Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

The impact of short-term changes in sleeping and eating patterns on glucometabolic health and gut microbiota in healthy young adults: a proof-of-concept controlled feeding study

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ARTICLE INFO

Article history:
Received 6 February 2023
Received in revised form 31 March 2023
Accepted 16 April 2023

Keywords:
Circadian rhythm
Glucose metabolism
Gut microbiota
Dietary patterns
Sleep schedule
Psychological status

ABSTRACT

Epidemiological studies showed that night workers are at higher risk of developing chronic metabolic diseases. However, no study has investigated the changes in circadian rhythms caused by a combined effect of sleep and diet in a real-life setting on cardiometabolic health, gut microbiota, and psychological status in healthy people. A 4-week step-wise misaligned-realigned controlled-feeding trial with a 2 × 2 factorial design (sleep and diet) was conducted on healthy young adults. At first, subjects experienced a one-week circadian rhythm misalignment with a high-fat fast-food diet, extended eating window, and delayed sleep schedules, then gradually transited to a complete circadian rhythm realignment with a high-fiber balanced diet, 8-h time-restricted eating, and normal sleep schedules. Circadian rhythm misalignment led to significantly higher levels of fasting glucose and homeostatic model assessment for insulin resistance (HOMA-IR) of subjects compared to baseline and failed to recover to the baseline level in circadian rhythm realignments. Notably, the incremental area under the curve (iAUC) of postprandial glucose decreased with circadian rhythm adjustments as compared to that in circadian rhythm misalignment, suggesting circadian rhythm realignment by sleep or/and diet could partly restore glucose metabolism impaired by a short-term circadian rhythm misalignment. However, circadian rhythm changes did not result in overall perturbations of gut microbiota diversities.

1. Introduction

Epidemiological studies showed that night or shift workers are at high risk of developing type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVD), obesity, metabolic syndrome (MetS),

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Peer review under responsibility of Tsinghua University Press.

SciOpen Publishing services by Tsinghua University Press

http://doi.org/10.26599/FSHW.2023.9250038

and cancer[1-2]. Circadian rhythms drive humans to follow periodic cycles approximately 24 h daily and govern physical, mental, and behavioral changes. The human circadian system is hierarchically structured and comprises 2 major types of circadian clocks-the master clock and the peripheral clock. They both respond to a stimulus and synchronize to maintain a coherent circadian system. The master clock is the suprachiasmatic nuclei (SCN) of the hypothalamus, responding to environmental light-dark cycles and orchestrating activities of peripheral circadian clocks as a central conductor to ensure the body functions in the appropriate phase[3-4]. The peripheral clocks found in organs and tissues, such as the liver, muscles, and fatty tissue, are coordinated by the SCN and responsive to behavioral time cues, such as light/dark exposure, feeding/fasting cycles, and food intake[3]. The mysterious circadian clock known as the food-entrainable oscillator is entrained by meal timing and food intake and implicated in harmonizing the digestive and absorptive processes. Evidence suggests that those with disrupted peripheral clocks induced by dysregulated eating times are at higher risk of developing metabolic disorders such as T2DM and obesity[5-7].

Night and shift workers exposed to irregular lifestyle schedules are likely to experience a misalignment between peripheral clocks and the master clock[8], inducing glucose, lipid, and protein/amino acid metabolism changes[4]. A study conducted on 10 healthy young adults (5 female) in the laboratory for 10 days reported that circadian misalignment systemically decreased leptin, sleep quality and duration, increased glucose, insulin, and mean arterial pressure when eating and sleeping schedules of the subjects were around 12 h out of phase from their habitual times[3]. In addition, people with a circadian rhythm misalignment are associated with a higher incidence of psychiatric symptoms, especially depression, compared to the healthy population[7].

It was reported that delayed meal timing could directly delay the plasma glucose rhythms and thus impair glucose metabolism[6]. Recent evidence supported that implementing time-restricted eating (TRE) improved glucose tolerance in MetS patients and people at risk of T2DM and reduced non-HDL cholesterol levels in MetS patients[5,9]. In addition, diet structure also plays a vital role in regulating metabolism, and diet composition changes can dramatically alter lipid metabolism in only 4 days in 10 healthy young adults[10]. It is worth noting that TRE was also shown to partially restore daily cyclical fluctuations of gut microbiota, increasing its diversity and thus protecting against obesity and metabolic diseases in a mouse study[11].

To our knowledge, no studies have investigated how disruption to the circadian rhythm caused by a combined effect of sleep and diet may affect the cardiometabolic health, gut microbiota, and psychological states in healthy young adults. Therefore, we developed a short-term circadian rhythm misaligned-realigned study combining diet and sleep to provide insights on developing strategies to realign disrupted circadian rhythm. In the present study, healthy adults with habitual bedtime of 22:00–00:00 followed a 4-week step-wise intervention protocol with 4 stages, from Stage 1–circadian rhythm misalignment transited gradually to Stage 4–circadian rhythm alignment. We hypothesized that cardiometabolic profiles, the composition of gut microbiota, and psychological status would be improved during the reconstruction from the disrupted circadian rhythm to a normal state.

2. Material and methods

The study was approved by the Chinese Ethics Committee of Registering Clinical Trials (ChiECRCT20200376) and was pre-registered in the Chinese Clinical Trial Registry (ChiCTR2000040944).

2.1 Study design

The 4-week step-wise misaligned-realigned controlled-feeding trial with a 2 × 2 factorial design (sleep and diet) was conducted in the Shenzhen People's Hospital between December 2020 and January 2021. Subjects lived in Shenzhen for more than a year. All subjects were instructed to follow a one-week baseline period (Stage 0) and 4 consecutive intervention stages (Fig. 1). Each intervention stage is comprised of 6 days, with the 7th day being a clinical investigation

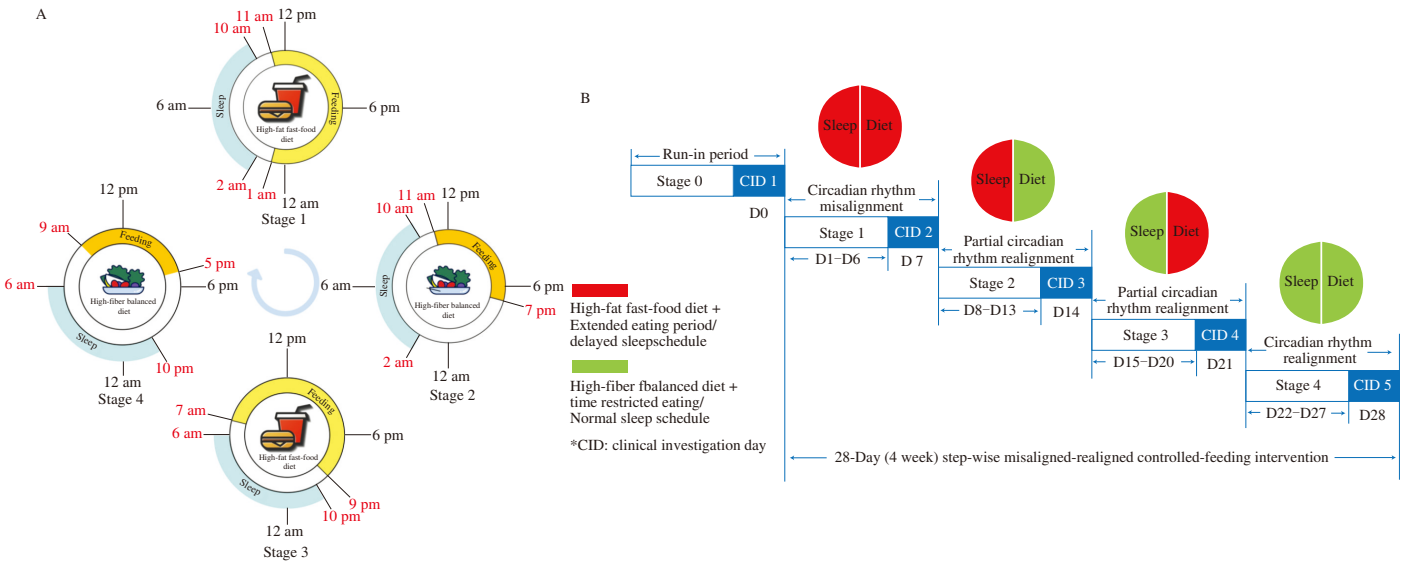

Fig. 1 Study design. (A) Sleep and diet patterns in the study. (B) Timeline of the study. Each intervention stage was 6 days, with the 7th day a CID. Each participant attended 5 clinical investigation days CIDs on days 0, 7, 14, 21, and 28 throughout the intervention period.

day (CID) without intervention. Each participant attended 5 CIDs on days 0, 7, 14, 21, and 28 throughout the intervention period after an overnight fast. In the baseline period, Fitbit watches were distributed to the subjects who were instructed to get accustomed to wearing the watch while sleeping. In addition, subjects were required to take photos before and after their meals to document their diets in the last three days of the baseline period.

Stage details are shown in Table 1.

Stage 0 (S0)—baseline period: subjects continued with their habitual diet and sleep patterns;

Stage 1 (S1)—circadian rhythm misalignment: postponed sleep schedule (2:00 am–10:00 am) combined with a high-fat fast-food diet (HFD) with an extended eating window of 14 h (11:00 am–(+1) 1:00 am);

Stage 2 (S2)—partial circadian rhythm realignment: postponed sleep schedule (2:00 am–10:00 am) combined with a healthy high-fiber balanced diet (BD) with TRE of 8 h (11:00 am–7:00 pm);

Stage 3 (S3)—partial circadian rhythm realignment: normal sleep schedule (10:00 pm–(+1) 6:00 am) combined with HFD with an extended eating window of 14 h (7:00 am–9:00 pm);

Stage 4 (S4)—circadian rhythm realignment: normal sleep schedule (10:00 pm–(+1) 6:00 am) combined with BD with TRE of 8 h (9:00 am–5:00 pm).

2.2 Subjects

The study was advertised through posters and social media for 3 months, from October 2020 to December 2020, in the Shenzhen People's Hospital and communities in Shenzhen, China. In total 289 volunteers were screened for eligibility *via* in-person meetings. 127 human subjects were renounced from joining the study because of the conflicts between their daily work schedules with the research schedules. In addition, 151 subjects did not meet at least one inclusion criteria and were excluded. One subject withdrew from the study during the baseline period due to being unable to fall asleep when wearing a Fitbit Watch which is mandatory for monitoring the daily activities of participants. 10 healthy young subjects (5 males and 5 females, matched for age and body mass index (BMI)), who met the inclusion and exclusion criteria and gave consent, were enrolled (Fig. 2). The inclusion and exclusion criteria are as follows: (1) previously or currently with chronic or severe gastrointestinal diseases; (2) currently with metabolic diseases; (3) currently with severe liver, gallbladder, and kidney diseases; (4) currently with cancers or recovered from cancers less than 5 years; (5) currently suffering from infectious diseases; (6) currently or previously suffering from mental illness; (7) currently taking prescriptive medications; (8) currently or during the past 3 months participating in other intervention trials; (9) currently with tobacco or drinking habits (alcohol intake ≥ 5 drinks/week, 100 g of Chinese Baijiu or 250 g of rice wine or 150 g of red wine per drink) or extreme dietary or exercise patterns; (10) other reasons that the researchers consider unsuitable to participate in the study.

