Effects of Frequent Eating on Human Glucose and Lipid Metabolism and Biological Rhythm Expression: Postprint

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Abstract

Background: The incidence of glucose and lipid metabolism-related diseases is increasing annually, and dietary patterns are widely recognized as closely associated with their development. Improper diet can lead to disruption of circadian rhythms, a process regulated by multiple genes. Clarifying the effects of different dietary patterns is crucial for preventing metabolic diseases; however, current epidemiological data and evidence from population studies remain limited.

Objective: To investigate the effects of frequent eating on glucose and lipid metabolism and circadian rhythm expression in humans, and to provide insights for research on dietary frequency and disease risk in healthy populations.

Methods: Healthy volunteers aged 18–29 years with regular sleep schedules, consistent dietary habits, and moderate snack consumption were recruited from April to May 2022. Twenty selected healthy volunteers were randomly divided into a three-meal group (n=10) and a six-meal group (n=10) for a crossover intervention. The three-meal group consumed three main meals at 7:00, 12:00, and 18:00. The six-meal group received, in addition to the three main meals, 300 mL of water containing 75 g of anhydrous glucose powder at 10:00, 15:00, and 20:00. The single intervention period lasted one day for both groups. Blood samples were collected at eight time points (7:00, 8:00, 10:00, 12:00, 13:00, 16:00, 20:00, and 02:00) to measure serum glucose and lipid metabolism-related indicators [total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), blood glucose, insulin, leptin] and mRNA expression levels of circadian genes (Clock, Bmal1, Per2, Cry1, Pparα, and Sirt1).

Results: Group and time showed an interaction effect on TG levels (F_{interaction}=2.277, P_{interaction}=0.032). Group demonstrated

a significant main effect on LDL-C levels (F_{between}-group=4.803, P_{between}-group=0.030). Time showed a significant main effect on TC levels (F_{time}=2.092, P_{time}=0.048). No statistically significant differences were observed between groups in TC, TG, LDL-C, or HDL-C levels at any time point (P>0.05). Group and time showed an interaction effect on blood glucose levels (F_{interaction}=3.926, P_{interaction}=0.001). Group demonstrated a significant main effect on insulin levels (F_{between}-group=12.240, P_{between}-group<0.001). Time showed significant main effects on blood glucose, insulin, and leptin levels (F_{time}=10.840, 2.399, 4.347; P_{time}<0.05). The six-meal group exhibited higher blood glucose levels at 12:00 and 20:00, higher insulin levels at 10:00 and 16:00, and lower leptin levels at 16:00 compared with the three-meal group (P<0.05). Group and time showed an interaction effect on Cry1 mRNA expression levels (F_{interaction}=30.250, P_{interaction}<0.001). Group demonstrated significant main effects on mRNA expression levels of Clock, Bmal1, Per2, Cry1, Pparα, and Sirt1 genes (P_{between}-group<0.05). Time showed significant main effects on mRNA expression levels of Bmal1, Per2, Cry1, and Sirt1 genes (P_{time}<0.05). The six-meal group showed higher Clock mRNA expression levels at 8:00 and 13:00, higher Per2 mRNA expression levels at 8:00, 16:00, and 02:00, and higher Cry1 mRNA expression levels at 7:00, 8:00, and 10:00 compared with the three-meal group (P<0.05). The six-meal group exhibited lower Bmal1 mRNA expression levels at 10:00, 12:00, and 13:00, and lower Cry1 mRNA expression levels at 20:00 and 02:00 compared with the three-meal group (P<0.05). No statistically significant differences were observed between groups in Pparα or Sirt1 mRNA expression levels at any time point (P>0.05).

Conclusion: The six-meal group with frequent eating led to elevated insulin and blood glucose levels, disrupted regulatory mechanisms, and caused homeostatic imbalance in glucose metabolism. Simultaneously, it affected the expression, phase, and amplitude of various clock genes, resulting in circadian rhythm disruption.

