weight-loss responsiveness.

RESULTS

Study overview and heterogeneous weight loss effect of TRE intervention

Our cohort was comprised fifty-one participants completing the 12-month TRE intervention and providing fecal samples at both baseline and 12-month (Figure S1A). The relative changes of body weight at 12 months in participants receiving TRE intervention ranged from −33.9% to 6.7% (Figure S1B). We further classified these participants into moderate weight loss group (MG, n = 27) and distinct weight loss group (DG, n = 21) based on the degree of weight loss (Figure 1A). There were no significant differences in baseline characteristics between the MG and DG groups (Table 1). During the whole 12 months intervention, dietary compliance, energy intake calculated from daily dietary logs and physical activity level were comparable between the MG and DG groups (Figures 1B–1D). The participants in DG group demonstrated more decrease of body weight, BMI, waist circumference, and body fat than that in MG group (Figures 1E–1J). Meanwhile, participants in DG group showed more reduction in blood pressure, fasting glucose level, and insulin resistance than that in MG group (Figures 1K–1N). In contrast to a significant decrease of lipid profiles in DG group, no obvious change was observed in MG groups (Figures 1O and 1P). Given that the perturbation of gut microbiota homeostasis is closely associated with the improvement of metabolic parameters in humans, we next explored whether it was involved in the heterogeneous weight-loss effects of TRE in our trial.

Microbial composition is a determining factor of distinct weight loss efficacy under TRE intervention

We assessed the structure of gut microbiota in our cohort via shotgun metagenomic sequencing. No significant differences in alpha diversity, including richness, Shannon, and Simpson index, or beta diversity between the microbiome before and after TRE intervention were observed, suggesting TRE treatment may not lead to dramatic alterations in the overall structure of gut microbiome (Figures S2A–S2C, and S3). The overall composition and abundance of the bacterial community at the phylum level in each group were comparable between several visits during the 12-month TRE intervention (Figure S2D).

To explore whether the gut microbiota composition was different in patients with different efficacy in TRE intervention, we investigated the microbiome signatures in the MG and DG groups. The community diversity estimated by the Shannon index, Simpson index, and richness index were significantly higher in DG group then MG group at baseline, however, no difference between the two groups was observed at 3 months, 6 months, or 12 months assessment (Figures 2A–2C). We found that beta diversity was generally comparable between the MG and DG groups at baseline or after 12-month intervention (Figure 2D). The relative abundance of the bacterial community at the phylum level and the Firmicutes/Bacteroidetes ratio in each group were not significantly different at baseline or after a 12-month TRE intervention (Figures 2E and S4). At genus level, the abundance of *Clostridiales_noname*, *Clostridium*, and *Ruminococcaceae_noname* was found selectively increased in DG group after 12-month TRE intervention. *Coprobacillus* and *Megamonas* were found selectively decreased in DG group after 12-month TRE intervention. Meanwhile, MG group was characterized by the selectively increased abundances of *Citrobacter*, *Enterobacter*, *Holdemania*, *Faecalibacterium*, and *Burkholderiales_noname* genus (Figure 2F). Notably, abundances of seven species, including *Ruminococcaceae_bacterium_D16*, *Parabacteroides_goldsteinii*, *Lachnospiraceae_bacterium_1_1_57FAA*, *Clostridium_leptum*, *Clostridium_asparagiforme*, *Clostridiales_bacterium_1_7_47FAA*, and *Bacteroides_intestinalis*, were significantly increased after 12-month TRE intervention in DG group. While abundances of another seven species were decreased in DG group: *Megamonas_unclassified*, *Megamonas_hypermegale*, *Enterobacter_cloacae*, *Dorea_unclassified*, *Coprobacillus_unclassified*, and *Bacteroides_eggerthii*. Meanwhile, we observed 14 significantly altered species caused by TRE intervention in MG group, including 2 species with decreased amount (*Pseudomonas_mendocina* and *Klebsiella_pneumoniae*) and 12 species with increased abundances (*Megasphaera_unclassified*, *Lachnospiraceae_bacterium_5_1_63FAA*, *Holdemania_filiformis*, *Faecalibacterium_prausnitzii*, *Eubacterium_ventriosum*, *Escherichia_unclassified*, *Enterobacter_cloacae*, *Collinsella_aerofaciens*, *Clostridium_asparagiforme*, *Citrobacter_unclassified*, *Citrobacter_freundii*, *Burkholderiales_bacterium_1_1_47*) (Figure 2G). To illustrate the importance of the species alterations for weight loss efficacy during TRE, we constructed a random forest classifier model that could identify DG group from MG group based on all 149 species. The importance of the species alterations was ranked according to their contribution to the classifier model (Figure 2H). Among 25 significantly altered species, six species including *Ruminococcaceae_bacterium_D16*, *Lachnospiraceae_bacterium_5_1_63FAA*, *Coprobacillus_unclassified*, *Bacteroides_intestinalis*, *Collinsella_aerofaciens*, *Clostridium_leptum*, were ranked within the top 30 species in the classifier model (Figure 2H).

Moreover, the network interaction complexity of gut microbiota, assessed by co-abundance analysis, was decreased in both MG and DG groups with TRE intervention for 12 months. However, the DG group exhibited stronger and broader network interaction complexity than the MG group, suggesting an enhanced community interaction in participants with effective weight loss during the 12-month TRE intervention (Figure 3). Collectively, these findings indicate that other than the difference in composition and diversity, microbial differences in structure and complexity could partially explain the inconsistent outcomes of TRE.

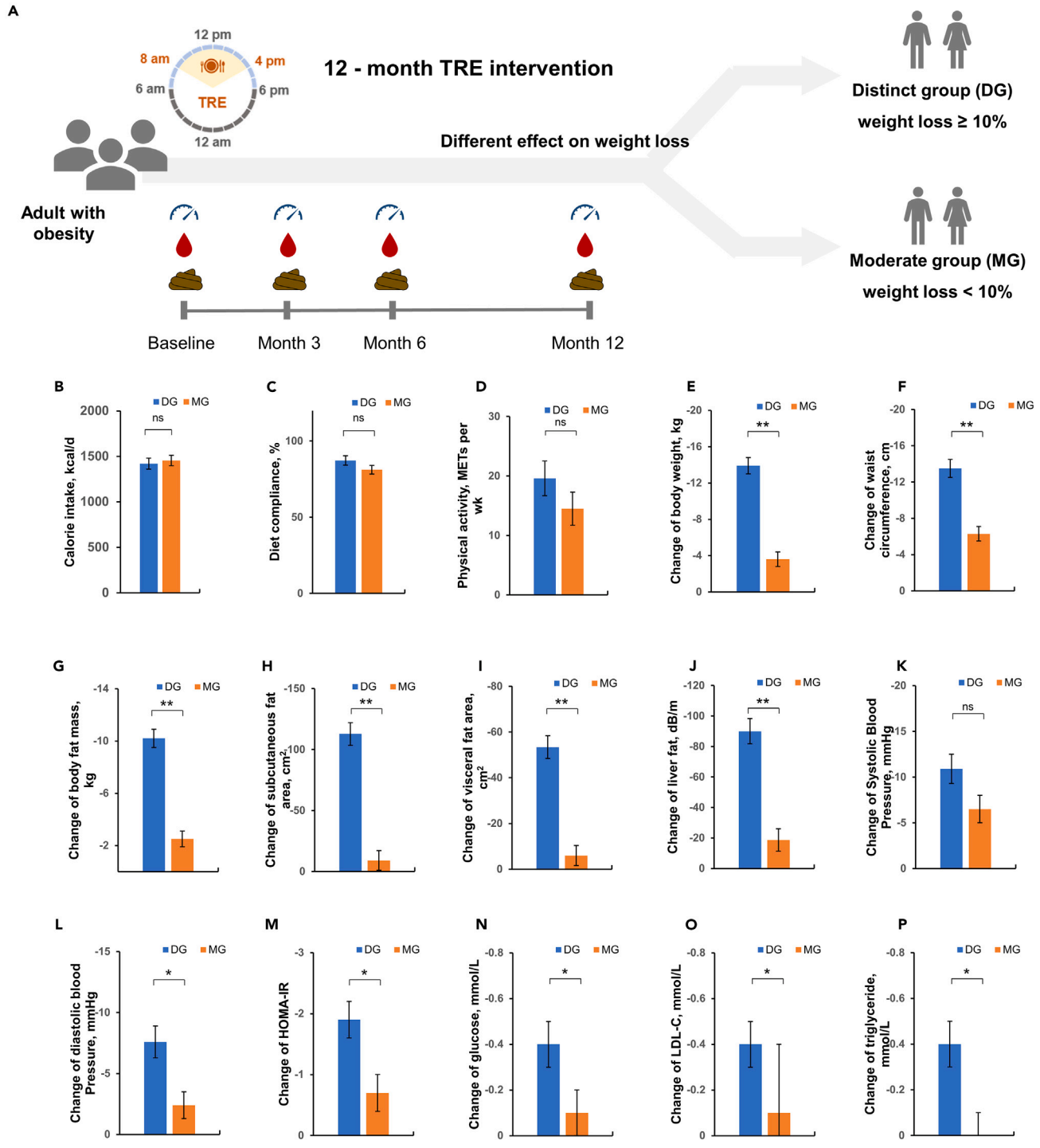


Figure 1. Variation in the metabolic parameters change during a 12-month trial period

(A) Schematic diagram of the study design.

