

Time restricted feeding attenuates metabolic dysregulation and demonstrates legacy effect in high fat diet rat model

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The global rise in obesity, particularly in developing countries, has become a critical public health concern due to its strong association with diabetes, hypertension, cardiovascular diseases, and certain cancers. The limited long-term efficacy of current nutritional and pharmaceutical therapies underscores the need for durable, efficient approaches that address underlying circadian disturbances and metabolic disorders. Core circadian genes like *Per1* and *Bmall*, which control daily cycles of fat absorption, storage, and the metabolism of glucose, lipids, and cholesterol, are disrupted in obesity. A promising lifestyle modification that synchronises food intake with circadian rhythms and may enhance metabolic outcomes is time-restricted feeding (TRF). This study sought to understand how TRF affects metabolic profiles and gene expression in obese Wistar rats at the physiological and molecular levels. Fifteen rats were split into two groups: an experimental group (n=9) that was rendered obese by a high-fat diet (HFD; *ad libitum*) for two months, and a control group (n=6) that was fed a chow diet for six months. Six of the obese rats were converted to TRF (12-hour fasting from 8:00 pm to 8:00 am, followed by 12-hour HFD access) for three months, while three were put down for baseline evaluations. The findings revealed that, in comparison to controls, HFD-fed rats had significantly higher blood glucose and body weight ($P=0.0263$, $P=0.0089$), lower levels of melatonin and insulin ($P=0.006$), lower HDL, and higher levels of total cholesterol (TC), triglycerides (TG), and LDL. The TRF intervention increased insulin, melatonin, TC, and HDL while decreasing body weight, blood glucose, TG, and LDL. Significantly, TRF exhibited circadian modulation by modulating the expression of *Per1* and *Bmall*, and its metabolic benefits continued even after the return to *ad libitum* feeding. These results imply that TRF is a workable, non-pharmacological method of reducing metabolic and circadian disruptions associated with obesity, deserving of more research as a human preventive and treatment approach.

Keywords: *Bmall*, *Per1*, High fat diet, TRF, Life style modification

Obesity is a major public health concern in both developed and developing countries¹. In the United States, one-third of adults are obese², while in India, the prevalence is 40%³. According to WHO, 39% of the global population is overweight and 13% is obese. It is a significant risk factor for diabetes, cancer, cardiovascular disease (CVD) and non-alcoholic fatty liver disease (NAFLD)⁴. Nutritional imbalance is a major contributor, disrupting pathways that affect the liver, adipose tissue, and muscles, which regulate glucose, amino acids, and lipids⁵. Alongside genetic and epigenetic influences, lifestyle factors such as altered eating habits, reduced physical activity, and shortened sleep contribute to obesity⁶. The first-line therapeutic strategy is behavioural intervention, focusing on reducing calorie intake and increasing physical activity⁷.

The 24-hour circadian rhythm regulates physiology, metabolism, and behaviour in a tissue-specific manner. Feeding and fasting cycles significantly impact body function and health⁸. The interplay between circadian systems and feeding patterns helps prevent metabolic disorders by activating nutrient-sensing pathways, which maintain nutrient homeostasis during feeding–fasting cycles at both transcriptional and post-transcriptional levels⁹. Hence, this study was undertaken to evaluate the effects of time-restricted feeding (TRF) on metabolic parameters and circadian clock gene expression (*Per1*, *Cry1* and *Bmall*) in high-fat diet-induced obese Wistar rats.

Materials and Methods

Computational based Study

The genes associated with the Circadian Rhythm were submitted to the DAVID platform for Kyoto Encyclopedia of Genes and Genomes (KEGG)

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pathway enrichment analysis. Subsequently, gene ontology (GO) enrichment analysis was performed to assess molecular function, biological process, and cellular component, with results presented ($-\log_{10}$ *P*-value). The common genes were further analysed using the String database (<https://cn.string-db.org>) to elucidate protein-protein interactions (PPI) and co-expression patterns among circadian genes.

Experimental study

Animal study

A total of 15 healthy Wistar male rats of age between 8-12 weeks with 150-200g were procured from CSIR-Central Drug Research Institute, Lucknow. included. The animals were maintained under controlled room temperature ($22\pm 3^{\circ}\text{C}$), humidity ($55\pm 5\%$) and light/dark (12/12 hours) with proper diet and water. The animals used in the present study were maintained in accordance with the principles and guidelines of the Canadian Council on Animal Care as outlined in the Guide for the Care and Use of Laboratory Animals. Ethical approval was approved from Animal Ethics Committee of this University, with No-123/IAEC/2019 dated September 28, 2019 and adhering to Committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines and norms for the care and use of animals in scientific research.