Full Text

Effects of Frequent Diets on Glucolipid Metabolism and Biorhythmic Expression in Humans

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Abstract

Background: The prevalence of glycolipid metabolism disorders is rising annually, and dietary patterns are recognized as key modulators of their pathogenesis. Improper eating habits cause circadian disruption, which is mediated by multiple genes. Clarifying the role of different dietary methods is crucial for preventing metabolic diseases, yet relevant epidemiological and population-based evidence remains scarce.

Objective: To investigate the effects of dietary frequency on human glycolipid metabolism and circadian gene expression, thereby providing insights into the relationship between dietary frequency and disease risk in healthy populations.

Methods: Healthy volunteers aged 18–29 years with regular sleep-wake cycles, consistent dietary patterns, and moderate snack intake were recruited between April and May 2022. Twenty eligible participants were randomly assigned to either a three-meal group (n=10) or a six-meal group (n=10) for a crossover intervention. The three-meal group consumed main meals at 7:00, 12:00, and 18:00. The six-meal group received three additional glucose challenges (75 g anhydrous glucose dissolved in 300 mL water) at 10:00, 15:00, and 20:00. Each intervention lasted 24 hours. Blood samples were collected at eight timepoints (7:00, 8:00, 10:00, 12:00, 13:00, 16:00, 20:00, and 02:00) to analyze serum metabolic markers including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), glucose, insulin, and leptin. mRNA expression levels of circadian genes (Clock, Bmal1, Per2, Cry1, Pparα, and Sirt1) were also measured.

Results: A significant group $\times$ time interaction effect was observed for TG levels (Finteraction = 2.277, Pinteraction = 0.032). Group showed a significant main effect on LDL-C (Fgroup = 4.803, Pgroup = 0.030), while time exhibited a significant main effect on TC (Ftime = 2.092, Ptime = 0.048). No significant differences in TC, TG, LDL-C, or HDL-C were found between groups at any timepoint ($P > 0.05$). A significant group $\times$ time interaction was identified for blood glucose (Finteraction = 3.926, Pinteraction = 0.001). Group showed a significant main effect on insulin (Fgroup = 12.240, Pgroup < 0.001), and time demonstrated significant main effects on glucose, insulin, and leptin (Ftime = 10.840, 2.399, and 4.347, respectively; Ptime < 0.05). The six-meal group exhibited higher glucose levels at 12:00 and 20:00, elevated insulin at 10:00 and 16:00, and lower leptin at 16:00 compared to the three-meal group ($P < 0.05$). A significant group $\times$ time interaction was observed for Cry1 mRNA expression (Finteraction = 30.250, Pinteraction < 0.001). Significant group main effects were detected for Clock, Bmal1, Per2, Cry1, Pparα, and Sirt1 mRNA expressions (Pgroup < 0.05), while significant time main effects were noted for Bmal1, Per2, Cry1, and Sirt1 (Ptime < 0.05). The six-meal group displayed significantly higher Clock expression at 8:00 and 13:00, Per2 at 8:00, 16:00, and 02:00, and Cry1 at 7:00, 8:00, and 10:00 compared to the three-meal group ($P < 0.05$). Conversely, significantly lower expressions of Bmal1 at 10:00,

12:00, and 13:00, and Cry1 at 20:00 and 02:00 were observed in the six-meal group ($P < 0.05$). No significant differences in Pparα or Sirt1 expression were detected between groups at any timepoint ($P > 0.05$).

Conclusion: Frequent six-meal consumption elevates insulin and glucose levels, disrupts metabolic homeostasis, and alters circadian clock gene expression in both phase and amplitude. These changes induce glucose metabolism dysregulation and circadian rhythm disruption.

Keywords: Metabolism; Glycolipid metabolism; Biorhythm; Dietary frequency; Circadian clock genes; Crossover intervention; Multivariate analysis of variance

Introduction

Modern societal acceleration and diversified food culture have gradually shifted eating patterns from regular three-meal structures to high-frequency, fragmented consumption. Frequent eating not only alters the temporal distribution of energy intake but may also disrupt endogenous circadian rhythm systems and affect glucolipid metabolic homeostasis. While existing research has focused primarily on how single nutrients or total energy intake influence metabolism, few studies have examined how meal timing distribution and frequency impact circadian rhythms and glucolipid metabolism.