(B–D) Average (B) energy intake, (C) diet compliance, and (D) physical activity level during the 12-month intervention. Compliance to prescribed diets was calculated as adherent/intervention days.

(E–P) The change of (E) body weight (E), (F) waist circumference, (G) body fat mass, (H) abdominal subcutaneous fat area, (I) abdominal visceral fat area, (J) liver fat, (K) systolic blood pressure, (L) diastolic blood pressure, (M) HOMA-IR, (N) fasting glucose level, (O) LDL-C, and (P) triglyceride after 12-month TRE intervention. Data were shown as mean ± standard error. *, $p < 0.05$; **, $p < 0.01$; ns, not significant using the PROC MIXED procedure controlling for the baseline measurements.

Table 1. Characteristic of the participants at baseline

Variables	MG (*N* = 29)	DG (*N* = 22)	*p* value	*p value*[a]
Age, years	33.2 (8.5)	31.8 (8.4)	0.57	0.61
Male, n (%)	17 (58.6)	9 (40.9)	0.21	
Weight, kg	88.2 (9.2)	84.5 (9.0)	0.15	0.42
BMI, kg/m2	31.5 (2.5)	31.1 (2.2)	0.55	0.67
Waist circumference, cm	98.6 (6.3)	97.9 (8.1)	0.72	0.68
Total caloric intake, kcal/day	2060 (397.5)	2030.8 (290.7)	0.77	0.91
Daily eating window, hr: min	10:20 (1:16)	10:26 (1:28)	0.81	0.85
Body fat mass, kg	32.5 (4.7)	32.5 (6.1)	1.00	0.78
Body fat, %	37.3 (4.5)	38.9 (6.2)	0.28	0.84
Abdominal fat area, cm^2				
Visceral	129.9 (101.3–161.0)	103.8 (82.8–135.9)	0.093	0.16
Subcutaneous	305.6 (263.0–346.6)	319.0 (264.5–386.3)	0.26	0.43
Liver fat, dB/m	313.9 (37.0)	301.2 (32.2)	0.21	0.22
Blood pressure, mmHg				
Systolic	127.7 (10.6)	122.8 (13.0)	0.14	0.36
Diastolic	75.8 (8.1)	71.0 (11.1)	0.079	0.21
Glucose, mg/dL	5.1 (0.5)	4.9 (0.5)	0.15	0.17
Glycated hemoglobin, %	5.3 (0.4)	5.1 (0.3)	0.14	0.16
Cholesterol, mmol/L	5.3 (1.0)	4.8 (0.7)	0.077	0.17
HDL-C, mmol/L	1.1 (0.2)	1.2 (0.2)	0.12	0.23
LDL-C, mmol/L	3.6 (0.9)	3.2 (0.7)	0.096	0.19
Triglycerides, mmol/L	1.70 (1.14–2.15)	1.23 (0.78–1.77)	0.10	0.24
Physical activity, METs*hour/week	11.6 (8.8–21.2)	15.4 (11.0–24.0)	0.26	0.20

Data were mean (standard deviation) or median (interquartile range).
MG, moderate weight loss group; DG, distinct weight loss group; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; METs, metabolic equivalents.
[a]Adjusted for age and sex.

Associations of taxonomic alterations with changes in clinical parameters induced by TRE intervention

We next conducted correlation analysis to investigate whether the compositional changes in microbiota between MG and DG were associated with improvements in clinical parameters. We found that alterations of *Lachnospiraceae_bacterium_1_1_57FAA*, *Bacteropdes_intestinails*, *Clostridium_leptum*, and *Ruminococcaceae_bacterium_D16* were significantly associated with the changes of the body composition parameters, including whole body weight, BMI, waist circumference, body fat rate, and abdominal fat area. The change of *Parabacteroides_goldsteinii* was correlated with the change of serum cholesterol level (Figure 4). Taken together, the previous findings imply that distinct changes of these species may underline the difference in the effects of weight loss in participants receiving a standard TRE intervention.

TRE promotes divergent functional shifts in gut microbiome between MG group and DG group

To further understand how the changes of gut microbiota between MG and DG modulate host metabolism, we annotated microbial genes to Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KOs). Principal coordinate analysis (PCoA) of Bray-Curtis dissimilarity showed that the distribution patterns of KOs were not significantly different between the MG and DG groups at baseline or a 12-month TRE intervention (Figure 5A). We then applied Wilcoxon rank-sum test ($p < 0.001$) to identify the differential KOs before and after 12-month TRE intervention. A total of 57 differential KOs were identified in the DG group, with 56 KOs decreased and 1 KO increased (Figure S5). Meanwhile, 17 increased KOs and 1 decreased KO were identified in the MG group. We further analyzed and compared the altered KOs of the two groups through the statistics of KEGG pathway abundances. In the MG group, 18 pathways were found to be significantly increased, such as "glycolysis/gluconeogenesis", "citrate cycle (TCA cycle)", "pyruvate metabolism", etc. In DG group, we found 53 pathways, including "citrate cycle", "pentose phosphate pathway", "starch and sucrose metabolism", "fatty acid biosynthesis", "lysine biosynthesis", "histidine metabolism", etc., were significantly decreased (Figure 5B).

A Spearman correlation analysis was performed between differential KOs and changes in clinical parameters (Figure S6). Nearly half of the differential KOs, such as K06896 (maltose 6′-phosphate phosphatase), K01496 (phosphoribosyl-AMP cyclohydrolase), K16148(alpha-maltose-1-phosphate synthase), K11263(acetyl-CoA/propionyl-CoA carboxylase), K00886 (polyphosphate glucokinase), etc., were found to be

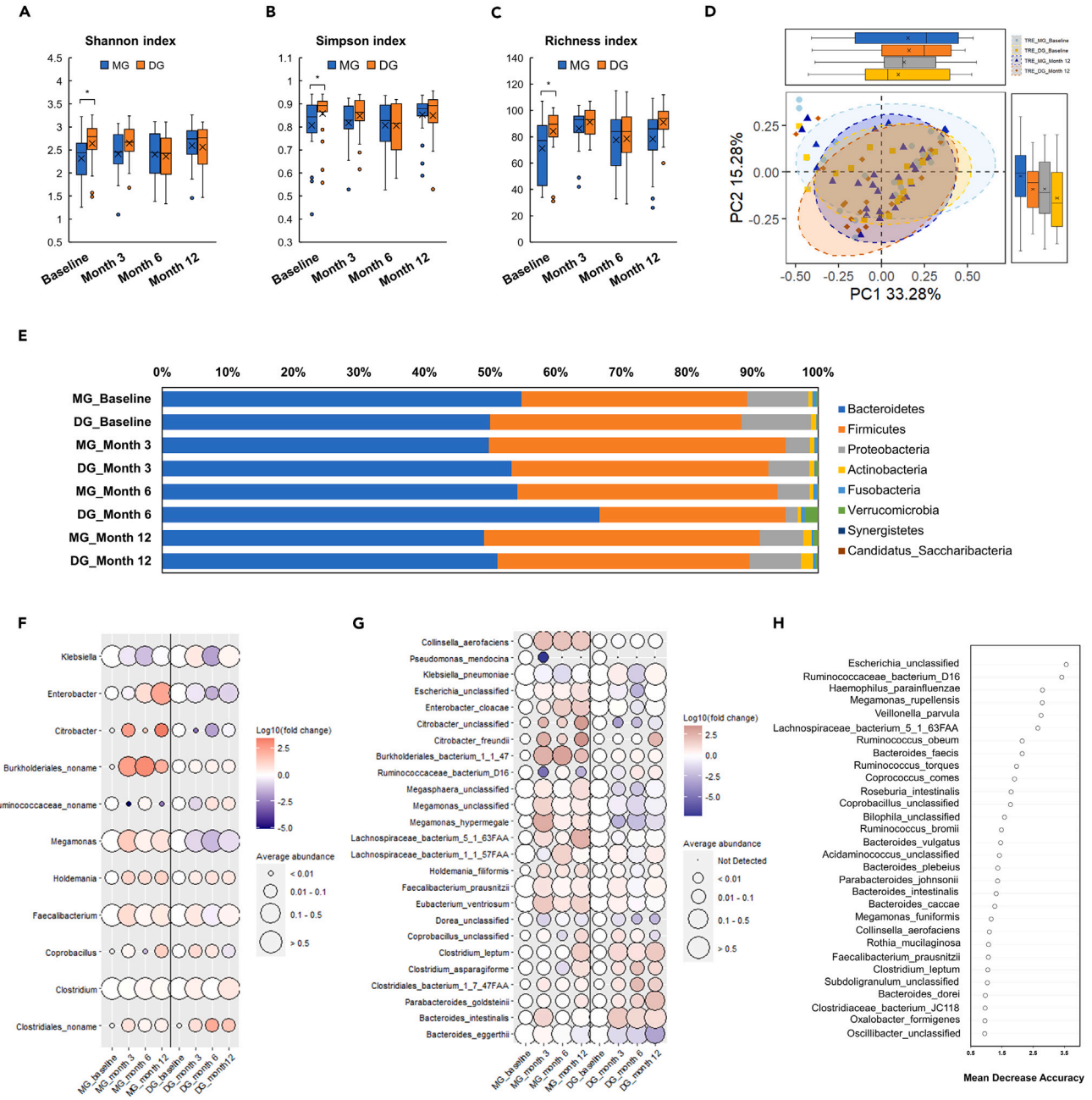


Figure 2. Gut microbial characteristics in moderate and distinct weight loss groups during a 12-month trial period

(A–C) Boxplots of the alpha-diversity index including (A) Shannon, (B) Simpson, and (C) richness index between the moderate weight loss group (MG) group and distinct weight loss group (DG) group at 0, 3, 6, or 12 months of TRE intervention. In the boxplot, the cross indicates the mean, the horizontal bar indicates median; the top and bottom borders of the box mark the first and third quartiles; the error bars indicate the 5th and 95th percentiles, and the dots indicate the individuals whose values were outside the 5th or 95th percentiles.