Control group

This group comprised of six Wistar rats that were administered a chow diet (306.2 kcal/100 g with 48.8% as carbohydrate, 21% as protein, and 3% as fat) at *ad libitum* for six months prior to euthanasia and subsequent sample collection.

Experimental obese model

The second group comprised nine Wistar rats that had been obese for two months due to *ad libitum* consumption of a HFD (HA). The HFD contained 414.0 kcal/100g of carbohydrates, 17% protein, and 40% fat. After two months three obese rats were euthanized, and their anthropometric and biochemical data were analysed.

TRF intervention

The remaining six Wistar rats in this cohort were transitioned to TRF, which involved a 12-hour fasting period i.e 8:00 am to 8:00 pm followed by a pre-existing high-fat diet (HT). They adhered to this regimen for a duration of three months after which

three rats from this cohort were euthanized for further examination. The three remaining rats were reinstated to an *ad libitum*, high-fat diet (24-hour feeding) regimen (HAT) for period of one month. Upon completion of the experimental study, the surviving rats were euthanized and samples were collected. (Fig. 1)

Sample collection

A 2 mL blood samples were collected in EDTA and plain vials for molecular study and biochemical parameter assessment, respectively. For RNA study, blood and tissue samples were collected in TRIzol reagent and preserved at -20°C for subsequent analysis.

Anthropometrics parameters

Body weight was measured monthly by using a weighing balance. BMI of rats was measured as body weight in grams and height in cm^2 .

Biochemical estimation

Fasting blood glucose was measured with a glucometer, and lipid profile and insulin levels were analysed with an autoanalyser. The melatonin level was quantified using the protocol of Lin *et al.* using the HPLC (High Performance Liquid Chromatography) method¹⁰ (Agilent 1200).

RNA isolation and qRT-PCR

RNA extraction from whole blood was carried out utilising TRIzol reagent (Thermo Fisher Scientific^R, Cat no. 15-596-026). Quality control (QC) of isolated RNA was performed using NanoDrop spectrophotometry for purity (A260/A280) and concentration assessment. All RNA samples showed A260/A280 ratios in the range of 1.9–2.1, indicating high purity suitable for downstream applications. cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, UK; Cat. No. K2563) according to the manufacturer's instructions. The expression of isolated mRNA for *Per1* (forward: ACACCACTGCCGATCTAAAG; reverse: CAGGTGAAGGATGAGACACATAG), *Cry1* (forward: CTTGAAGCTCTCGGTAGAAGAAG; reverse: AGTC-CCTTCGGAACCAATG), and *Bmal1/Arntl* (forward: CGGGTGAAGTCTATGGAGTATG; reverse: ACCT-AGAAGTTCCTGTGGTAGA) was quantified using quantitative PCR (qPCR) on an Applied Biosystems 7500 Real-Time PCR System. The primers for qPCR were designed using the Integrated DNA Technologies (IDT) platform. qPCR was performed with an initial