Studies indicate that increased snack consumption beyond three standard meals (breakfast, lunch, and dinner) correlates with elevated type 2 diabetes mellitus (T2DM) risk [1]. Consequently, some researchers propose reducing meal frequency to lower fasting blood glucose [2] and decrease the risk of obesity, hypercholesterolemia, and glucose intolerance [3]. However, Alencar et al. [4] found that fasting glucose and insulin levels remain unaffected by two-meal versus six-meal patterns. Other studies propose that intermittent or periodic fasting states may help prevent and treat metabolic diseases [5]. Thus, findings on how meal frequency affects metabolism remain inconclusive.

Circadian rhythms, functioning as endogenous biological clocks, respond not only to environmental cycles but also regulate metabolic activities including insulin secretion, sensitivity, and cholesterol synthesis, serving as a “core hub” for glucolipid metabolism [6-8]. Clock gene mutant mice develop obesity, hyperglycemia, hepatic steatosis, and other metabolic syndromes [8], while circadian misalignment in humans leads to prediabetic glucose abnormalities and elevated metabolic markers [9,10], revealing the intimate connection between biological rhythms and energy metabolism [11]. Beyond light exposure, diet serves as a key regulator of circadian rhythms, with irregular meal timing disrupting digestive system clocks and affecting glycemic fluctuations [12-14]. Animal experiments confirm that regular feeding restores hepatic gene rhythm expression [15], while disrupted feeding patterns trigger gastrointestinal and metabolic dysfunction [16-18], demonstrating that synchronization between diet and biological rhythms is essential for maintaining metabolic homeostasis.

Improved living conditions have led to rising incidences of diabetes and hyperlipidemia. Could this be related to frequent eating behaviors in modern life? Does frequent eating cause metabolic disorders by disrupting biological rhythms? This study employs a crossover intervention trial to explore how different meal frequencies affect glucolipid metabolism and circadian gene expression in healthy individuals, aiming to provide new insights into the relationship between dietary frequency and metabolic disorders. Against the backdrop of increasing global burden from glucolipid metabolic diseases, elucidating the regulatory role of dietary rhythms on metabolic health carries important public health significance for prevention and precision nutrition management.

Methods

Study Subjects

Healthy volunteers aged 18–29 years with regular sleep-wake cycles, consistent dietary patterns, and moderate snack intake were recruited between April and May 2022. All volunteers provided informed consent, and the study was approved by the Ningxia Medical University Ethics Committee (Approval No. 2019-108).

Inclusion criteria: (1) BMI 18–24 kg/m^2; (2) no smoking or alcohol consumption; (3) regular sleep schedule (wake time 7:00–9:00 for the past year); (4) bedtime 21:00–23:00; (5) regular meals (breakfast 7:00–9:00, lunch 11:00–13:00, dinner 17:00–19:00) for the past year; (6) moderate snack intake (consuming snacks, fruits, or sugary beverages 2–4 days per week).

Exclusion criteria: (1) waist circumference $ $80 cm for females or $ $85 cm for males; (2) endocrine, metabolic, gastric, hepatobiliary, or other chronic diseases; (3) acute infection, medication use, or surgical history within the past 2 months; (4) family history of diabetes.

Sample Size Estimation

This study primarily examined changes in circadian gene Clock (circadian locomoter output cycles kaput) and Bmal1 (brain and muscle Arnt-like protein-1) expression. Sample size was calculated using the formula for comparing two means in epidemiological studies: $N = 2(Z\alpha + Z\beta)^2\sigma^2/d^2$. Based on previous experimental data [19], the difference (d) in Clock and Bmal1 protein expression levels at 210 minutes postprandial in healthy individuals was 0.325, with a standard deviation of 0.192 ($\sigma = 0.192$). With $\alpha = 0.05$, $\beta = 0.1$, and two-tailed testing, the calculated N 8, leading to a final sample size of 10 per group.