(D) Principal coordinate analysis (PCoA) of beta-diversity based on Bray-Curtis distance between the MG and DG groups. The boxplot of samples discrete distribution on the principal component (PC)1 and PC2 axis were also shown.

(E) The overall composition and relative abundance of the bacterial community in each group at the phylum level.

(F and G) The relative abundance of bacterium significantly altered by a 12-month TRE intervention ($p < 0.05$ by Wilcoxon signed-rank test) at the (F) genus and (G) species level in MG and DG groups at 0, 3, 6, or 12 months.

(H) The importance of the species alterations ranked according to their contribution to the distinguishing of MG and DG groups in the classifier model built by Random Forest.

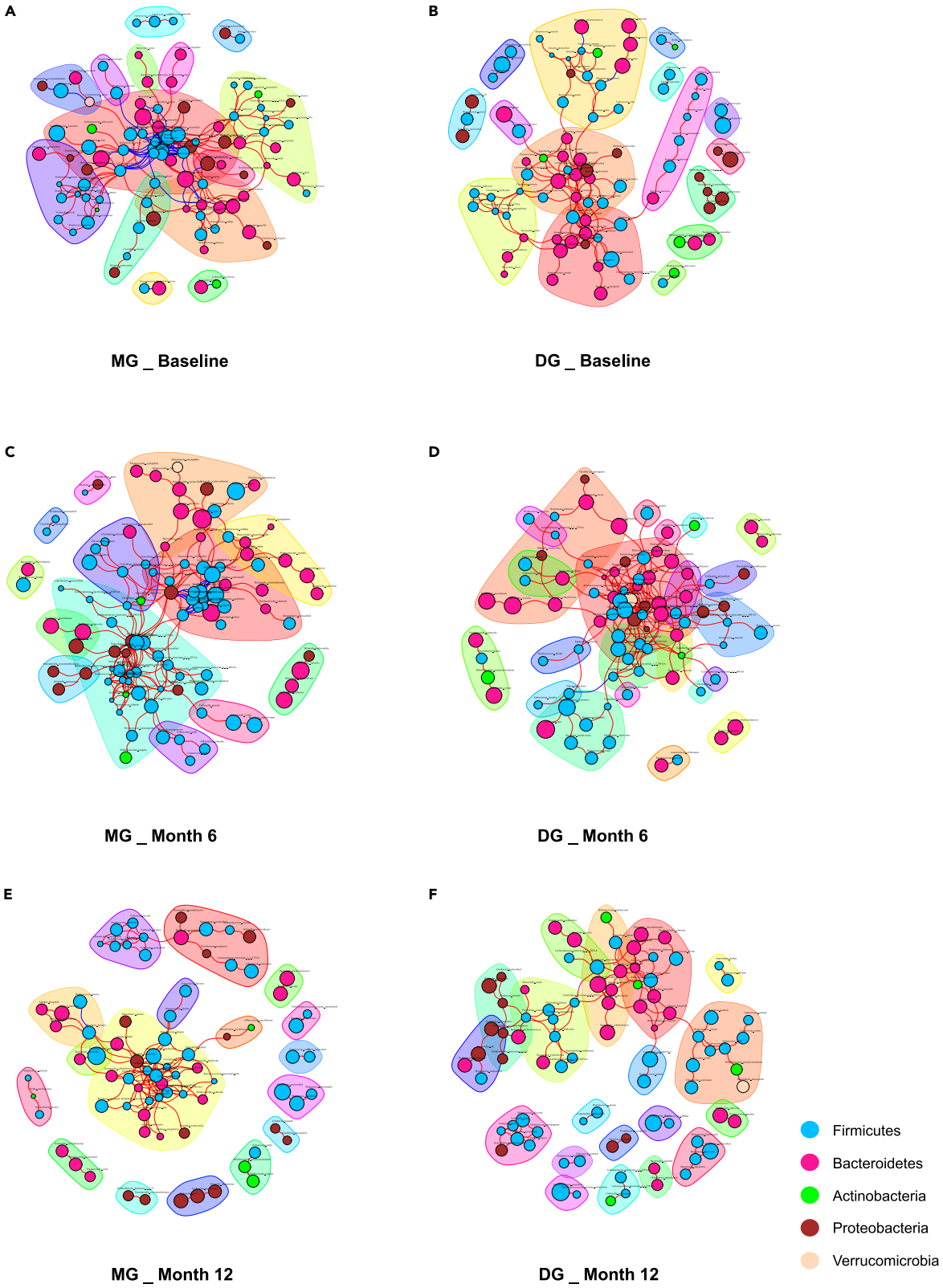

A
B
MG _ Baseline
DG _ Baseline
C
D
MG _ Month 6
DG _ Month 6
E
F
MG _ Month 12
DG _ Month 12
Firmicutes
Bacteroidetes
Actinobacteria
Proteobacteria
Verrucomicrobia

Figure 3. Co-abundance network before and after dietary intervention

The cooccurrence networks in (A) MG group and (B) DG group at 0, 6, and 12 months of TRE intervention reflect network interaction complexity. All nodes were colored at the phylum level (isolated nodes were excluded), and edges were estimated by Spearman's rank correlation coefficient (abs[r] > 0.6, $p < 0.05$). The node size represented the average relative abundance of each species.

positively correlated with the changes of body weight and body composition parameters like fat mass, body fat, abdominal fat area, and liver fat. Meanwhile, another KOs like K00616 (transaldolase), K07173 (S-ribosylhomocysteine lyase), K05823 (N-acetyldiaminopimelate deacetylase), etc. were found to be negatively correlated with the changes of lipid profiles including triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C). Only a few KO alterations were correlated with the changes of glucometabolic index (fasting glucose, OGTT-2h glucose, HOMA-IR, and HbA1c) and blood pressure.

Spearman correlation analysis was also performed to explore the association between KO changes and the gut microbiota alterations (Figure 5C). Among the 21 species alterations significantly correlated with the changes of KO abundances, *Ruminococcaceae_bacterium_D16*, *Parabacteroides_goldsteinii*, *Megasmonas_hypermegale*, and *Klebsiella_pneumoniae*, showed the strongest associations (Figure 5C). Specifically, *Ruminococcaceae_bacterium_D16* with increased abundance in DG group, was found to be negatively associated with K11263 (acetyl-CoA/propionyl-CoA carboxylase) and K16148 (alpha-maltose-1-phosphate synthase). *Parabacteroides_goldsteinii*, another bacterium with increased abundance in DG group, was found to be negatively correlated to K00886 (polyphosphate glucokinase), K016148 (alpha-maltose-1-phosphate synthase), K00616 (transaldolase), K11263 (acetyl-CoA/propionyl-CoA carboxylase), and K07173 (S-ribosylhomocysteine lyase). *Megamonas_unclassified*, decreased abundance in DG group, was found to be positively associated with K06896 (maltose 6′-phosphate phosphatase). *Bacteroides_intestinali* with increased abundance in DG group was found to be negatively associated with K05823 (N-acetyldiaminopimelate deacetylase). Meanwhile, *Klebsiella_pneumoniae* with decreased abundance in MG group, was found to be positively correlated with K00886 (polyphosphate glucokinase) and K01496 (phosphoribosyl-AMP cyclohydrolase). K11381 (2-oxoisovalerate dehydrogenase E1 component), the only increased KO in DG group, was positively correlated with *Clostridium_leptum*, whose abundance was increased in DG group. These findings suggest that the underlying mechanisms of the gut microbiome influencing weight loss efficacy of TRE intervention might be driven by specific bacterial species involved in different metabolic pathways.

Notably, the differential KOs, mostly enzymes, were enriched in carbon metabolism and amino acid metabolism (Figure 5D). The decreased genes in the DG group involved in glycolysis (K00886), glycogen biosynthesis (K06896), pentose phosphate pathway (K00616), citrate cycle (K01902), and fatty acid biosynthesis (K11263). Furthermore, altered genes in DG group were also found to participate in amino acid metabolism. The decreased of K07173 involving in the cysteine biosynthesis from methionine, K01496 involving in the histidine biosynthesis from pentose phosphate pathway metabolites, K05823 encoding enzymes responsible for lysine biosynthesis from aspartate, were significantly decreased in DG group. While K11381, a gene participating in the catabolism of branched-chain amino acids including valine, leucine, and isoleucine, was selectively increased in DG group. Taken together, these data demonstrate that differential capacity for carbon metabolism and amino acid metabolism shaped by TRE intervention might contribute to the difference in the weight loss efficacy of TRE intervention.