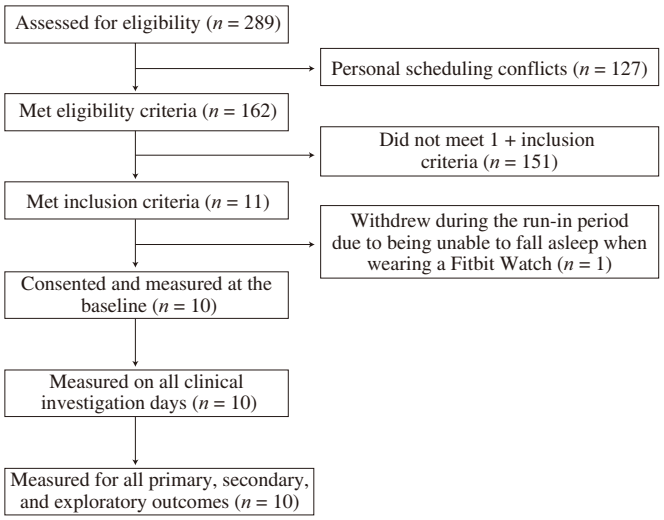

Fig. 2 Flow chart of the recruitment.

2.3 Intervention strategies

2.3.1 Diet

Meals were provided to subjects 3 times a day during the intervention. All foods were prepared by researchers and delivered to the subjects daily. Energy levels for subjects throughout the trial were determined by professional dietitians based on basal metabolic rates measured by Vmax Encore System (CareFusion, USA) and 24 h dietary records at the baseline level of the subjects. Energy levels were thus set at 2 000 kcal for males and 1 800 kcal for females. Two different types of isoenergetic diets were implemented in the trial, an HFD and a BD diet. The macronutrient composition (protein:fat:carbohydrate) of HFD was adapted to 17:43:40, whereas that of BD was 17:23:60. Disrupted dietary pattern and schedule—HFD combined with an extended eating window (14 h) was designed for S1 (eating window: 11:00 am−(+1) 1:00 am) and S3 (eating window: 7:00 am−9:00 pm). On the other hand, an optimized dietary pattern and schedule, BD combined with a TRE (8 h), was designed for S2 (eating window: 11:00 am−7:00 pm) and S4 (eating window: 9:00 am−5:00 pm). Menus throughout the trial are shown in Table S2. Calorie-free drinks, such as water and sugar-free tea, were allowed during the eating window of the day. Messages such as "Please hold off from eating between 1:00 am tonight to 11:00 am tomorrow" or "It is fine to eat from now till 1:00 am after midnight" were sent to the subjects before each eating/fasting period to encourage compliance. Subjects were instructed to take photos before and after each meal and send them to the researchers right after each meal for energy intake

Table 1
Diet and sleep patterns in different stages.

Stages	Diet pattern	Sleep pattern
S0	Habitual diet pattern (predominantly high carbohydrate diet with an eating window of 10−12 h)	Habitual sleep pattern (Bedtime 10:00 pm–00:00 am)
S1	HFD with an extended eating window of 14 h (11:00 am–(+1) 1:00 am)	Postponed sleep schedule (2:00 am–10:00 am)
S2	BD with TRE of 8 h (11:00 am–7:00 pm)	Postponed sleep schedule (2:00 am–10:00 am)
S3	HFD with an extended eating window of 14 h (7:00 am–9:00 am)	Normal sleep schedule (10:00 pm–(+1) 6:00 am)
S4	BD with TRE of 8 h (9:00 am–5:00 pm)	Normal sleep schedule (10:00 pm–(+1) 6:00 am)

assessment and compliance monitoring. Their actual energy intake was assessed by researchers based on the energy of the provided meals as well as the consumed portion sizes estimated by pre- and post-meal photos.

2.3.2 Sleep

The present study implemented 2 types of sleeping schedules: late-night sleep schedule (S1 and S2, scheduled sleeping period: 2:00 am–10:00 am) and normal sleep schedule (S3 and S4, scheduled sleeping period: 10:00 pm–6:00 am). Messages, such as "Sleeping period will be started in 10 min" or "Now it is time to go to sleep" were sent to the subjects 10 min before each sleeping period. Subjects were required to wear provided eye patches and earplugs during sleep to avoid light and sound interferences. Subjects were also instructed to wear Fitbit Watch to record their sleep status, including bedtime and sleeping duration. Fitbit Watch data was analyzed daily to monitor compliance with sleep schedules.

2.3.3 Physical activity

Subjects were instructed to refrain from daily physical activity over 10 000 walking steps and avoid intensive activity during the trial. Fitbit Watch recorded actual daily walking steps and intensive activities, if any, of the subjects. Fitbit Watch data were analyzed daily. Subjects would be kindly reminded if their physical activities surpassed 10 000 walking steps or equivalent exercises.

2.4 Clinical assessment on clinical investigation day

2.4.1 The challenge meal test

During each CID, subjects were instructed to consume a standard challenge meal—a 400 mL high-fat, high-sugar milkshake which contained 60 g palm oil, 75 g glucose, and 20 g milk protein (manufactured by Tetra Pak, China, based on the provided formula) within 5 min after the collection of fasting blood samples. Consumption of the challenge milkshake would induce metabolic responses in the subjects. Postprandial blood samples were collected *via* a cannula by research nurses at 30, 60, 90, 120, and 180 min. During the postprandial period, subjects remained at rest in the designated resting room.

2.4.2 Anthropometric assessment and resting energy expenditure (REE)

Anthropometric parameters and REE measurements were conducted at each CID at a fasting state of the subject. Height and weight were measured by the SK-CK Ultrasonic Weight Measurement machine (Sonka, China), and BMI was calculated as the body weight (in kg) divided by the square of the height (in m). During the testing, subjects were instructed to maintain the minimum clothing possible, therefore shoes, socks, and coats were taken off. Subjects were required to stand on the machine barefoot, keep shoulders in a relaxed position, arms hanging freely and head in the Frankfort horizontal plane. Blood pressure was measured by an HBP-9021 aneroid sphygmomanometer (Omron, Japan), including systolic blood pressure (SBP) and diastolic blood pressure (DBP). Subjects were required to take the measurement in a sitting position after 15 min of rest. Body composition analysis, including fat mass, lean mass, and visceral fat mass, was conducted using the bioelectrical impedance analysis (BIA) device (Inbody S10, Inbody, Korea). REE was measured using a Vmax Encore pulmonary function device (CareFusion, USA) under the resting state of the subject in a cozy and quiet environment at a room temperature of 18−20 °C. All measurements except for REE were done in duplicates (or triplicates if the first two measurements differed by more than 1%) for each subject. All measurements were recorded, and the mean of the two nearest measures was calculated.

2.4.3 Fecal samples collection

Sterile stool collection kits (TinyGene, China) were provided to subjects to collect fecal samples. Subjects were provided with written and oral instructions and videos on the standard methods to collect fecal samples before the trial. The fecal samples were collected the day before each CID. After collection, samples were sent to the laboratory and stored at −80 °C prior to analysis.

2.5 Qualitative assessment via questionnaires

2.5.1 Appetite

The appetites of the subjects were evaluated weekly by the Nutrition Appetite questionnaire with a visual analogue scale (VAS) of 100[12]. Subjects were required to complete the questionnaire the night before each CID.

2.5.2 Sleep quality

Sleep quality was assessed weekly by the Pittsburgh Sleep Quality Index (PSQI), a standardized sleep questionnaire consisting of 19 individual items creating 7 components that produce one global score[13]. The 7 components of the PSQI include subjective sleep quality, sleep latency (i.e., how long it takes to fall asleep), sleep duration, habitual sleep efficiency (i.e., the percentage of time in bed that one is asleep), sleep disturbances, use of sleeping medication, and daytime dysfunction. Subjects were required to complete the questionnaire the night before each CID.

2.5.3 Depression, anxiety, stress levels, and mood states

Profile of Mood States (POMS) and the Depression Anxiety Stress Scale-21 (DASS-21) were questionnaires for the weekly evaluation of the psychological status of the subjects, including depression, anxiety, stress, and mood. POMS is a psychological rating scale used for assessing the mood states measuring changes in total mood disturbance, anger, confusion, depression, esteem, tension, vigor, and fatigue levels of the subjects during the intervention period[14]. DASS-21, a scale that proved to be effective in reflecting the levels of depression, anxiety, and stress, was also implemented in the study[15]. Subjects were required to complete the questionnaires the night before each CID.

2.6 Sample analysis

2.6.1 Biochemical analysis

Concentrations of blood glucose, insulin, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density cholesterol (LDL-C), and triglyceride (TG) were analyzed using commercial-available reagent cassettes (Roche Diagnostics GmbH, Mannheim, Germany, Table S3) with Cobas® 8000 modular analyzer series (Roche Diagnostics GmbH, Mannheim, Germany). Analysis of blood TC, HDL-C, LDL-C, and TG levels was performed by the c702 module whereas blood glucose and insulin levels were determined by the c701 and e602 modules of the Cobas® 8000 modular analyzer. The incremental area under the curve (iAUC) for postprandial blood glucose (iAUC of glucose) and blood lipids (iAUC of TC, iAUC of TG, iAUC of HDL-C, and iAUC of LDL-C) were calculated based on the measurement results. In addition, fasting glucose levels and fasting insulin levels were used to calculate the homeostatic model assessment for insulin resistance (HOMA-IR) for the assessment of insulin resistance: HOMA-IR = [fasting insulin level (μU/mL) × fasting glucose level (mmol/L)]/22.5.

2.6.2 DNA extraction, 16S rRNA sequencing of fecal samples

DNA was extracted using MagPure Stool DNA KF kit B (Magen, China), following the manufacturer's instructions. DNA was quantified with a Qubit Fluorometer by using a Qubit® dsDNA BR Assay kit (Invitrogen, USA) and its quality was checked by running an aliquot on 1% agarose gels.

The variable regions V3−V4 of the 16S rRNA gene were amplified using the polymerase chain reaction (PCR) amplification method with the degenerate primer 341F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Both forward and reverse primers were tagged with Illumina adapter, pad, and linker sequences. A reaction (50 μL) composed of a template (30 ng), fusion PCR primer, and PCR master mix was used for performing PCR enrichment. PCR cycling conditions were as follows: 94 °C for 3 min, 94 °C for 30 s (30 cycles), 56 °C for 45 s, 72 °C for 45 s followed by a final extension for 10 min at 72 °C. After all the PCR reactions had been finished, the PCR products were purified with AmpureXP beads and eluted in the Elution buffer. The validated libraries qualified by the Agilent 2100 bioanalyzer (Agilent, USA) were sequenced and analyzed by Beijing Genomics Institute (Shenzhen, China) and with the Illumina MiSeq platform (BGI, Shenzhen, China, following the standard pipelines of Illumina and generating 2 × 300 bp paired-end reads.