Intervention Protocol

Twenty participants were randomly assigned using Excel-generated random numbers to either a three-meal group (n = 10) or a six-meal group (n = 10). The three-meal group consumed three main meals daily. The six-meal group

received identical main meals with equivalent energy content after accounting for the glucose drinks, with identical side dishes between groups to ensure comparable total energy intake (see dietary content in Table 1). To prevent short-term lifestyle habits from affecting results, participants were instructed to maintain consistent wake times (7:00–9:00), bedtimes (21:00–23:00), and meal times for one week prior to the intervention. On the day before intervention, participants consumed normal three-meal diets without snacks and began fasting at 20:00.

This crossover intervention trial consisted of a single 24-hour intervention period followed by a one-week washout period before crossover. The three-meal group ate at 7:00, 12:00, and 18:00, with each meal lasting 30–40 minutes. The six-meal group consumed three additional glucose challenges (75 g anhydrous glucose dissolved in 300 mL water, providing 300 kcal) at 10:00, 15:00, and 20:00, with each consumption period lasting 30–40 minutes and the glucose solution required to be consumed within 15 minutes.

Sample Collection and Processing

Peripheral venous blood samples were collected before and 1 hour after the 7:00 meal, and subsequently at 10:00, 12:00, 13:00, 16:00, 20:00, and 02:00 (8 total collections). Each collection yielded two coagulation-promoting tubes and two anticoagulant tubes (4–5 mL each). Coagulation tubes were left at room temperature for 10–20 minutes; anticoagulant tubes were inverted 4–5 times for thorough mixing. Samples were centrifuged at 1,000×g for 15 minutes. Serum (from coagulation tubes), plasma (from anticoagulant tubes), and blood cells (anticoagulant tube sediment) were aliquoted into 300 L portions and stored at -80°C.

Detection Indicators

Serum glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using an automatic biochemical analyzer. Insulin and leptin levels were determined by enzyme-linked immunosorbent assay (ELISA). mRNA expression of Clock, Bmal1, Cry1 (cryptochrome 1), Per2 (period 2), Sirt1 (sirtuin-1), and Pparα (peroxisome proliferator-activated receptor α) was detected by quantitative polymerase chain reaction (qPCR).

Statistical Analysis

SPSS 24.0 software was used for statistical analysis. Normally distributed data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and compared between groups using independent samples t-tests. Non-normally distributed data were expressed as median (P25, P75) and analyzed using nonparametric rank-sum tests. Repeated measures multivariate analysis of variance was used for time-course comparisons between groups. GraphPad Prism 10 was used for figure

preparation. $P < 0.05$ was considered statistically significant.

Results

Effects of Meal Frequency on Lipid Metabolism

A significant group × time interaction effect was observed for TG levels (Finteraction = 2.277, Pinteraction = 0.032). No significant interaction effects were found for TC, LDL-C, or HDL-C (Pinteraction > 0.05). Group showed a significant main effect on LDL-C (Fgroup = 4.803, Pgroup = 0.030), while time exhibited a significant main effect on TC (Ftime = 2.092, Ptime = 0.048). However, no significant differences in TC, TG, LDL-C, or HDL-C were detected between groups at any timepoint ($P > 0.05$, Table 2).

Dyslipidemia plays an important role in diabetes pathogenesis, with elevated TC, TG, and LDL-C increasing T2DM risk [20,21], while HDL-C serves as a beneficial lipoprotein [20,22]. Kahleova et al. [23] demonstrated that under equivalent daily energy restriction, TG and LDL-C reductions were comparable between two-large-meal and six-small-meal regimens, with no significant changes in TC or HDL-C observed in either protocol. A short-term study in obese women similarly found that meal frequency did not affect TC or LDL-C [4]. Our crossover intervention likewise showed no significant effects on TC, TG, LDL-C, or HDL-C, suggesting that meal frequency has minimal impact on lipid metabolism.