Prediction of host responses to TRE intervention using baseline microbiota

Considering the differential alterations between MG and DG at taxonomic and functional levels after TRE intervention, we next explored whether differences of gut microbiome at baseline could predict the effects of weight loss during TRE. To this end, a random forest integrating the baseline microbial abundance was applied. Among the 30 most informative species contributing to this classifier (Figure 6A), only nine bacterial biomarkers showed a significant difference in relative abundance at baseline ($p < 0.05$, Figures 6B–6J). The nine discriminatory species, including *Escherichia_unclassified*, *Lachnospiraceae_bacterium_5_1_63FAA*, *Megamonas_hypermegale*, *Megamonas_unclassified*, *Subdoligranulum_unclassified*, *Veillonella_unclassified*, *Bacteroidales_bacterium_ph8*, *Collinsella_aerofaciens*, and *Ruminococcus_lactaris*, were enriched in DG group versus MG group at baseline. The relative abundance of microbial species at baseline was significantly correlated with the change of metabolic parameters after a 12-month TRE (Figure S7). Then the nine microbial species factors were assessed in the univariate and multivariate predictive linear regression models. The predictive linear regression model based on the relative abundance of single species could achieve an area under the curve (AUC) value ranging from 0.64 to 0.77 (Figure S8). While the predictive linear regression model based on the combination of the nine species could achieve an AUC of 0.83 with a confidence interval (CI) of 0.71–0.94 between MG and DG groups to predictive the outcome of weight loss efficacy (Figure 6K). We then constructed predictive models for body weight change based on the combination of nine discriminatory species and the predicted value and measured weight change had Spearman's R of 0.77 ($p < 0.001$, Figure 6L). These data suggested that the relative abundance of gut microbiota at baseline can be a powerful predictor of weight loss outcome after TRE intervention.

DISCUSSION

In this study, we assessed the role of gut microbiota in the heterogeneity of weight-loss effect during a 12-month TRE intervention. Based on the shotgun metagenomic data, the results demonstrated significant alterations in the composition and functions of gut microbiota between MG and DG. More importantly, our prediction model revealed that the baseline gut microbiota profiles can predict the individual weight loss outcomes before TRE interventions.

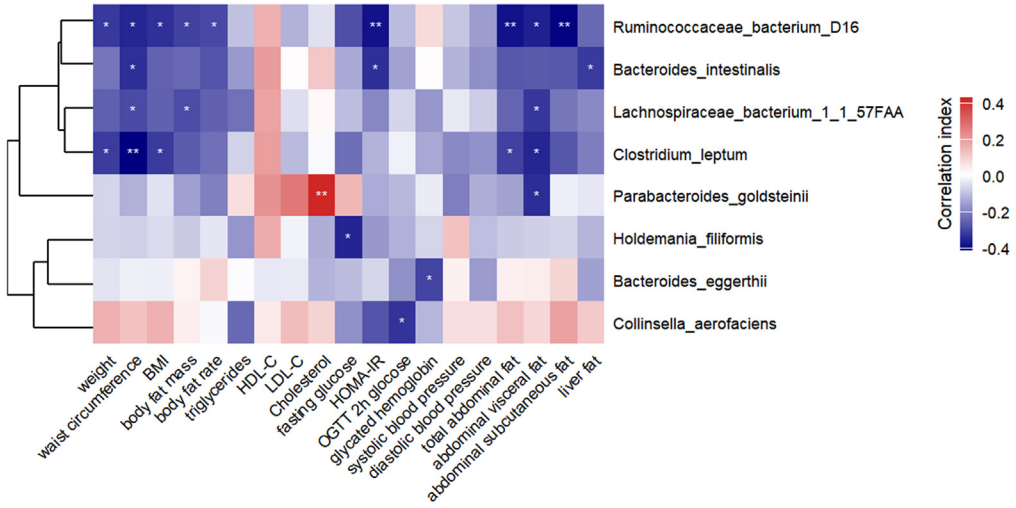


Figure 4. Associations between alterations of microbial species and changes of clinical indices

Heatmap of the Spearman's correlation coefficients between changes in different clinical indices and taxonomic alterations caused by a 12-month TRE intervention after adjustment for baseline measurements. *$p < 0.05$, and **$p < 0.01$. Only species significantly altered by a 12-month TRE intervention ($p < 0.05$ by Wilcoxon signed-rank test) and with significant correlations (at least one p value <0.05) with changes of clinical indices were shown.

TRE refers to time-restricted food consumption for a period of counted hours and allows daily fasting duration greater than 12 h, irrespective of altering the quality and quantity of nutrients.[23] In fact, the host's circadian and eating behavior might influence the community compositions and metabolic functions of gut microbiome.[24] Human studies show that TRE enhances the richness of gut microbes and increases abundances of many specific species correlated with weight control.[17,18] In the present study, we failed to find the changes of diversity of gut microbiota in post-TRE compared to pre-TRE. However, weight change was extremely different between individuals in our study. These participants were divided into MG and DG based on their weight loss during TRE. We proposed that gut microbiota might influence the individual's responses to TRE intervention. Therefore, we further analyzed the compositions and functions in both MG and DG.

In our study, in line with the significant reductions of body weight in DG, the alterations of gut microbiota from baseline to 12-month in DG were different from that in MG. The species *Bacteroides intestinalis*, *Parabacteroides goldsteinii*, *Lachnospiraceae bacterium_1_1_57FAA*, *Ruminococcaceae bacterium_D16*, and *Clostridium leptum* enriched in DG were positively associated with the reduction of body weight and obesity-related disorders.[25–28] Previous study demonstrated that Ramadan-associated intermittent fasting was especially associated with an upregulation of *Ruminococcaceae* and butyric acid—producing *Lachnospiraceae* in a manner that correlates to improvement in human physiologic surrogate markers such as BMI.[29] *Lachnospiraceae* and *Clostridium leptum* were the major bacterial species which could produce butyrate in the human intestine.[30] The microbiota-generated butyric acid, a highly bioactive compound, is known to promote metabolic benefits through the modulation of gut-brain neural circuits.[31] The alteration of microbiome may provide an explanation for at least some of the different weight change among participants with intermittent fasting. Meanwhile, higher abundance of *Dorea_ unclassified*, *Bacteroides eggerthii*, *Coprobacillus_ unclassified*, and *Megamonas hypermegale*, which were decreased in DG, were reported to be correlated with the development of obesity and T2DM.[32–34] In contrast to the beneficial change of microbial composition in DG, *Coprobacillus_ unclassified*, and *Escherichia_ unclassified* that increased in MG positively correlated with obesity and T2DM development. Proteobacteria phylum enriched in MG was associated with most metabolic diseases, such as obesity.[35,36] Previous study indicates that Proteobacteria can affect the production of metabolites as these bacteria contain enzymes necessary for the breakdown of galactosides.[37]

To inferring how different gut microbes modulate the responses to weight-loss during TRE, we found that gut microbes in DG and MG groups exhibited a clear segregation of functional variations and divergent pathway enrichment. Pathways involved in fatty acid biosynthesis, glycogen biosynthesis, and nucleotide metabolism were preferentially decreased in DG and enhanced in MG. Glycogen is a predominant carbohydrate reserve in various organisms, which provides energy for different life activities. Glucose and fatty acid metabolism plays a crucial role in energy metabolism, which provides the energy required for life processes, such as growth and development.[38] However, great rates of glycogen and fatty acids synthesis are exhibited in obesity and plasma free fatty acids might cause the hepatic glycogen content that is associated with obesity and insulin resistance.[39] Similar with the study of high-fat diet fed mice,[40] we observed that the serum lipids levels were significantly decreased in TRE-DG and the expressions of genes involved in the bioenergetic pathway were reversed in DG after TRE intervention. Furthermore, the role of amino acids metabolism is emerged to be involved in the development of obesity and its related metabolic disorders. Blood amino acid concentrations of some essential amino acids and their derivatives, in particular branch chain amino acids (BCAA), sulfur amino acids, tyrosine, and phenylalanine, are associated with obesity and insulin resistance.[41] Increased plasma concentration of histidine was also observed in cohort of obese T2DM women.[42,43] Of note, we found the histidine synthesis was increased in MG but decreased in DG. Meanwhile, gene related to cysteine biosynthesis was