Raw reads were filtered to remove adaptors and low-quality and ambiguous bases for quality control. The Fast Length Adjustment of Short reads program (FLASH, Version 1.2.11, http://ccb.jhu.edu/software/FLASH/) was used to add paired-end reads to tags. The software UPARSE (version 7.0.1090, http://drive5.com/uparse/) was used to assign the 97% similarity tags to the same operational taxonomic units (OTU). Chimera sequences were compared with the Gold database (Version 20110519) using UCHIME (Version 4.2.40, http://drive5.com/usearch/manual/uchime_algo.html) to detect. OTU representative sequences were then classified using Ribosomal Database Project (RDP, Version 2.2) with a minimum confidence threshold of 0.6 and trained on the Greengene database (Version 201305) by QIIME (Version 1.80, http://qiime.org). Each sample's OTU abundance statistics table was acquired by comparing all Tags back to OTU using USEARCH_global.

Subsequent analysis of α-diversity and β-diversity was performed using MOTHUR (Version 1.31.2, https://mothur.org) and QIIME (Version 1.80, http://qiime.org), respectively, at the OTU level. The sample cluster was conducted by QIIME (Version 1.80, http://qiime.org) based on UPGMA.

2.6.3 Analysis of short-chain fatty acids (SCFAs)

A modified version based on a previously reported method[16-17], was used in the present study to determine the contents of SCFAs in fecal samples using gas chromatography-mass spectrometry (GC-MS), including acetic acid (AA), propionic acid (PrA), isobutyric acid (IbA), butyric acid (BA), isovaleric acid (IvA) and pentanoic acid (PeA). Total SCFAs were calculated as the sum of the 6 SCFAs measured.

Totally 200 mg of each fecal sample were mixed with distilled water (1.0 mL) and centrifuged at 13 000 r/min for 10 min. The supernatant (0.5 mL) was added with 0.5 mL of 25 mmol/L 2-ethylbutyric acid solution (internal standard) and centrifuged at 8 000 r/min for 10 min. The supernatant was later filtered with the PES membrane (diameter: 13 mm, pore size: 0.22 μm). The filtered supernatant was loaded onto GC-MS (7890B-5977B, Agilent) equipped with a DB-FFAP capillary column (30 m × 0.25 mm × 0.25 μm, Agilent) using helium as carrier gas (flow rate: 1 mL/min). The injection volume was 1 μL. The column temperature was initially maintained at 80 °C for 3 min, then increased at a rate of 10 °C/min to 170 °C and further maintained for 12 min. The injector and detector temperatures were both set at 250 °C. Contents of SCFAs were determined based on standard curves. The content of total SCFAs was calculated as the sum of 6 SCFAs measured.

2.7 Adverse events

Subjects were informed to report any physiological discomforts or psychological conditions to the researchers. Researchers also enquired about the subjects' physiological or psychological status daily *via* phone calls or messages. Adverse events were recorded according to the protocol.

2.8 Statistical analysis

Based on the iAUC of glucose of healthy adults reported in Jensen et al.[18], we hypothesized a 25% decrease in glucose iAUC from S1 to S4, given a 25% dropout rate, the sample size was calculated as 10 subjects for the present study. Statistical analysis was performed with the use of R (version 4.0.3). All data were presented as means ± standard deviations (SDs). The Shapiro-Wilk test was applied to test the normality of the data. If the data was not normally distributed, the pairwise Wilcoxon rank-sum test was performed for the comparison between 2 different stages. The Friedman test was used for the comparison among all stages. Pairwise *t*-test and one-way repeated ANOVA were conducted if the data were normally distributed. To determine the significance of the microbiota composition, β-diversity measures-unweighted and weighted Unifrac

distance, were calculated between samples. The principal components analysis (PCoA) analysis and PERMANOVA were conducted based on the above β-diversity measures. The pairwise Wilcoxon rank-sum test was also used for the comparison of α-diversity indexes (the number of observed species, Chao 1 index, Shannon index, and Simpson index) between stages. Furthermore, the abundance of the microbiota was rarefied to the lowest sequencing depth, and then the pairwise Wilcoxon rank-sum test was conducted to detect the difference between each taxon. The correlation between different variables was determined by the Spearman correlation test. All *P*-values were adjusted by Benjamini-Hochberg (BH) procedure. In addition, we also used the two-way repeated ANOVA to explore the sleep × diet interaction effects for different parameters. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Subject characteristics and protocol adherence

Ten healthy subjects (5 males and 5 females) completed the 4-week step-wise misaligned-realigned controlled-feeding trial. No gender difference was observed in the markers investigated. During the intervention, 3 subjects experienced a one-day delay in one of their CIDs due to menstruation (n = 2, S1 and S2, respectively) and cold (n = 1, S4). No discomforts, loss of appetite, or sleep difficulties were reported by the 3 participants despite of menstruations and a very mild cold without fever. Their inflammatory markers were within the normal range on their respective CIDs. The baseline characteristics of subjects are shown in Table 2. Walking steps in the baseline period and the 4 trial stages were recorded by a Fitbit Watch (Table S1) and were kept at less than 10 000 steps per day during the intervention as instructed to minimize energy expenditure. No intense exercise was recorded on Fitbit Watch or reported by the subjects throughout the trial. The subjects were found excellent adherence to the provided meals. The average energy level for males was (2 014 ± 96) kcal, whereas that for females was (1 870 ± 113) kcal.

Table 2
Baseline characteristics.

Characteristics	All (n = 10)	Male (n = 5)	Female (n = 5)
Age (years)	20.5 ± 2.2	19.4 ± 1.95	21.6 ± 1.95
Height (cm)	167.5 ± 6.4	172.3 ± 4.6	162.7 ± 3.5
Weight (kg)	58.9 ± 7.8	63.5 ± 8.9	54.2 ± 8.9
BMI (kg/m^2)	20.9 ± 1.8	21.3 ± 2.4	20.5 ± 1.0
SBP (mm Hg)	108.0 ± 11.0	115.4 ± 8.6	101.4 ± 9.4
DBP (mm Hg)	64.0 ± 13.0	66.4 ± 18.3	61.0 ± 6.5
Heart rate (times/min)	80.0 ± 12.0	85.4 ± 11.1	75.0 ± 12.4
REE (kcal)	1 214.0 ± 133.0	1 326.6 ± 77.5	1 100.6 ± 41.2

Note: Data shown as Mean ± SD.

3.2 Short-term circadian misalignment led to changes in glucose metabolism

The fasting glucose of subjects was significantly higher during the intervention period than at baseline (Fig. 3A, S0 *vs.* S1/S2/S3/S4, $P < 0.05$), indicating that short-term circadian misalignment could increase fasting glucose level. Moreover, disturbed fasting glucose of subjects in S1 did not recover to the baseline level in the short term after the restoration of diet and sleep rhythm. Compared to sleep adjustment in S3 and S4, the effect that benefited from diet adjustment (S2) seemed more significant. Fasting glucose of subjects decreased slightly in S2 (BD) while slightly increased back in S3 when implemented HFD again though no significant differences were observed. No significant sleep × diet interaction was displayed in fasting glucose, although there was a tendency toward an interaction effect (P = 0.06).

Postprandial glucose and lipids were evaluated by a high-fat, high-sugar challenge meal test. Postprandial glucose response was shown in Fig. 3A. Results indicated that changes in iAUC of postprandial glucose in S1 when circadian rhythm was disturbed were insignificant as compared to the baseline (Figs. 3B-J). However, postprandial glucose response was significantly improved and a decrease in iAUC

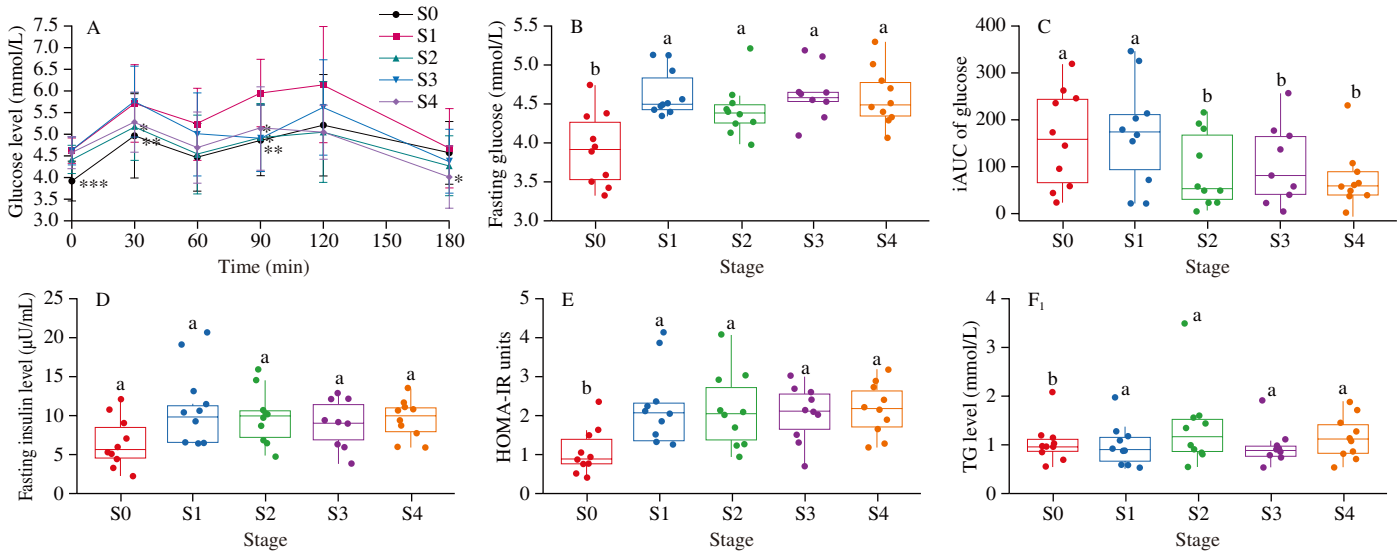

Fig. 3 Impact of circadian rhythm changes on cardiometabolic health. (A) A plot of postprandial glucose response to the challenge meal in 180 min (n = 10). Values of different time points were compared against S1, *indicates statistically significant as compared to S1, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Box plots of levels of (B) fasting glucose, (C) iAUC of postprandial glucose, (D) fasting insulin, (E) HOMA-IR units, (F) fasting lipids profiles, (G) iAUC of postprandial lipids profiles, (H) DBP, SBP, (I) heart rates, and (J) REE, respectively, across all the stages in the study. Values with different letters (a-d) denote significant differences ($P < 0.05$) between stages. Color-coded points represent individual subjects (n = 10).