Effects of Meal Frequency on Glucose, Insulin, and Leptin

A significant group × time interaction effect was identified for blood glucose (Finteraction = 3.926, Pinteraction = 0.001). No significant interaction effects were found for insulin or leptin ($P > 0.05$). Group showed a significant main effect on insulin (Fgroup = 12.240, Pgroup < 0.001), while time demonstrated significant main effects on glucose, insulin, and leptin (Ftime = 10.840, 2.399, and 4.347, respectively; Ptime < 0.05). The six-meal group exhibited higher glucose levels at 12:00 and 20:00, elevated insulin at 10:00 and 16:00, and lower leptin at 16:00 compared to the three-meal group ($P < 0.05$). No significant differences were observed at other timepoints ($P > 0.05$, Table 3).

In a randomized crossover trial of individuals with impaired fasting glucose, researchers found that short-term increased meal frequency improved glucose metabolism and insulin secretion when energy intake was equivalent [24]. Another randomized clinical trial in T2DM patients showed that isocaloric small frequent meals reduced blood glucose and insulin levels [25]. However, a randomized controlled study in T2DM found that three meals versus six isocaloric meals significantly reduced HbA1c, fasting glucose, and 24-hour average glucose [26]. Another trial demonstrated that increased meal frequency led to hepatic fat accumulation, decreased insulin sensitivity, and increased obesity and binge-eating risk [27]. In healthy individuals, blood glucose rises postprandially and

returns to baseline after 2 hours. Our findings show that both groups exhibited postprandial glucose elevations, but the six-meal group maintained higher glucose balance with increased meal frequency, suggesting that the regulatory mechanism gradually becomes imbalanced. This indicates that three daily meals may be more beneficial for glycemic control than six meals. Although insulin trends were not significantly different, serum insulin levels increased in the six-meal group, possibly representing a protective mechanism whereby the body secretes more insulin to cope with sudden increases in meal frequency. These results suggest that frequent eating overstimulates insulin secretion. Leptin, derived from adipocytes, reduces food intake and aids weight loss [28]. Lower leptin levels at 16:00 in the six-meal group may reflect reduced leptin secretion due to frequent eating, increasing obesity risk. Therefore, while increased meal frequency may improve glucose metabolism in individuals with impaired glucose tolerance, irregular eating patterns disrupt insulin secretion rhythms even in healthy individuals, causing metabolic dysregulation within a single day.

Effects of Meal Frequency on Circadian Gene Expression

A significant group × time interaction effect was observed for Cry1 mRNA expression ($F_{interaction} = 30.250$, $P_{interaction} < 0.001$). No significant interaction effects were found for Clock, Bmal1, Per2, Pparα, or Sirt1 ($P > 0.05$). Group showed significant main effects on mRNA expression of Clock, Bmal1, Per2, Cry1, Pparα, and Sirt1 ($P_{group} < 0.05$), while time demonstrated significant main effects on Bmal1, Per2, Cry1, and Sirt1 ($P_{time} < 0.05$). The six-meal group exhibited significantly higher Clock expression at 8:00 and 13:00, Per2 at 8:00, 16:00, and 02:00, and Cry1 at 7:00, 8:00, and 10:00 compared to the three-meal group ($P < 0.05$). In contrast, Bmal1 expression at 10:00, 12:00, and 13:00, and Cry1 at 20:00 and 02:00 were significantly lower in the six-meal group ($P < 0.05$). No significant differences in Pparα or Sirt1 expression were detected between groups at any timepoint ($P > 0.05$, Table 4).

Discussion

This study achieved important findings regarding the effects of frequent eating on glucolipid metabolism and circadian rhythms, though several limitations must be acknowledged. The intervention duration was relatively short, focusing only on acute changes without tracking long-term effects of frequent eating. Our study suggests that long-term frequent eating may reduce insulin sensitivity, disrupt glucose regulation, and increase metabolic disease risk, but these potential effects require future validation. Additionally, different populations may respond differently to frequent eating; future studies should include obese individuals, diabetic patients, and other special populations to provide more comprehensive and accurate information about diet-metabolism relationships.