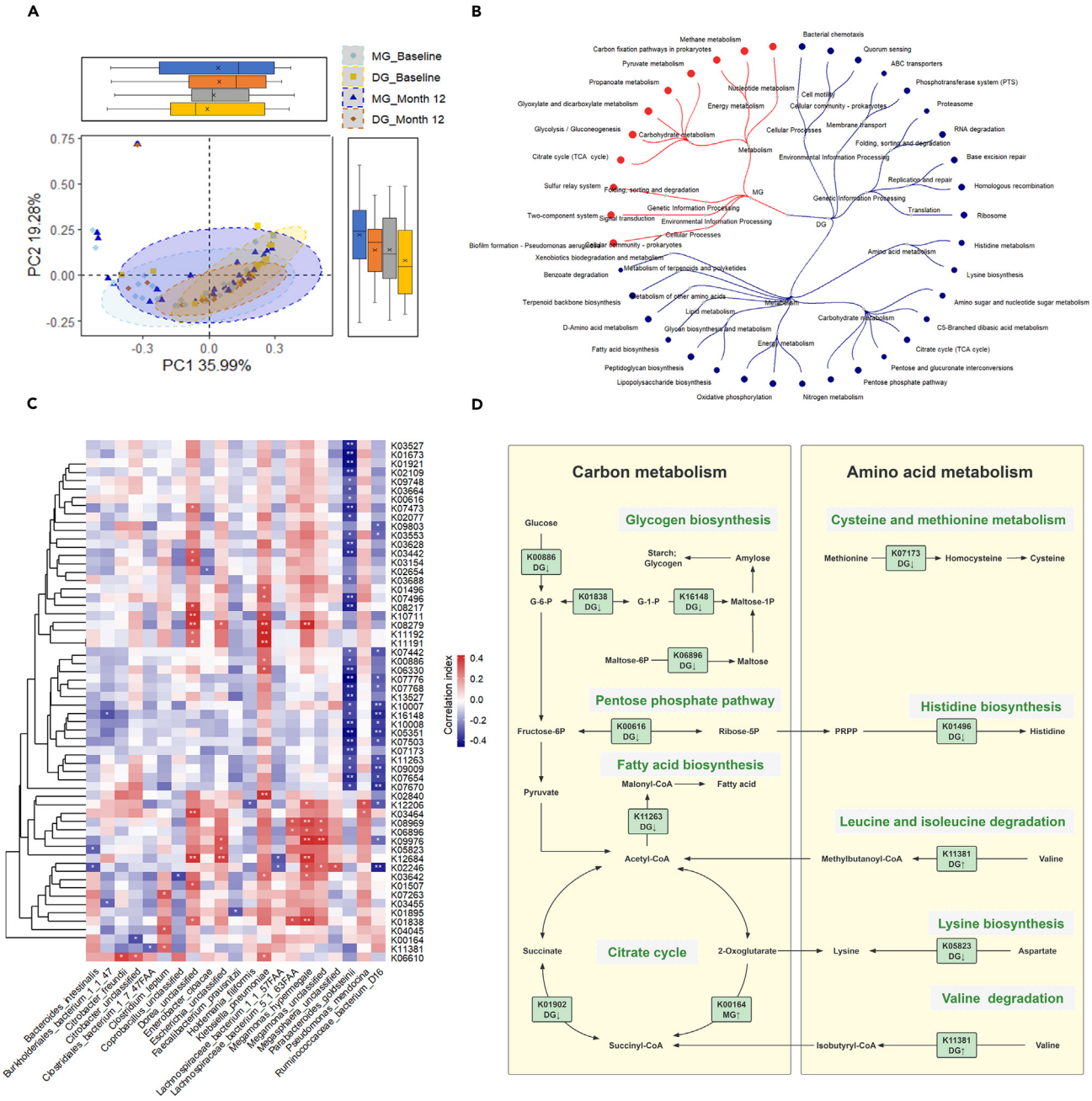


Figure 5. Functional shifts of gut microbiota in moderate and distinct weight loss groups

(A) Principal coordinates analysis (PCoA) plot based on the Bray-Curtis distance in KEGG orthology(KO). The boxplot of samples discrete distribution on the principal component (PC)1 and PC2 axis in KOs were also shown.

(B) Significantly altered pathways ($p < 0.05$) induced by TRE intervention in MG and DG, respectively. The tree demonstrates a functional hierarchy in the KEGG pathway maps. Red and blue indicate increased and decreased relative abundance, respectively. The size of outer nodes reflected the magnitude of the change.

(C) Heatmap of the Spearman's correlation coefficients between the differential KOs and taxonomic alterations caused by TRE intervention. $*p < 0.05$, and $**p < 0.01$.

(D) Altered KOs involved in carbon metabolism and amino acids metabolism.

decreased in DG, and did not change in MG, consisting with previous findings that increased cysteine availability may promote the development of obesity.[44,45]

Due to the inconsistently responsiveness to the same weight loss strategy, many studies previously reported that the individual's metabolic response to diets depended partly on baseline gut microbiome structures.[46–48] High abundance of *Blautia wexlerae* and *Bacteroides dorei* at

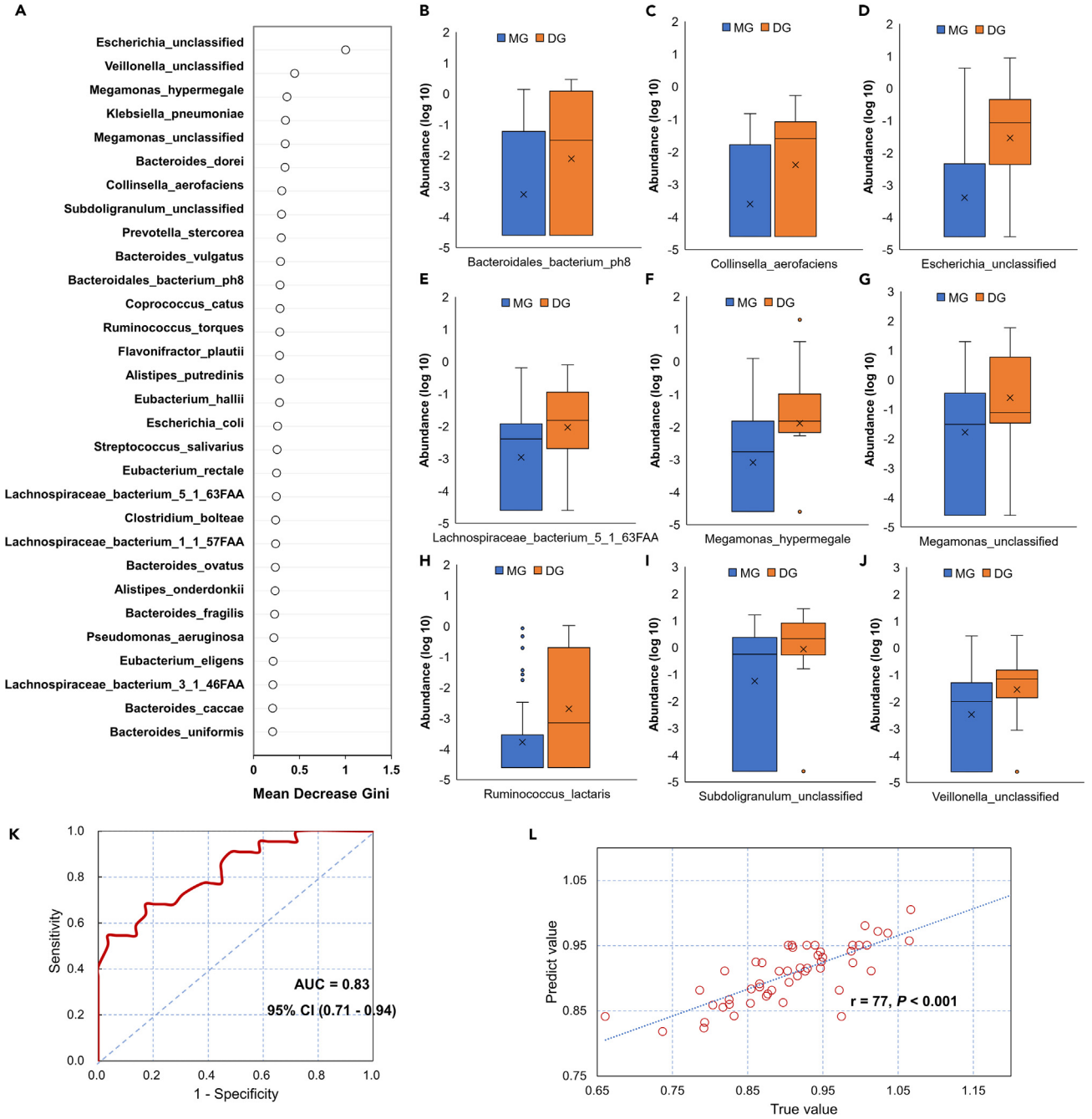


Figure 6. Baseline microbial composition contributing to the classifier of moderate and distinct weight loss groups

(A) The importance of the species ranked according to their contribution to the predictive model built by Random Forest. (B–J) The relative abundance of (B) *Bacteroidales_bacterium_ph8*, (C) *Collinsella_aerofaciens*, (D) *Escherichia_unclassified*, (E) *Lachnospiraceae_bacterium_5_1_63FAA*, (F) *Megamonas_hypermegale*, (G) *Megamonas_unclassified*, (H) *Ruminococcus_lactaris*, (I) *Subdoligranulum_unclassified*, and (J) *Veillonella_unclassified* at baseline. *, $p < 0.05$ by Wilcoxon rank-sum test.

(K) The receiver operating characteristic (ROC) curves and area under curve (AUC) of the nine-species-based algorithm for the discrimination between moderate and distinct weight loss efficacy.

(L) Comparison of the association strength between the true and predicted body weight change based on the nine species.

baseline were regarded as important predictors for weight loss in calorie restriction intervention.[46] The baseline *Prevotella-to-Bacteroides* (P/B) ratio was validated to be an important biomarker associated with dietary weight loss.[49] Recently, higher relative abundance of *Bacteroidaceae Bacteroides* at baseline was proposed to contribute to superior weight loss after low-carbohydrate diet intervention.[47] However,

data from the study of low-energy diet suggested that baseline microbiota was not predictive of changes in clinical measures related to glucose metabolism.[50] In our study, we found that gut microbe profiles were different between participants in MG and DG at baseline, and we built a predictive linear regression model to predict weight loss efficacy according to the baseline microbial signatures. The differential microbial species between MG and DG at baseline were the contributors to the prediction model that achieved an AUC value of 0.83 to predict the outcome of weight loss efficacy. Among these, baseline abundance of *unclassified Escherichia*, *Megamonas hypermegale*, and *unclassified Megamonas* were present negatively correlated with body weight, body fat, and waist circumference change. Some studies have also proved that an obvious increase in *Megamonas* abundance was observed in the gut microbial profile in obesity,[51,52] and some members of *Megamonas* can produce acetic and propionic acids, which have been discovered to be substrates for the formation of lipogenesis and cholesterol in rodents.[53] Especially, the *Megamonas* species had positive relationships with K01838 and K06896, which contribute to the pathways responsible for the biosynthesis of glycogen.