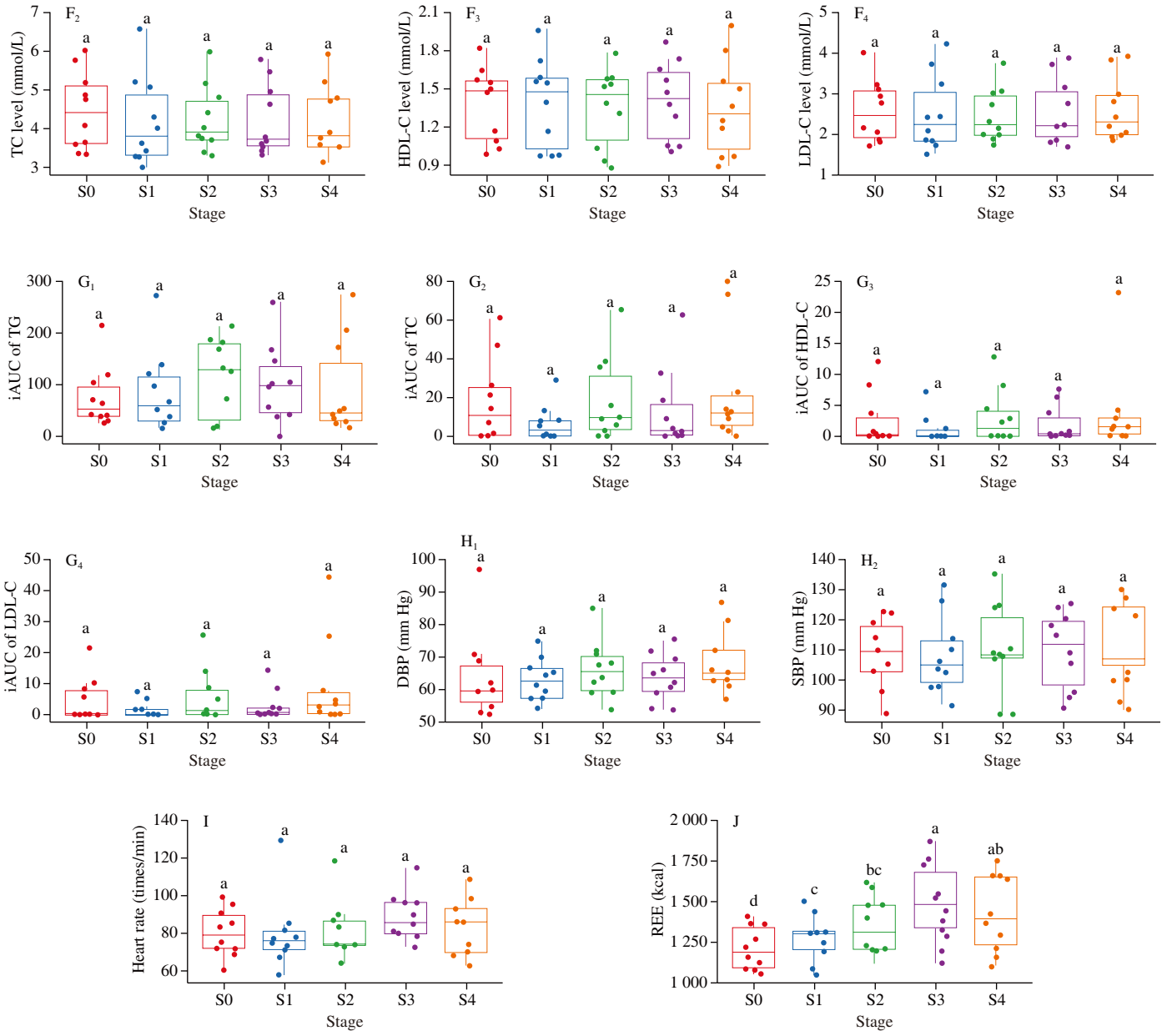

Fig. 3 (*Continued*)

of glucose was observed with the adjustment of circadian rhythm in S2-S4 as compared to S1 (Figs. 3B-J; S1 *vs*. S2/S3/S4, $P < 0.05$), indicating that diet or sleep adjustment after a short-term circadian misalignment could regulate blood glucose metabolism. However, there was no significant difference in the iAUC of postprandial glucose among these 3 stages (S2-S4). In addition, no interaction between sleep and diet was observed in iAUC.

Fasting insulin of subjects did not differ significantly between different stages. However, there was an upward trend in the intervention period compared to the baseline (Fig. 3D). Similar to fasting glucose response, the insulin resistance of the subjects indicated by HOMA-IR increased significantly in the intervention period compared to baseline (Fig. 3E), suggesting that circadian rhythm misalignment induced insulin resistance could not restore to the original level by a short-term realignment adjustment.

3.3 Impact of short-term circadian misalignment on other vital signs

Blood pressure, heart rates as well as fasting lipid profiles, including TC, HDL-C, LDL-C, and TG, showed no significant differences between the intervention period and the baseline, as well as between different intervention stages (Figs. 3F, H, and I). Additionally, iAUC for postprandial blood lipids (iAUC of TC, iAUC of TG, iAUC of HDL-C, and iAUC of LDL-C) demonstrated no significant difference between stages (Fig. 3G). Our results implied that the intervention had minimal impact on these parameters.

3.4 Circadian rhythm realignment increased REE

The average REE of subjects was systematically higher in the intervention period than that in the baseline (Fig. 3J; S0 *vs*. S1/S2/S3/S4,

$P < 0.05$), indicating that REE is sensitive to circadian phase changes. After S3 (normal sleeping schedule with the extended eating period of HFD), the average REE of participants was significantly increased compared to baseline, S1 and S2. Following S4 intervention, the participants' REE decreased, however, without statistical significance. Sleep adjustment in S3 and a combination of sleep and diet adjustment in S4 contributed to a higher REE than S1 (S1 *vs*. S3/S4, $P < 0.05$). Additionally, REE in S3 was higher than that in S2, suggesting that short-term sleep adjustment could be a more effective strategy than diet adjustment in improving REE. Nevertheless, REE displayed a significant sleep × diet interaction ($P = 0.017$), suggesting that both diet and sleep play key roles in modulating REE.

3.5 Effect of circadian rhythm realignment on body weight and body composition

Overall, circadian rhythm misalignment and realignment did not induce significant changes in the bodyweights of subjects (Fig. 4A). Interestingly, improvement was observed in the body composition of subjects in S3 (Figs. 4B-D). In S3 (normal sleep schedules and HFD), the body fat of subjects was significantly lower compared to that in S4 (Fig. 4B; S3 ((20.08 ± 5.45)%) *vs*. S4 ((22.2 ± 6.12)%), $P < 0.05$). Similarly, visceral fat was significantly lower in S3 ((45.78 ± 16.29) cm^2) than in S2 ((50.47 ± 17.40) cm^2) and S4 ((53.64 ± 19.89) cm^2) (S3 *vs*. S2/S4, $P < 0.05$). Fat-free mass was increased in S3 ((47.29 ± 7.27) kg) as compared to that in the baseline ((46.37 ± 7.27) kg) and S4 ((46.32 ± 7.22) kg). We speculated that a short-term high-fat, low-carb isoenergetic diet combined with a proper sleep schedule (S3) could be beneficial for reducing body fat and visceral fat. However, there were significant sleep × diet interactions in body composition parameters, including percent of body fat, fat-free mass, and visceral fat area ($P < 0.05$).

3.6 Circadian rhythm realignment led to a better control of fatigue

The mood states of the subjects were assessed weekly by the POMS questionnaire (Fig. 5C). Notably, the fatigue scores were significantly lower in S2, S3, and S4 as compared to that in S1 and baseline (Fig. $5C_5$, $P < 0.05$), suggesting that circadian rhythm realignment *via* diet or/and sleep schedules could lead to better control of subjective fatigue levels. But of note, a significant interaction between sleep and diet was observed in fatigue levels ($P < 0.05$). Other components of mood states, including total mood disturbance, anger, confusion, depression, esteem, tension, and vigor, were not affected by the intervention (Fig. 5C). In addition, no observable effect was found in appetite and stress levels assessed by the DASS-21 throughout the trial (Figs. 5A and D, $P > 0.05$).

3.7 Optimized sleep schedule contributes to extended sleep durations

Sleep quality during the trial was assessed weekly by the PSQI combined with the data collected by the Fitbit Watch. No significant difference was observed in PSQI scores between different stages (Fig. $5B_1$, $P > 0.05$). However, the sleep duration of subjects was markedly extended with normal sleep schedules (Fig. $5B_2$; S3 ((538 ± 48) min) and S4 ((573 ± 59) min)) compared to stages with postponed sleep schedules (S1 ((426 ± 50) min) and S2 ((428 ± 47) min)).

3.8 Fecal microbiota diversity was not changed by circadian system misalignment or realignment

To investigate the effect of circadian rhythm disturbance on gut microbiota, 16S rRNA sequencing was used to examine the impact of short-term circadian rhythm misalignment-realignment on the diversity and composition of gut microbiota. α-Diversity (observed species, Chao 1 index, Shannon index, and inverse Simpson index)

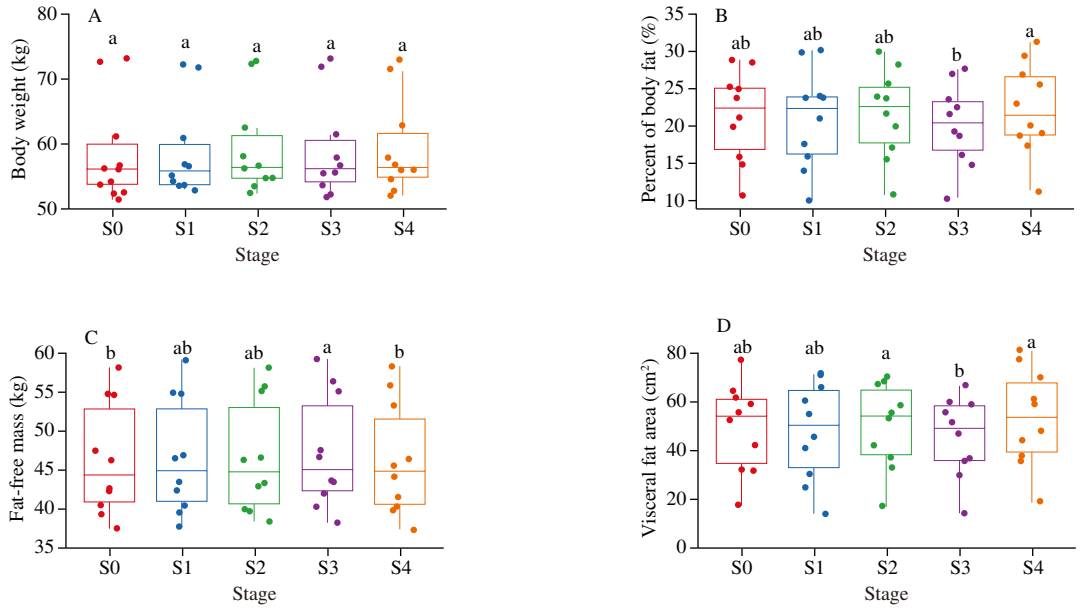

Fig. 4 Impact of circadian rhythm changes on body weight and body composition. (A) Body weight; (B) Body fat; (C) Fat-free mass; (D) Visceral fat mass. Values with different letters (a,b) denote significant differences ($P < 0.05$) between stages. Color-coded points represent individual subjects ($n = 10$).