Our findings demonstrate that frequent eating alters circadian gene expression in terms of phase and amplitude. The six-meal group showed enhanced rhythmic

expression of Clock, Pparα, Sirt1, Cry1, and Per2, with increased variation amplitude, indicating that clock genes sense daily changes in cellular metabolism and systemic signals to predictively modify the circadian transcriptome [33]. Unlike Clock, Bmal1 expression showed oscillation but remained lower overall in the high-frequency eating condition. *Nature* has reported the importance of circadian clocks in dietary metabolism [34]. Researchers have found that pancreatic islet cells possess their own clocks regulated by Clock and Bmal1 genes; when these genes are absent, the islet clock is disrupted, breaking insulin secretion patterns across sleep-wake cycles and causing hypoinsulinemia and diabetes. Our study shows that six-meal eating affects Clock and Bmal1 expression, influencing insulin sensitivity.

Mouse experiments demonstrate that hepatic overexpression of Cry1 reduces blood glucose and improves insulin sensitivity in insulin-resistant db/db mice [35], suggesting that Cry1 expression correlates with glucose homeostasis, insulin secretion, and sensitivity, and that Cry1 overexpression may benefit T2DM prevention and treatment. In our study, Cry1 mRNA expression in the six-meal group was lower than in the three-meal group after 12:00, particularly at 20:00 and 02:00, indicating that frequent eating reduces Cry1 expression, which may decrease insulin sensitivity. Reduced Bmal1 and Cry1 expression decreases insulin sensitivity and disrupts glucose regulatory mechanisms, consistent with our insulin findings. Per2 showed the most dramatic increase in mRNA expression in the six-meal group. Research indicates that circadian resetting involves acute upregulation of Per1 and Per2 [36], suggesting Per2 is a crucial component of clock-resetting mechanisms. To cope with frequent eating-induced circadian disruption, Per2 expression increases acutely to restore normal rhythms before the next transcriptional cycle.

Our results show identical Sirt1 expression patterns between groups. This may be because Sirt1 has target gene specificity [37], and our short intervention period may have been insufficient to affect Sirt1 expression. A 2012 study from the Shanghai Institutes for Biological Sciences further revealed that Clock and Bmal1 regulation of insulin sensitivity depends on the key regulatory protein Sirt1 [38]. In our study, the six-meal group showed higher insulin levels at 10:00 and 16:00 and higher Clock mRNA expression at 8:00 and 13:00, while both Clock and Sirt1 peaked at 12:00–13:00 and 20:00, with insulin levels showing a stable upward trend at these times. This suggests that abnormal insulin elevation from frequent eating interacts with Clock and Sirt1 rhythmic regulation.

Among clock genes, Pparα functions as a nutrient sensor that adapts rates of fatty acid oxidation, lipogenesis, and ketogenesis in response to feeding and fasting states, and serves as a transcriptional regulator of hepatic glucose production [39]. Our significant group main effect on Pparα mRNA expression suggests that frequent eating activates Pparα expression to convey "saturation" signals and prompt cessation of eating.

In summary, one day of frequent eating in healthy individuals increased serum

insulin, disrupted glucose regulation, reduced leptin secretion, and caused abnormal circadian gene expression patterns including oscillation, enhanced expression, or reduced expression. Frequent eating modulates both glucolipid metabolism and circadian gene expression, with bidirectional interactions between metabolic and circadian signaling pathways.

Conclusion

Frequent six-meal consumption elevates insulin and glucose levels, disrupts metabolic homeostasis, and alters circadian clock gene expression in phase and amplitude. These changes induce glucose metabolism dysregulation, leading to circadian rhythm disruption.

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Author Contributions

YANG Jun conducted the primary experiments, data analysis, statistical graphing, and manuscript writing. MAIBUBAIMU · Aisikaer performed partial experiments. YANG Qianqian collected and organized data. LI Kai implemented the survey and selected participants. YIN Gaojun revised the manuscript and conducted quality control. CAI Huizhen conceived and designed the study, supervised the overall project, and provided funding.

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Note: Figure translations are in progress. See original paper for figures.