This study illustrated that gut microbiota might contribute to different individual responsiveness during a TRE intervention. After a 12-month intervention, the changes of gut microbial composition were different between DG and MG. Of note, we found baseline gut microbiota signatures might be a strong predictor to evaluate individualized weight-loss efficacy before TRE intervention. In the future, gut microbiota may help to guide the clinical application of TRE intervention to develop personalized weight loss strategies.

Limitations of the study

The strengths of this study were relatively long duration of TRE intervention and the high percentage diet adherence of participants. This study also has some limitations. Firstly, some samples missed data of the information about stool collection timing, we cannot exclude the possibility that natural temporal fluctuation of the gut microbiota. Secondly, a relatively small sample size of this study due to some participants did not provide stool samples or exhausted samples. The findings should be further validated in a larger cohort in the future. Thirdly, although alterations of gut microbiota in DG were observed after TRE intervention, further animal study is needed to validate the beneficial effects of these bacteria based on our findings. Fourthly, metabolomics profiling should be performed to explore the mechanisms of how gut microbes modulating the responses of weight-loss during TRE.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Study population and ethics approval
- METHOD DETAILS
  - Diet
  - Anthropometric and clinical measurements
  - Responsiveness of weight loss over 12 months
  - Fecal DNA extraction and sequencing
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Metagenomics analysis
  - Other statistical analysis
- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110202.

ACKNOWLEDGMENTS

The authors would like to thank the participants of the study. This study was supported by a grant (no. 82325011) from National Science Fund for Distinguished Young Scholars; a grant (U22A20288) from the Joint Funds of the National Natural Science Foundation of China; grants (no.LC2019ZD010 and 2019CR022) from Key-Area Clinical Research Program of Southern Medical University; a grant (no. 81970736, no. 81970725, and no. 82170716) from National Natural Science Foundation of China; and a grant (no.2018YFA0800404) from the National Key Research and Development Project; a grant (no. 2022M711513) from China Postdoctoral Science Foundation. The funding organizations played no role in the design of study, choice of enrolled participants, review and interpretation of data, preparation of manuscript, final approval of manuscript or decision to submit for publication.

AUTHOR CONTRIBUTIONS

C.H., D.L., and H.Z. analyzed and drafted the manuscript. C.H., D.L., J.L., and H.Z. were involved in the study conception and design. C.H., D.L., S.Y., X.W., and Y.H. recruited and followed up patients. J.L. and H.Z. obtained funding for the study. C.H., D.L., P.Z., J.L., B.X., and Y.L. participated in the interpretation of the results and critical revision of the manuscript. Y.L. and D.G. give administrative, technical, or material support. H.Z. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final manuscript for submission.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: January 19, 2024
Revised: April 16, 2024
Accepted: June 1, 2024
Published: June 6, 2024

REFERENCES

1. Bluher, M. (2019). Obesity: global epidemiology and pathogenesis. Nat. Rev. Endocrinol. *15*, 288–298. https://doi.org/10.1038/s41574-019-0176-8.
2. Jensen, M.D., Ryan, D.H., Apovian, C.M., Ard, J.D., Comuzzie, A.G., Donato, K.A., Hu, F.B., Hubbard, V.S., Jakicic, J.M., Kushner, R.F., et al. (2014). 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. J. Am. Coll. Cardiol. *63*, 2985–3023. https://doi.org/10.1016/j.jacc.2013.11.004.
3. Liu, D., Huang, Y., Huang, C., Yang, S., Wei, X., Zhang, P., Guo, D., Lin, J., Xu, B., Li, C., et al. (2022). Calorie Restriction with or without Time-Restricted Eating in Weight Loss. N. Engl. J. Med. *386*, 1495–1504. https://doi.org/10.1056/NEJMoa2114833.
4. Bray, G.A., Ryan, D.H., Johnson, W., Champagne, C.M., Johnson, C.M., Rood, J., Williamson, D.A., and Sacks, F.M. (2017). Markers of dietary protein intake are associated with successful weight loss in the POUNDS Lost trial. Clin. Obes. *7*, 166–175. https://doi.org/10.1111/cob.12188.
5. Greenberg, I., Stampfer, M.J., Schwarzfuchs, D., and Shai, I.; DIRECT Group (2009). Adherence and success in long-term weight loss diets: the dietary intervention randomized controlled trial (DIRECT). J. Am. Coll. Nutr. *28*, 159–168. https://doi.org/10.1080/07315724.2009.10719767.
6. Lean, M., Brosnahan, N., McLoone, P., McCombie, L., Higgs, A.B., Ross, H., Mackenzie, M., Grieve, E., Finer, N., Reckless, J., et al. (2013). Feasibility and indicative results from a 12-month low-energy liquid diet treatment and maintenance programme for severe obesity. Br. J. Gen. Pract. *63*, e115–e124. https://doi.org/10.3399/bjgp13X663073.
7. Maruvada, P., Leone, V., Kaplan, L.M., and Chang, E.B. (2017). The Human Microbiome and Obesity: Moving beyond Associations. Cell Host Microbe *22*, 589–599. https://doi.org/10.1016/j.chom.2017.10.005.
8. Ridaura, V.K., Faith, J.J., Rey, F.E., Cheng, J., Duncan, A.E., Kau, A.L., Griffin, N.W., Lombard, V., Henrissat, B., Bain, J.R., et al. (2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science *341*, 1241214. https://doi.org/10.1126/science.1241214.
9. Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.M., Kennedy, S., et al. (2013). Richness of human gut microbiome correlates with metabolic markers. Nature *500*, 541–546. https://doi.org/10.1038/nature12506.
10. Leong, K.S.W., Jayasinghe, T.N., Wilson, B.C., Derraik, J.G.B., Albert, B.B., Chiavaroli, V., Svirskis, D.M., Beck, K.L., Conlon, C.A., Jiang, Y., et al. (2020). Effects of Fecal Microbiome Transfer in Adolescents With Obesity: The Gut Bugs Randomized Controlled Trial. JAMA Netw. Open *3*, e2030415. https://doi.org/10.1001/jamanetworkopen.2020.30415.
11. Lahtinen, P., Juuti, A., Luostarinen, M., Niskanen, L., Liukkonen, T., Tillonen, J., Kössi, J., Ilvesmäki, V., Viljakka, M., Satokari, R., and Arkkila, P. (2022). Effectiveness of Fecal Microbiota Transplantation for Weight Loss in Patients With Obesity Undergoing Bariatric Surgery: A Randomized Clinical Trial. JAMA Netw. Open *5*, e2247226. https://doi.org/10.1001/jamanetworkopen.2022.47226.
12. Depommier, C., Everard, A., Druart, C., Plovier, H., Van Hul, M., Vieira-Silva, S., Falony, G., Raes, J., Maiter, D., Delzenne, N.M., et al. (2019). Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat. Med. *25*, 1096–1103. https://doi.org/10.1038/s41591-019-0495-2.
13. Zmora, N., Suez, J., and Elinav, E. (2019). You are what you eat: diet, health and the gut microbiota. Nat. Rev. Gastroenterol. Hepatol. *16*, 35–56. https://doi.org/10.1038/s41575-018-0061-2.
14. Beam, A., Clinger, E., and Hao, L. (2021). Effect of Diet and Dietary Components on the Composition of the Gut Microbiota. Nutrients *13*, 2795. https://doi.org/10.3390/nu13082795.
15. Li, G., Xie, C., Lu, S., Nichols, R.G., Tian, Y., Li, L., Patel, D., Ma, Y., Brocker, C.N., Yan, T., et al. (2017). Intermittent Fasting Promotes White Adipose Browning and Decreases Obesity by Shaping the Gut Microbiota. Cell Metabol. *26*, 672–685.e4. https://doi.org/10.1016/j.cmet.2017.08.019.
16. Khan, M.N., Khan, S.I., Rana, M.I., Ayyaz, A., Khan, M.Y., and Imran, M. (2022). Intermittent fasting positively modulates human gut microbial diversity and ameliorates blood lipid profile. Front. Microbiol. *13*, 922727. https://doi.org/10.3389/fmicb.2022.922727.
17. Zeb, F., Wu, X., Chen, L., Fatima, S., Ijaz Ul, H., Chen, A., Xu, C., Jianglei, R., Feng, Q., and Li, M. (2020). Time-restricted feeding is associated with changes in human gut microbiota related to nutrient intake. Nutrition *78*, 110797. https://doi.org/10.1016/j.nut.2020.110797.
18. Ozkul, C., Yalinay, M., and Karakan, T. (2019). Islamic fasting leads to an increased abundance of Akkermansia muciniphila and Bacteroides fragilis group: A preliminary study on intermittent fasting. Turk. J. Gastroenterol. *30*, 1030–1035. https://doi.org/10.5152/tjg.2019.19185.
19. Xie, Z., Sun, Y., Ye, Y., Hu, D., Zhang, H., He, Z., Zhao, H., Yang, H., and Mao, Y. (2022). Randomized controlled trial for time-restricted eating in healthy volunteers without obesity. Nat. Commun. *13*, 1003. https://doi.org/10.1038/s41467-022-28662-5.
20. Zeb, F., Wu, X., Chen, L., Fatima, S., Haq, I.U., Chen, A., Majeed, F., Feng, Q., and Li, M. (2020). Effect of time-restricted feeding on metabolic risk and circadian rhythm associated with gut microbiome in healthy males. Br. J. Nutr. *123*, 1216–1226. https://doi.org/10.1017/S0007114519003428.
21. Ferrocino, I., Pellegrini, M., D'Eusebio, C., Goitre, I., Ponzo, V., Fadda, M., Rosato, R., Mengozzi, G., Beccuti, G., Merlo, F.D., et al. (2022). The Effects of Time-Restricted Eating on Metabolism and Gut Microbiota: A Real-Life Study. Nutrients *14*, 2569. https://doi.org/10.3390/nu14132569.
22. Gabel, K., Marcell, J., Cares, K., Kalam, F., Cienfuegos, S., Ezpeleta, M., and Varady, K.A. (2020). Effect of time restricted feeding on the gut microbiome in adults with obesity: A pilot study. Nutr. Health *26*, 79–85. https://doi.org/10.1177/0260106020910907.
23. Manoogian, E.N.C., Chow, L.S., Taub, P.R., Laferrère, B., and Panda, S. (2022). Time-restricted Eating for the Prevention and Management of Metabolic Diseases. Endocr. Rev. *43*, 405–436. https://doi.org/10.1210/endrev/bnab027.
24. Thaiss, C.A., Levy, M., Korem, T., Dohnalová, L., Shapiro, H., Jaitin, D.A., David, E., Winter, D.R., Gury-BenAri, M., Tatirovsky, E., et al. (2016). Microbiota Diurnal Rhythmicity

Programs Host Transcriptome Oscillations. Cell *167*, 1495–1510.e12. https://doi.org/10.1016/j.cell.2016.11.003.