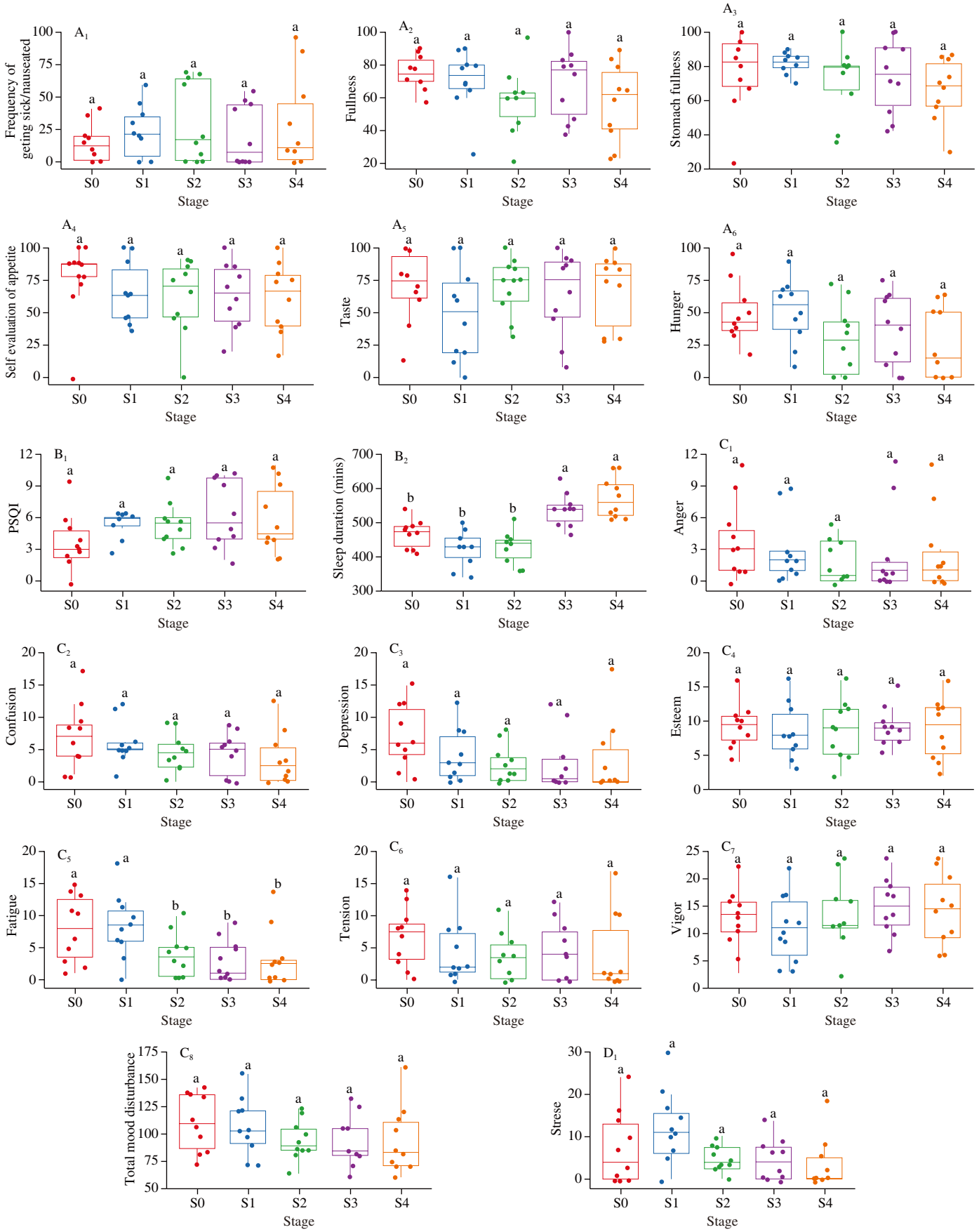

Fig. 5 Impact of circadian rhythm changes on appetite, sleep quality, mood states, and stress levels. (A) Appetite with a visual analogue scale of 100; (B) sleep quality assessed by the PSQI; (C) mood states assessed by the POMS questionnaire; (D) stress levels assessed by the DASS-21. Values with different letters (a, b) denote significant differences ($P < 0.05$) between stages. Color-coded points represent individual subjects ($n = 10$).

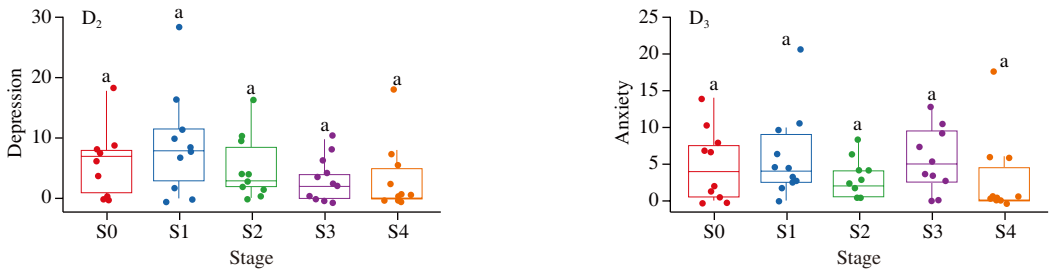

Fig. 5 (*Continued*)

and β-diversity, which was evaluated by weighted and unweighted Unifrac distance, exhibited no significant changes among stages (Figs. 6A and B). However, a significant drop in unweighted Unifrac distance in S3 was observed compared to S0 (Fig. 6B_2).

The changes in the gut microbiome at different phylogenetic levels from phylum to species were comprehensively determined at different stages. The key phylotypes were distributed among 3 bacteria phyla, Bacteroidetes, Firmicutes, and Proteobacteria, followed by Fusobacteria and Actinobacteria (Fig. 6C). Our data showed an increase in Bacteroidetes and a decrease in Firmicutes in S1 and S3 when subjects were provided HFD were observed as compared to that in S0, as well as S2 and S4 when BD was provided (Fig. 6D). Similar findings were reflected by the changes in the ratio of Firmicutes to Bacteroidetes (F/B) (Fig. 6E), in which decreased F/B was found in S1 and S3. Our result suggested that the macronutrient composition of diet plays a crucial role in modulating gut microbiota composition. In addition, a significantly lower abundance of Proteobacteria is shown in S2 as compared to the baseline and S1 (Fig. 6D).

At the phylum level, Spearman correlation test identified that changes in Bacteroidetes and Fusobacteria are associated with glucose metabolism (Fig. 6F). Bacteroidetes was negatively correlated with HOMA-IR when circadian rhythm was misaligned (S1) whereas positively correlated with HOMA-IR when circadian rhythm was partially or completely reconstructed (S2-S4). However, a significant positive association was only observed in S0 ($P < 0.05$), indicating the fluctuation of Bacteroidetes levels in response to insulin resistance during the intervention. On the other hand, Fusobacteria was positively correlated with the iAUC of postprandial glucose, with significance observed in S2 and S3 ($P < 0.05$). The second most abundant phyla Firmicutes was negatively associated with REE in the study, but a significant association was only observed in S4 ($P < 0.05$). Interestingly, Firmicutes was negatively associated with sleep duration except for S2, which in S2 demonstrated a significant positive correlation ($P < 0.05$). Furthermore, a negative correlation was identified between Proteobacteria and sleep duration during the intervention, but a significant association was only observed in S4 ($P < 0.05$). An inverse association between Actinobacteria and fatigue was detected, with significance observed in S4 ($P < 0.05$).

Bacteroides, *Prevotella*, *Faecalibacterium*, and Lachnospiraceae incertae sedis, which belong to Bacteroidetes and Firmicutes,

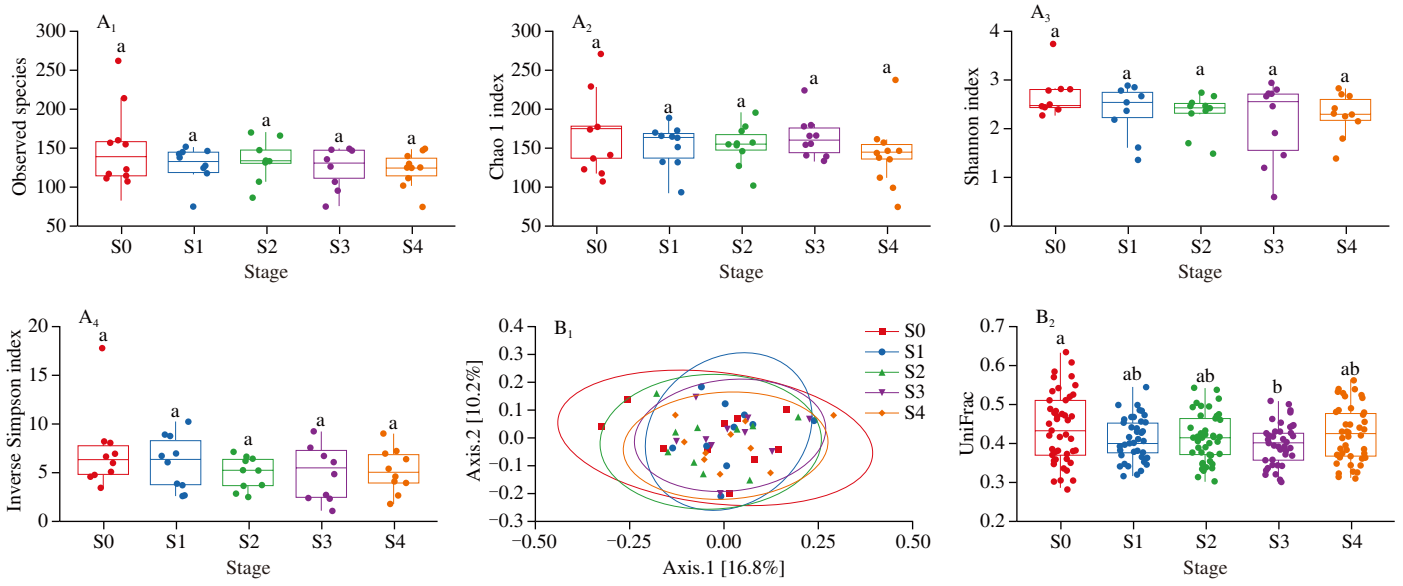

Fig. 6 Impact of circadian rhythm changes on gut microbiota. (A) α-Diversity evaluated by the number of observed species, Chao 1 index, Shannon index, and inverse Simpson index. (B) β-Diversity. Structural difference of bacteria was visualized by PCoA vectors based on an unweighted and weighted UniFrac distance matrix. (B_1) PCoA based on unweighted unifrac distance; (B_2) unweighed unifrac distance; (B_3) PCoA based on weighted unifrac distance; (B_4) weighted unifrac distance. (C) Relative abundance of bacteria in response to intervention at the phyla level. (D) Changes in the relative abundance of top 3 phyla (Bacteriodetes, Firmicutes, and Proteobacteria) in response to the intervention. (E) Ratio of F/B. (F) Relationship between the relative abundance of top 5 phyla (Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria, Actinobacteria) and 6 metabolic parameters (fasting glucose, HOMA-IR, iAUC of glucose, fatigue levels, REE, sleep duration) using Spearman correlation test. In Figs. 6A-E, values with different letters ((a-c)) denote significant differences ($P < 0.05$) between stages; color-coded points represent individual subjects ($n = 10$). In Fig. 6F, * indicates a significant difference ($P < 0.05$).