25. Wu, T.R., Lin, C.S., Chang, C.J., Lin, T.L., Martel, J., Ko, Y.F., Ojcius, D.M., Lu, C.C., Young, J.D., and Lai, H.C. (2019). Gut commensal Parabacteroides goldsteinii plays a predominant role in the anti-obesity effects of polysaccharides isolated from Hirsutella sinensis. Gut *68*, 248–262. https://doi.org/10.1136/gutjnl-2017-315458.
26. Wang, T., Han, J., Dai, H., Sun, J., Ren, J., Wang, W., Qiao, S., Liu, C., Sun, L., Liu, S., et al. (2022). Polysaccharides from Lyophyllum decastes reduce obesity by altering gut microbiota and increasing energy expenditure. Carbohydr. Polym. *295*, 119862. https://doi.org/10.1016/j.carbpol.2022.119862.
27. Deng, K., Shuai, M., Zhang, Z., Jiang, Z., Fu, Y., Shen, L., Zheng, J.-S., and Chen, Y.-m. (2022). Temporal relationship among adiposity, gut microbiota, and insulin resistance in a longitudinal human cohort. BMC Med. *20*, 171. https://doi.org/10.1186/s12916-022-02376-3.
28. Nie, X., Chen, J., Ma, X., Ni, Y., Shen, Y., Yu, H., Panagiotou, G., and Bao, Y. (2020). A metagenome-wide association study of gut microbiome and visceral fat accumulation. Comput. Struct. Biotechnol. J. *18*, 2596–2609. https://doi.org/10.1016/j.csbj.2020.09.026.
29. Su, J., Wang, Y., Zhang, X., Ma, M., Xie, Z., Pan, Q., Ma, Z., and Peppelenbosch, M.P. (2021). Remodeling of the gut microbiome during Ramadan-associated intermittent fasting. Am. J. Clin. Nutr. *113*, 1332–1342. https://doi.org/10.1093/ajcn/nqaa388.
30. Meehan, C.J., and Beiko, R.G. (2014). A phylogenomic view of ecological specialization in the Lachnospiraceae, a family of digestive tract-associated bacteria. Genome Biol. Evol. *6*, 703–713. https://doi.org/10.1093/gbe/evu050.
31. Belcheva, A., Irrazabal, T., Robertson, S.J., Streutker, C., Maughan, H., Rubino, S., Moriyama, E.H., Copeland, J.K., Surendra, A., Kumar, S., et al. (2014). Gut Microbial Metabolism Drives Transformation of Msh2-Deficient Colon Epithelial Cells. Cell *158*, 288–299. https://doi.org/10.1016/j.cell.2014.04.051.
32. Liu, Y., Zhu, J., Wang, H., Lu, W., Lee, Y.K., Zhao, J., and Zhang, H. (2022). Machine learning framework for gut microbiome biomarkers discovery and modulation analysis in large-scale obese population. BMC Genom. *23*, 850. https://doi.org/10.1186/s12864-022-09087-2.
33. Ottosson, F., Brunkwall, L., Ericson, U., Nilsson, P.M., Almgren, P., Fernandez, C., Melander, O., and Orho-Melander, M. (2018). Connection Between BMI-Related Plasma Metabolite Profile and Gut Microbiota. J. Clin. Endocrinol. Metab. *103*, 1491–1501. https://doi.org/10.1210/jc.2017-02114.
34. Wang, L., Yu, X., Xu, X., Ming, J., Wang, Z., Gao, B., Xing, Y., Zhou, J., Fu, J., Liu, T., et al. (2021). The Fecal Microbiota Is Already Altered in Normoglycemic Individuals Who Go on to Have Type 2 Diabetes. Front. Cell. Infect. Microbiol. *11*, 598672. https://doi.org/10.3389/fcimb.2021.598672.
35. Gu, Y., Liu, C., Zheng, N., Jia, W., Zhang, W., and Li, H. (2019). Metabolic and Gut Microbial Characterization of Obesity-Prone Mice under a High-Fat Diet. J. Proteome Res. *18*, 1703–1714. https://doi.org/10.1021/acs.jproteome.8b00945.
36. Zhang, X., Zhao, Y., Zhang, M., Pang, X., Xu, J., Kang, C., Li, M., Zhang, C., Zhang, Z., Zhang, Y., et al. (2012). Structural changes of gut microbiota during berberine-mediated prevention of obesity and insulin resistance in high-fat diet-fed rats. PLoS One *7*, e42529. https://doi.org/10.1371/journal.pone.0042529.
37. Acharya, K.D., Noh, H.L., Graham, M.E., Suk, S., Friedline, R.H., Gomez, C.C., Parakoyi, A.E.R., Chen, J., Kim, J.K., and Tetel, M.J. (2021). Distinct Changes in Gut Microbiota Are Associated with Estradiol-Mediated Protection from Diet-Induced Obesity in Female Mice. Metabolites *11*, 499. https://doi.org/10.3390/metabo11080499.
38. Zhang, J.B., Lu, Z.J., and Yu, H.Z. (2022). Silencing of Glycogen Synthase Kinase 3 Significantly Inhibits Chitin and Fatty Acid Metabolism in Asian Citrus Psyllid, Diaphorina citri. Int. J. Mol. Sci. *23*, 9654. https://doi.org/10.3390/ijms23179654.
39. Allick, G., Sprangers, F., Weverling, G.J., Ackermans, M.T., Meijer, A.J., Romijn, J.A., Endert, E., Bisschop, P.H., and Sauerwein, H.P. (2004). Free fatty acids increase hepatic glycogen content in obese males. Metabolism *53*, 886–893. https://doi.org/10.1016/j.metabol.2004.01.012.
40. Hatori, M., Vollmers, C., Zarrinpar, A., DiTacchio, L., Bushong, E.A., Gill, S., Leblanc, M., Chaix, A., Joens, M., Fitzpatrick, J.A.J., et al. (2012). Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metabol. *15*, 848–860. https://doi.org/10.1016/j.cmet.2012.04.019.
41. Adams, S.H. (2011). Emerging perspectives on essential amino acid metabolism in obesity and the insulin-resistant state. Adv. Nutr. *2*, 445–456. https://doi.org/10.3945/an.111.000737.
42. Huffman, K.M., Shah, S.H., Stevens, R.D., Bain, J.R., Muehlbauer, M., Slentz, C.A., Tanner, C.J., Kuchibhatla, M., Houmard, J.A., Newgard, C.B., and Kraus, W.E. (2009). Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. Diabetes Care *32*, 1678–1683. https://doi.org/10.2337/dc08-2075.
43. Fiehn, O., Garvey, W.T., Newman, J.W., Lok, K.H., Hoppel, C.L., Adams, S.H., and Adams, S.H. (2010). Plasma Metabolomic Profiles Reflective of Glucose Homeostasis in Non-Diabetic and Type 2 Diabetic Obese African-American Women. PLoS One *5*, e15234. https://doi.org/10.1371/journal.pone.0015234.
44. Elshorbagy, A.K., Smith, A.D., Kozich, V., and Refsum, H. (2012). Cysteine and obesity. Obesity *20*, 473–481. https://doi.org/10.1038/oby.2011.93.
45. Elango, R. (2020). Methionine Nutrition and Metabolism: Insights from Animal Studies to Inform Human Nutrition. J. Nutr. *150*, 2518S–2523S. https://doi.org/10.1093/jn/nxaa155.
46. Jie, Z., Yu, X., Liu, Y., Sun, L., Chen, P., Ding, Q., Gao, Y., Zhang, X., Yu, M., Liu, Y., et al. (2021). The Baseline Gut Microbiota Directs Dieting-Induced Weight Loss Trajectories. Gastroenterology *160*, 2029–2042.e16. https://doi.org/10.1053/j.gastro.2021.01.029.
47. Zhang, S., Wu, P., Tian, Y., Liu, B., Huang, L., Liu, Z., Lin, N., Xu, N., Ruan, Y., Zhang, Z., et al. (2021). Gut Microbiota Serves a Predictable Outcome of Short-Term Low-Carbohydrate Diet (LCD) Intervention for Patients with Obesity. Microbiol. Spectr. *9*, e0022321. https://doi.org/10.1128/Spectrum.00223-21.
48. Stanislawski, M.A., Frank, D.N., Borengasser, S.J., Ostendorf, D.M., Ir, D., Jambal, P., Bing, K., Wayland, L., Siebert, J.C., Bessesen, D.H., et al. (2021). The Gut Microbiota during a Behavioral Weight Loss Intervention. Nutrients *13*, 3248. https://doi.org/10.3390/nu13093248.
49. Hjorth, M.F., Blædel, T., Bendtsen, L.Q., Lorenzen, J.K., Holm, J.B., Kiilerich, P., Roager, H.M., Kristiansen, K., Larsen, L.H., and Astrup, A. (2019). Prevotella-to-Bacteroides ratio predicts body weight and fat loss success on 24-week diets varying in macronutrient composition and dietary fiber: results from a post-hoc analysis. Int. J. Obes. *43*, 149–157. https://doi.org/10.1038/s41366-018-0093-2.
50. Jian, C., Silvestre, M.P., Middleton, D., Korpela, K., Jalo, E., Broderick, D., de Vos, W.M., Fogelholm, M., Taylor, M.W., Raben, A., et al. (2022). Gut microbiota predicts body fat change following a low-energy diet: a PREVIEW intervention study. Genome Med. *14*, 54. https://doi.org/10.1186/s13073-022-01053-7.
51. Hiippala, K., Kainulainen, V., Kalliomäki, M., Arkkila, P., and Satokari, R. (2016). Mucosal Prevalence and Interactions with the Epithelium Indicate Commensalism of Sutterella spp. Front. Microbiol. *7*, 1706. https://doi.org/10.3389/fmicb.2016.01706.
52. Liu, Y., Jiang, Q., Liu, Z., Shen, S., Ai, J., Zhu, Y., and Zhou, L. (2021). Alteration of Gut Microbiota Relates to Metabolic Disorders in Primary Aldosteronism Patients. Front. Endocrinol. *12*, 667951. https://doi.org/10.3389/fendo.2021.667951.
53. Conterno, L., Fava, F., Viola, R., and Tuohy, K.M. (2011). Obesity and the gut microbiota: does up-regulating colonic fermentation protect against obesity and metabolic disease? Genes Nutr. *6*, 241–260. https://doi.org/10.1007/s12263-011-0230-1.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
QIAamp DNA stool mini kits	Qiagen	N/A
Illumina HiSeq 4000 platform	Illumina	N/A
Deposited data		
The datasets and metadata related to this study have been deposited in the NCBI Sequence Read Archive	This paper	BioProject number PRJNA988962
Software and algorithms		
R package	R CRAN	https://www.r-project.org/
SAS (Version 9.4)	SAS Viya	https://www.sas.com/