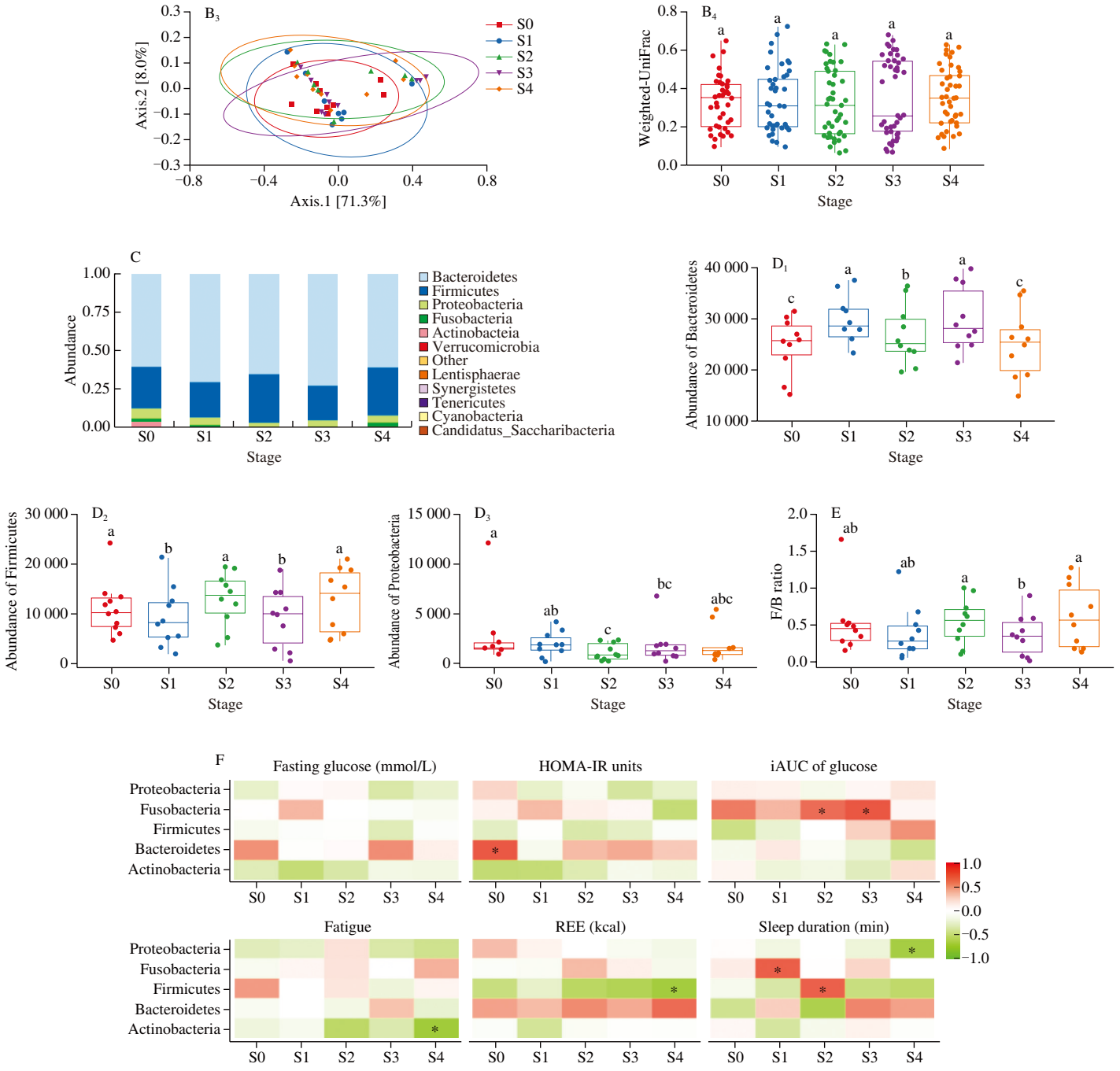

Fig. 6 (*Continued*)

were dominant at the genus level (Fig. 7). Within the phyla of Bacteroidetes, the abundance of *Bacteroides*, *Alistipes*, and *Parabacteroides* varied with diet, increased by HFD while decreased by BD; the level of *Butyricimonas* decreased during the realignment stages (S2-S4), with the lowest abundance observed in S4. Within the phyla of Firmicutes, the abundance of *Faecalibacterium*, in response to diet, increased in S2 and S4 as compared to S1 and S3; on the contrary, HFD in S1 and S3 led to decreased levels of fiber-fermenting bacteria such as Lachnospiraceae incertae sedis and unclassified Lachnospiraceae. In addition, misalignment of the circadian rhythm system (S1) led to a decrease in the abundance of *Clostridium* XIVa, which was recovered to the baseline level (S0) until fully realignment of circadian rhythm in S4. Furthermore, LEfSe analysis revealed that the family Porphyromonadaceae and the genus *Clostridum* IV in S1 showed a higher LDA score (> 3) as compared to S4 (Fig. S1).

As shown in Fig. 8, at the genus level, *Anaerostipes* (Firmicutes phyla) was negatively correlated with sleep duration in S3 and S4 when normal sleep schedules were implemented ($P < 0.05$) and positively associated with sleep duration in S1 and S2 but with no significance detected. An inverse association was observed between *Butyricimonas* (Bacteroidetes phyla) and sleep duration during S1-S3. However, a strong positive association was detected in S4 when circadian rhythm was restored ($P < 0.05$).

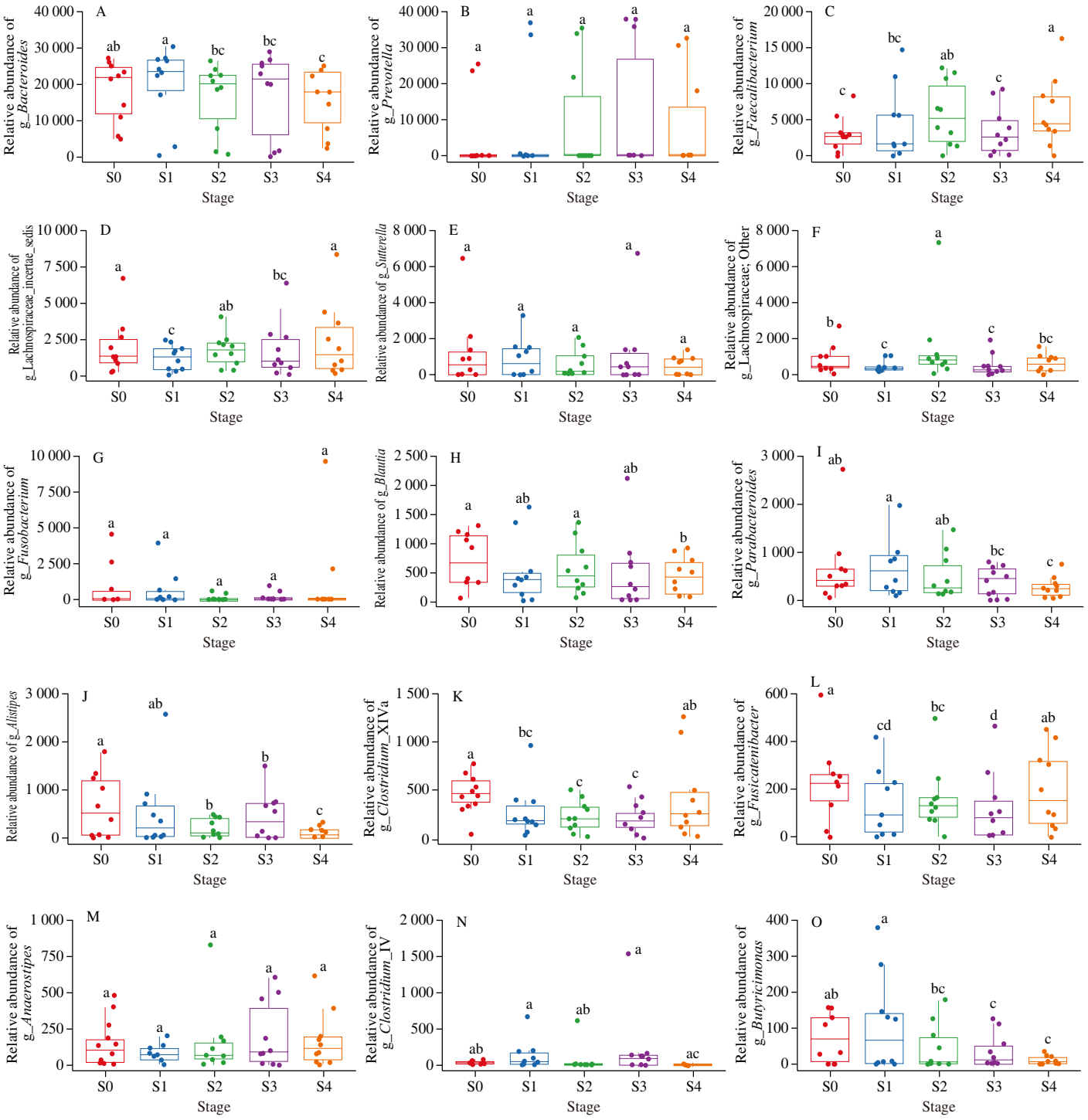

Fig. 7 Impact of circadian rhythm changes on gut microbiota at the genus level (top 15 genera). Values with different letters (a-d) denote significant differences ($P < 0.05$) between stages; color-coded points represent individual subjects ($n = 10$).

3.9 Changes in SCFA and correlation with metabolic parameters

There were no significant differences in total SCFAs, branched SCFAs (BCFAs, isobutyric and isovaleric acid), and the ratio of BCFA:SCFA among stages (Fig. 9). Of note, we observed an increase in valerate content in fecal samples during S1-S3, and it dropped back to the baseline level in S4 though a significant change was only found as compared to S2 (S2 ((5.10 ± 9.42) μmol/g) *vs*. S4 ((1.42 ± 3.18) μmol/g), $P < 0.05$). Propionate levels fluctuated in response to diet. HFD induced higher levels of propionate, whereas BD resulted in lower levels of propionate, though a significant change was only found in S3 compared to the baseline (S3 ((5.79 ± 6.49) μmol/g) *vs*. S0 ((0.78 ± 1.54) μmol/g), $P < 0.05$). In addition, sleep adjustment in S3 resulted in a decrease in percent of acetate and an increase in propionate as compared to other stages (S3 *vs*. S0/S1/S2/S4, $P < 0.05$), with a significant sleep × diet interaction observed in percentage of propionate ($P < 0.05$).

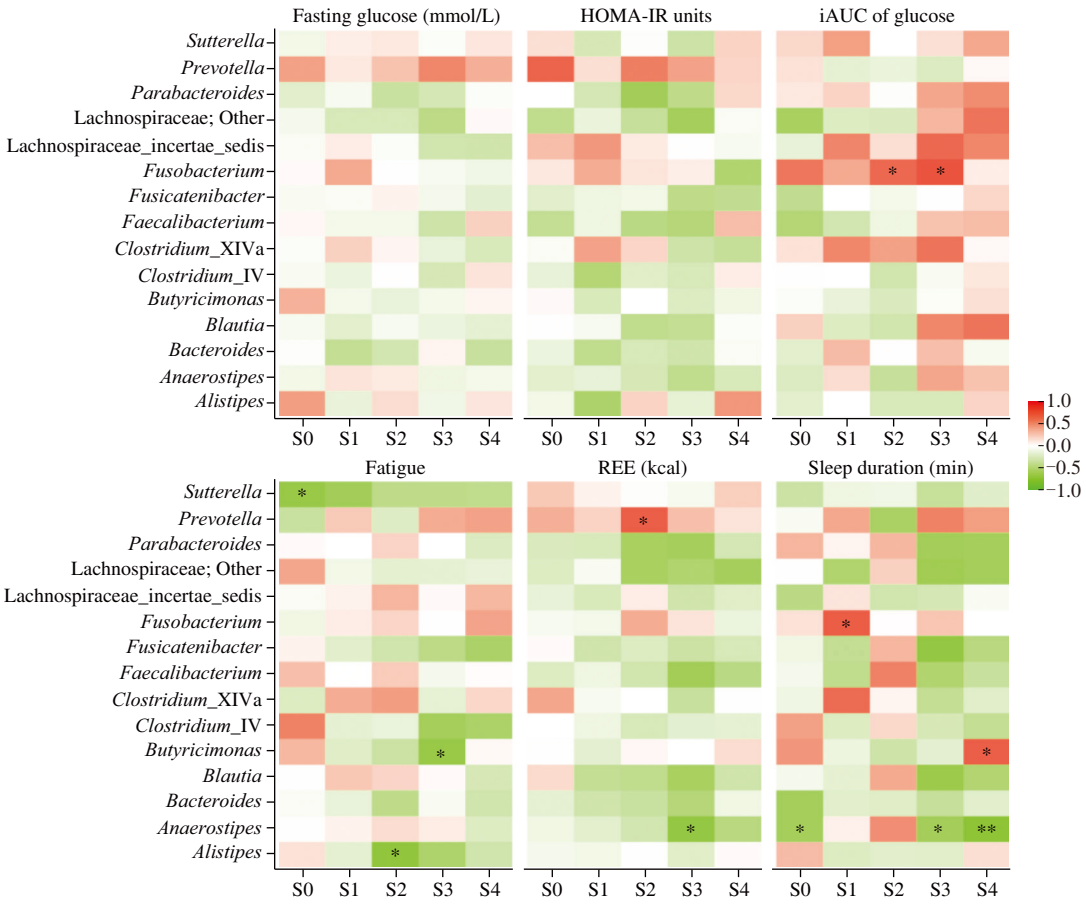

Fig. 8 Relationship between the relative abundance of top 15 genera and selected metabolic parameters. Selected metabolic parameters include fasting glucose, HOMA-IR, iAUC of glucose, fatigue levels, REE and sleep duration. Spearman correlation test was used to identify the correlation, $^{*}P < 0.05$, $^{**}P < 0.01$.

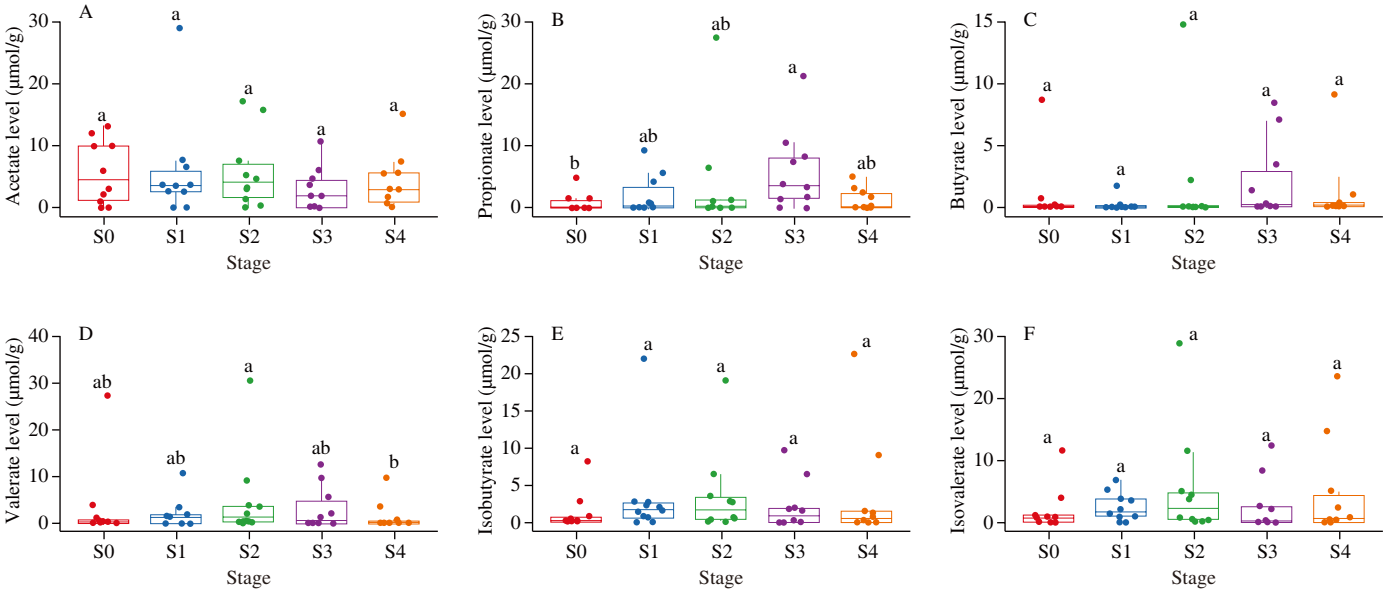

Fig. 9 Impact of circadian rhythm changes on fecal SCFAs associated parameters. SCFAs associated parameters include levels of 6 different kinds of SCFAs, total SCFAs, branched-SCFAs (BCFAs, isobutyric, and isovaleric acid), and the ratio of BCFA:SCFA, the percentage of acetate/propionate/butyrate to total SCFAs. Values with different letters (a, b) denote significant differences ($P < 0.05$) between stages; color-coded points represent individual subjects ($n = 10$).

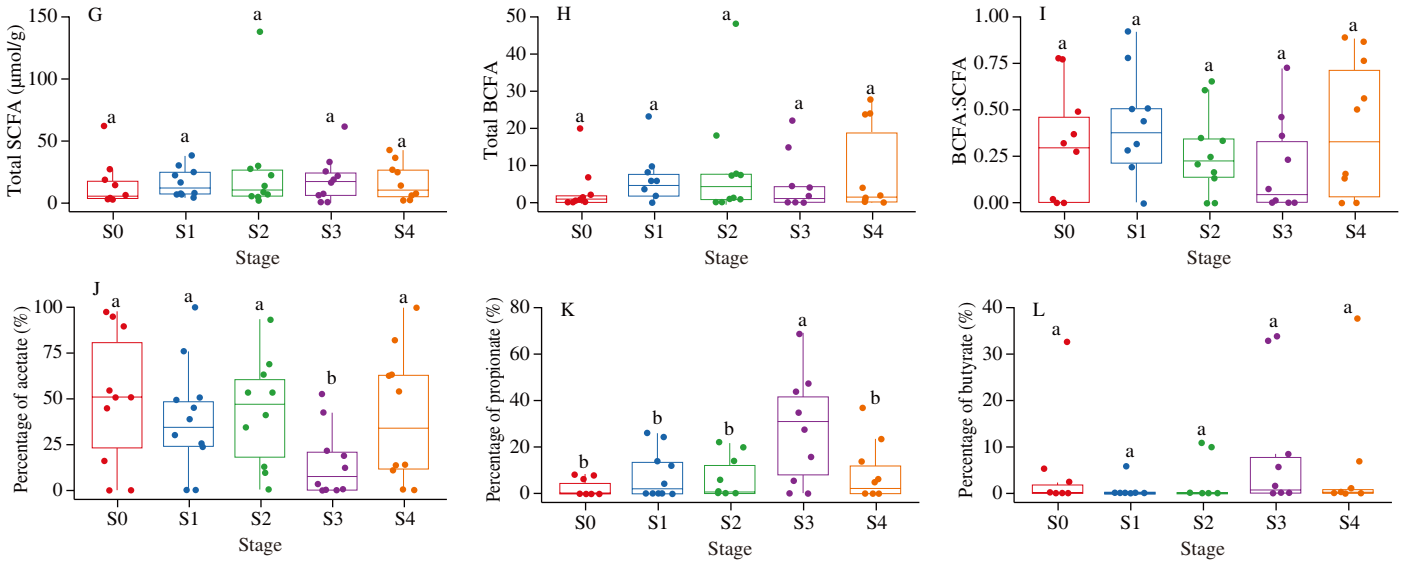

Fig. 9 (*Continued*)

As reflected in Fig. 10, we observed inverse associations in S3 between insulin resistance (indicated by HOMA-IR) and BCFAs, including levels of isovalerate, isobutyrate, total BCFAs, and BCFA:SCFA. Similar findings were also noticed in sleep durations. In addition, our results indicated that the butyrate level and percentage of butyrate are negatively correlated with sleep duration in S4 ($P < 0.05$). Propionate level and percentage of propionate were positively associated with fatigue levels in S0-S2, with significance only detected in S2. However, the associations were abruptly shifted to be negative in S3, suggesting the effect of sleep adjustment on the association.

4. Discussion

The present study developed a continuous controlled-feeding trial with a one-month circadian rhythm misaligned-realigned scheme combining both diet and sleep factors. This is the first study to examine the influence of acute circadian rhythm misaligned-realigned on healthy individuals' cardiometabolic health and gut microbiota in a real-life setting. We have adopted a widely recognized less healthy eating pattern (HFD + 14-h extended eating window) combined with a late-night sleep schedule (2 am–10 am) to induce circadian rhythm misalignment. On the other hand, an evidence-based healthy eating pattern (BD + 8-h TRE) combined with a normal sleep schedule (10 pm–6 am) was introduced in an attempt to restore circadian rhythm.