RESOURCE AVAILABILITY

Lead contact

Further information should be directed to and will be fulfilled by the lead contact, Huijie Zhang (huijiezhang2005@126.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All sample data of metagenome sequencing data have been deposited in BioProject database. Accession numbers are listed in the key resources table. They are publicly available as of the date of publication. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study population and ethics approval

TREATY trial was a randomized clinical trial that assessed the effects of 8-h time-restricted eating (TRE) with calorie restriction as compared with daily calorie restriction alone on weight loss among obese patients.[3] In this study, 69 obese participants assigned to TRE intervention were required to eat only between 8:00 a.m. to 4:00 p.m. for 12 months. These were aged 18–75 years, body mass index (BMI) between 28 and 45 kg/m^2, and predominantly Asian. Subjects were excluded if pregnant, smoking, or with acute or chronic viral hepatitis, dysregulated thyroid functions, malignant tumors, diabetes, severe liver dysfunction, and chronic kidney disease, subjects have serious cardiovascular or cerebrovascular disease within 6 months before randomization, or severe gastrointestinal diseases, gastrointestinal surgery within the past 12 months before randomization were also excluded. Finally, 51 participants who had provided stool were included in this analysis. The trial was registered at ClinicalTrial.gov (NCT03745612) and overseen by a steering committee. The protocol was approved by the institutional review board at Nanfang Hospital of Southern Medical University. All the participants provided written informed consent.

METHOD DETAILS

Diet

During the 12 months of the trial, the men were instructed to follow a diet of 1500–1800 kcal/day and the women to follow a diet of 1200–1500 kcal/day. According to the dietary guidelines of Chinese residents, the diet included 40–55% of calories from carbohydrates, 15–20% of calories from protein, and 20–30% of calories from fat. Participants received dietary counseling conducted by trained health coaches for the whole duration of the trial. Participants kept a dietary log, photographed the food they ate, and noted the time at which they ate via a custom mobile study application (app). Two researchers assessed participants' mealtimes and their dietary intake on the basis of the nutrient content shown on the Chinese Food Composition Table. Participants received follow-up telephone calls or an App message twice per week, and met with the health coach individually every 2 weeks to assess their adherence to the program and to achieve personalized energy targets for weight loss in the first 6 months. During the last 6-month intervention, participants were instructed to maintain their diet regimens and write their dietary log and record food picture and mealtime three times per week, and received follow-up telephone calls or an App message once per week and met with the health coach monthly. All participants were instructed to maintain their usual daily

physical activity throughout the trial. Compliance to the dietary intervention was defined according to the number of days that a participant met the requirements of the assigned diet. Participants in the TRE group were required both to eat within the prescribed time period and to meet the daily caloric-intake goal.

Anthropometric and clinical measurements

Anthropometric characteristics including body weight, height, circumference index, and blood pressure were measured by trained investigators according to standard operation procedures. Body composition was assessed by whole-body dual radiography system (Lunar iDXA, GE Healthcare). Abdominal fat areas (total, visceral and subcutaneous) were obtained by means of computed tomography (Revolution, GE Healthcare) at the level of the fourth and fifth lumbar vertebrae. Liver fats were evaluated by transient elastography (FibroScan 502 Touch, Echosens). Blood samples were taken for the determination of metabolic risk factors as follows: serum lipid profiles including high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, and total cholesterol; glucometabolic index including serum glucose and insulin, glycated hemoglobin and glucose 120 min after administration of a glucose product. Insulin resistance index was calculated using homeostatic model assessment for insulin resistance (HOMA–IR), defined as fasting insulin (mU/mL) × fasting glucose (mmol/L)/22.5.

Responsiveness of weight loss over 12 months

According to the guideline for the management of obesity in adults, a goal of approximately 5%–10% of initial weight was recommended to reduce cardiovascular risk factors, larger weight losses produce greater benefits.[2] Two groups were defined based on relative weight changes in 12 months: the moderate weight loss group (MG, weight loss <10%) and the distinct weight loss group (DG, weight loss ≥10%).

Fecal DNA extraction and sequencing

The fecal samples were frozen immediately at −80°C or briefly stored by participants in −20°C freezers before being transported to the study center within 12 h on ice, then stored at −80°C until processing. Microbial DNA extraction of fecal samples was performed according to the instructions of the QIAamp DNA stool mini kits (Qiagen, Germany). All samples were sequenced on the Illumina HiSeq 4000 platform (Illumina, San Diego, California, USA).

QUANTIFICATION AND STATISTICAL ANALYSIS

Metagenomics analysis

The metagenomics data were analyzed using R software. R package 'vegan' was used to calculate the alpha diversity (Shannon index, Simpson index and richness index). Beta diversity was computed on Bray-Curtis distance and estimated in two-dimensional space in the R packages 'vegan' and 'ggplot2'. Two-tailed Wilcoxon rank-sum tests or Wilcoxon signed-rank tests were used for unpaired and paired samples respectively and adjusted by Benjamini-Hochberg correction when multiple comparisons were applied. Multivariate analysis ADONIS test was performed using R vegan package for 999 permutations. The co-abundance network analysis was conducted at the species level for samples before and after 6-month, 12-month TRE intervention respectively. All nodes were colored at the phylum level (isolated nodes were excluded), and edges were estimated by Spearman's rank correlation coefficient (abs[r] > 0.6, $p < 0.05$). To estimate the importance of each taxon, random forest analysis was incorporated to probe key signature microbiota by the R package 'RandomForest'. Spearman's correlation analysis was performed to investigated the relationship between the abundance changes of significantly altered gut microbial species and changes in clinical indices. The functional annotation to Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology (KO) was performed through the combinatorial use of DIAMOND and KOBAS 2.0 annotate program. Bray-Curtis dissimilarity was used to evaluate functional diversity between samples. Pathway abundances were estimated by summing up the abundances of all genes present in the corresponding pathways (KEGG accessed in January 2023). Differentially altered KEGG pathways were identified using Wilcoxon signed rank test. All p values were two-tailed, and $p < 0.05$ was regarded to be statistically significant. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Other statistical analysis

Clinical data were analyzed using SAS software. Participants' baseline characteristics were presented as mean ± SD or median (interquartile range). The group comparison of baseline measurements was conducted with the general linear model for continuous variables and the chi-square test for categorical variables. Point estimates and standard errors of the changes from baseline were obtained and tested for differences between groups using the PROC MIXED procedure controlling for the baseline measurements in SAS statistical software, version 9.4 (SAS Institute). We use the mixed-effects model with an autoregressive correlation matrix to correct for the correlations of repeated measurements. Data were presented as least-squares means with 95% confidence intervals for continuous variables. All p values were two-tailed, and $p < 0.05$ was regarded to be statistically significant. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

ADDITIONAL RESOURCES

The trial was registered at ClinicalTrial.gov (NCT03745612).