The typical lifestyle of the night or shift workers—postponed feeding and sleep timing disrupts the circadian clock and potentially impacts short-term metabolic homeostasis. In accordance with previous studies[19], in which acute partial sleep deprivation resulted in elevated levels of fasting glucose and HOMA-IR, acute circadian rhythm misalignment (S1) in the present study led to impaired glucose metabolism, also represented by higher fasting glucose levels and

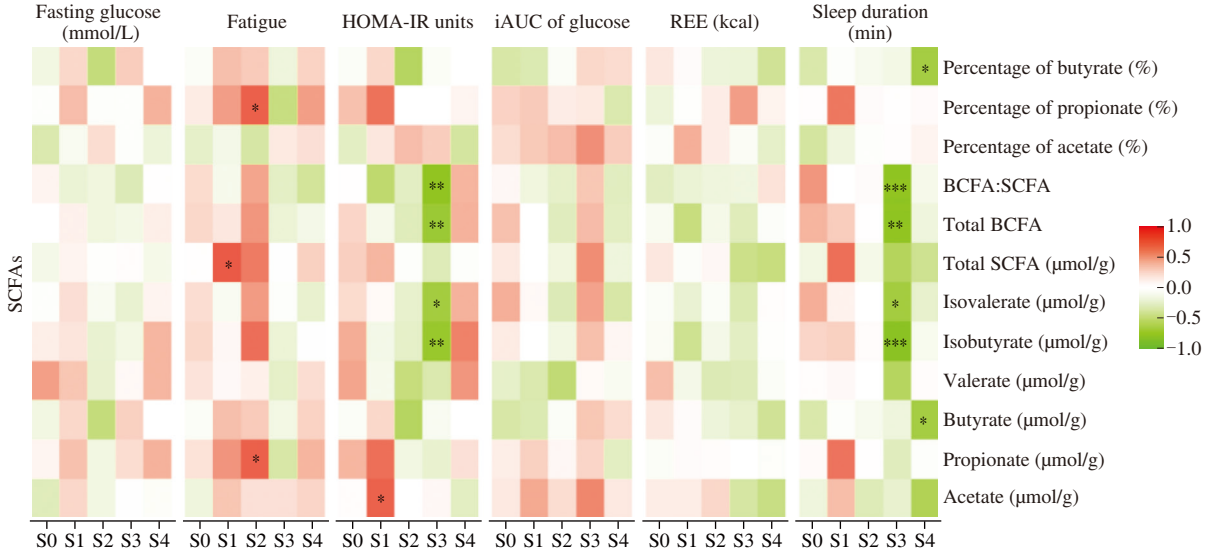

Fig. 10 Relationship between fecal SCFAs associated parameters and selected metabolic parameters. Selected metabolic parameters include fasting glucose, HOMA-IR, iAUC of glucose, fatigue levels, REE, and sleep duration. SCFAs associated parameters include levels of 6 different kinds of SCFAs, total SCFAs, branched-SCFAs (BCFAs, isobutyric, and isovaleric acid), and the ratio of BCFA:SCFA, the percentage of acetate/propionate/butyrate to total SCFAs. Spearman correlation test was used to identify the correlation, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

insulin resistance (indicated by HOMA-IR) which cannot be easily restored by circadian rhythm reconstruction within a short period (Fig. 3). Postprandial glucose was shown to be more resistant to circadian rhythm disruption (S1) but can be modulated by short-term partial/complete circadian rhythm realignments (S2-S4, Fig. 3). Overall, our results suggested that the disturbed glucose metabolism inflicted by acute circadian rhythm misalignment can be at least partially improved by circadian rhythm realignment.

Previous studies reported that BD played a beneficial role in glucose metabolism. A high-carbohydrate-low-saturated fat with a macronutrient composition similar to BD significantly lowered the levels of hemoglobin A1c (HbA1c), fasting blood glucose, and insulin markers (fasting insulin and HOMA-IR) in T2DM patients in a 2-year randomized controlled trial (RCT)[20]. In addition, a high-carb-low-fat diet, especially with high-fiber carbohydrates and low saturated fat, is strongly associated with weight loss and glycemic control, which may help the prevention of T2DM in high-risk individuals[21-22]. However, in the present study, diet alone without changes in sleep patterns did not induce changes in glucose metabolism. This could partly be attributed to the short intervention period.

In alignment with previous findings[23], we did not observe any significant changes or trends in fasting lipids and postprandial lipid profiles resulting from acute circadian rhythm misalignment or realignment. Additionally, in line with our findings on lipid profiles, most studies reported that both high-carb and high-fat diets exert no significant influence on the lipid profile and inflammatory markers in both healthy and high-risk individuals with metabolic dysfunction[20,24-26]. We speculated that the observed results in the present study might be attributed to that 1) lipid metabolism is more resilient in response to changes in circadian rhythm than the glucose regulatory system; 2) the excellent health conditions in the young subjects in the present study might contribute to some extent to tolerate the disturbance in systemic homeostasis. This could also explain, at least partially, the unaffected levels of blood pressure and heart rates in the present study.

Acute circadian rhythm changes have minimal impact on body weights and body compositions across all stages except S3. The decrease in both body fat and visceral fat mass while an increase in fat-free mass of the subjects in S3 could be due to the short-term high-fat, low-carbohydrate isoenergetic diet combined with a proper sleep schedule in this stage. Our result is in line with previous findings, suggesting that a carbohydrate-restricted diet could significantly reduce total fat and abdominal fat in 12 weeks[27-28]. But due to the significant sleep × diet interaction effect in body composition parameters, the interpretation of the results must be cautious.

It was reported that REE is susceptible to variations in macronutrient composition and can be increased by a high-fat-high-protein diet[29]. In addition, REE varies with the circadian phase and is lowest at the circadian phase 0°[30]. REE in the present study was raised during the intervention as compared to the baseline, with S3 being the highest, followed by S4, S2, and S1, with an interaction between sleep and diet reported. We speculated that the changes in circadian phases during the intervention increased REE, and the effect was enhanced by HFD, suggesting that REE is sensitive to sleep and diet.

Consistent with our findings, previous studies reported that optimizing sleep and dietary patterns could assist the subjects in managing subjective fatigue levels[31]. Sleep duration was significantly extended when normal sleep schedules were implemented in S3 and S4, suggesting that late-night sleep schedules (i.e., S1 and S2) could interfere with sleep cycles and thus potentially further induce sleep deprivation in the long run[3].

In the present study, short-term changes in sleep patterns did not lead to significant alterations in α- or β-diversity of the gut microbiota or SCFAs. Our findings align with the results obtained from a 2-day acute partial sleep deprivation study, which showed that the treatment has minimal impact on the diversity and composition of the microbial community in the gut[19]. However, as we can observe in the unweighted Unifrac distance figure (Fig. 6B), the mean values of S1 and S3 slightly decreased as compared to that of S0, though significance was only observed in S3. It suggested that HFD with extended eating windows of 14 h in S3 led to lower unweighted Unifrac distance, and the change of sleep schedules from a late-night sleep schedule to a normal one in S3 could probably further contribute to the lower unweighted Unifrac distance, indicating a specific type of diet and the change of sleep patterns could affect the composition of gut microbiota. Previous studies reported a positive association between Bacteroidetes and fat but a negative association with fibers, whereas a reverse association in Firmicutes was shown[32-33]. It was reported that the consumption of an animal-based diet or a high-fat fast food diet[34-38] increased the abundance of bile-tolerant microorganisms, such as *Bacteroides*, *Parabacteriodes*, and *Alistipes*, while decreased levels of fiber-fermenting bacteria belonging to the phyla Firmicutes such as Lachnospiraceae and *Butyricicoccus*. Consistently, we observed a similar trend in our study. In a 6-month RCT on young healthy adults, a low-fat diet (20% of energy from fat) resulted in a greater abundance of *Blautia* and *Faecalibacterium* (Firmicutes)[37], also in line with our findings in response to a low-fat BD diet (approximately 20% of energy from fat). Similarly, increased levels of *Faecalibacterium prausnitzii* were associated with a low-fat, high-complex carbohydrates diet in a 1-year RCT study on obese subjects[39]. Members of *Butyricimonas* were reported to be associated with the production of SCFAs[40]. In the present study, *Butyricimonas* decreased in S2-S4 compared to S0 and S1 and were positively associated with sleep duration in S4, suggesting the effect of sleep schedules on the taxa. However, the decreasing trend of *Butyricimonas* in S2-S4 was not reflected in SCFAs productions. Variations in diet led to changes in SCFA production. High-fat diet on healthy young subjects in a 6-month RCT induced the reduction of SCFAs compared to groups on low-fat and moderate-fat diets[37]. However, a high-saturated fat diet on subjects with MetS risks for 24 weeks increased the production of SCFAs compared to subjects who consumed a high mono-unsaturated fat diet and high-carbohydrate diet[41]. Acute partial sleep deprivation resulted in no changes in SCFAs[19]. In the present study, probably due to the accumulated effect of diet and sleep as well as the short-term intervention period, total SCFAs remained unchanged during the intervention.

The association between sleep conditions and F/B was observed in previous studies. Acute partial sleep deprivation was reported to lead to an increase in F/B[19]. However, we found that F/B was primarily

influenced by diet instead of short-term changes in sleep schedules in the present study.

5. Conclusion

This is the first study to examine the impact of short-term changes in sleeping and eating patterns on glucometabolic health and gut microbiota in healthy young adults *via* a controlled-feeding trial with a 1-month step-wise circadian rhythm misaligned-realigned scheme. Our results suggest that circadian rhythm realignment by sleep or/and diet could partly improve glucose metabolism impaired by a short-term circadian rhythm misalignment in healthy young people. However, short-term changes in sleep and dietary patterns did not lead to alterations in the diversities of gut microbiota or SCFAs though we observed changes in the abundance of certain species. Thus, the impact on gut microbiota requires further exploration.

6. Limitation

Our study provided valuable evidence to understand the impact of alterations in the human circadian rhythm system on metabolism and gut microbiota, and explored effective lifestyle preventive strategies on metabolic disorders for populations with disrupted circadian systems. However, our study has several limitations, and future measures shall be implemented to overcome the challenges. Firstly, this is a continuous intervention study without randomization of the stages. Therefore, a crossover study design with stage randomization could be considered to consolidate the results. Secondly, this is a within-subject controlled-feeding trial without a wash-out period which could otherwise interfere with the step-wise circadian rhythm misaligned-realigned schemes. Therefore, the inevitable carry-over effects must be considered when elucidating the results. Thirdly, a small sample size of subjects and a short period of each intervention stage were adopted in the present study. This study design is a small-scale proof-of-concept study to explore the impact of acute circadian disruption on health and the effective lifestyle intervention strategies to modulate it. Future studies with larger sample sizes and extended intervention periods are required to provide more concrete evidence to substantiate our findings. Last but not least, only young subjects were included in the study. Due to the demand for strict compliance with dietary and sleep arrangements and frequent weekly CIDs during the trial, only college students with flexible work schedules are available for recruitment. Therefore, the explanation of the results is restricted to young healthy individuals. Future studies on subjects with more diverse age ranges and health conditions are encouraged to expand the study scope.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgment

We would like to express our appreciation to all the subjects and the group of study coordinators involved in this study for their contributions and efforts in conducting the experiment.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2023.9250038.

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