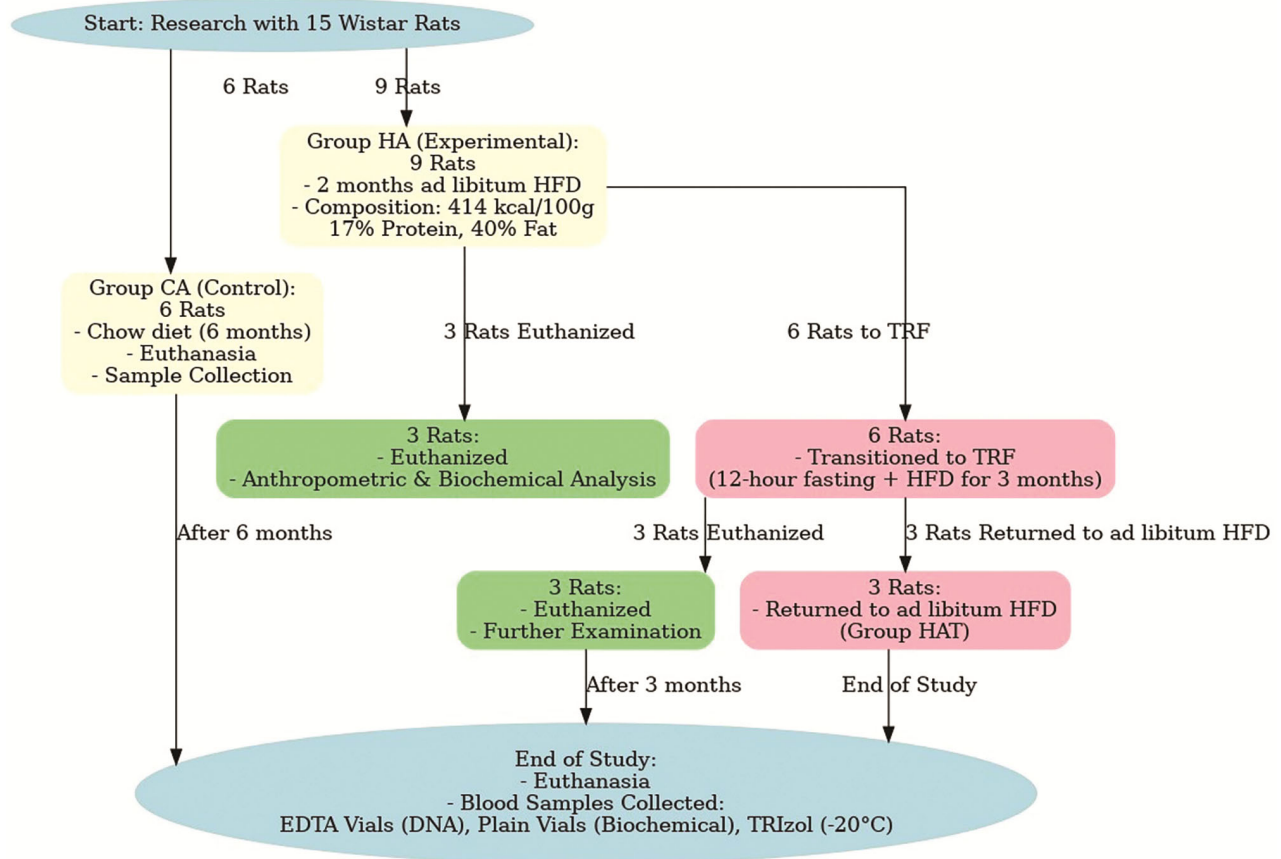


Fig. 1 — Experimental design outlining the study on the effects of high fat diet (HFD) and time-restricted feeding (TRF) in Wistar rats. A total of 15 male Wistar rats were divided into two groups: Control group (CA, $n = 6$) received a standard chow diet for 6 months, followed by euthanasia and sample collection. The experimental group (HA, $n = 9$) was fed an *ad libitum* HFD for 2 months. Three rats from the HA group were euthanized for anthropometric and biochemical analysis. The remaining six rats were transitioned to TRF (12-hour fasting + HFD) for 3 months (Group HT). After this intervention, three rats were euthanized for further examination, while the remaining three were returned to *ad libitum* HFD for one additional month (Group HAT). At the end of the study, all rats were euthanized, and blood samples were collected in EDTA vials (for RNA), plain vials (for biochemical assays), and TRIzol vials stored at -20°C (for molecular analysis).

denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 30 seconds. The qPCR data for gene expression was normalised using the comparative Ct ($\Delta\Delta\text{Ct}$) method, with **GAPDH** as the internal control to account for variation in RNA input and reverse transcription efficiency. Relative expression levels were calculated by comparing the Ct values of target genes to the reference gene, and results are expressed as fold changes which indicate upregulation or downregulation of the gene of interest.

Statistical analysis

The statistical analysis assessed significant criteria for computational-based findings, including pathway enrichment analysis and gene ontology results, using

$P < 0.05$. Protein-protein interactions and co-expression patterns of circadian genes were also investigated. The experimental investigation evaluated anthropometric and biochemical parameters between groups using statistical tests such as *t*-tests or analysis of variance (ANOVA) with a significance level of $P < 0.05$. Gene expression levels were evaluated using qRT-PCR, which took into account expression fluctuations and controlled for confounding factors. All analyses were carried out using GraphPad Prism software, version 5.

Results

KEGG pathway

The outcomes from the KEGG pathway analysis indicate that circadian genes participate in various cellular metabolic processes, specifically in circadian enrichment and MAPK signaling pathways. The

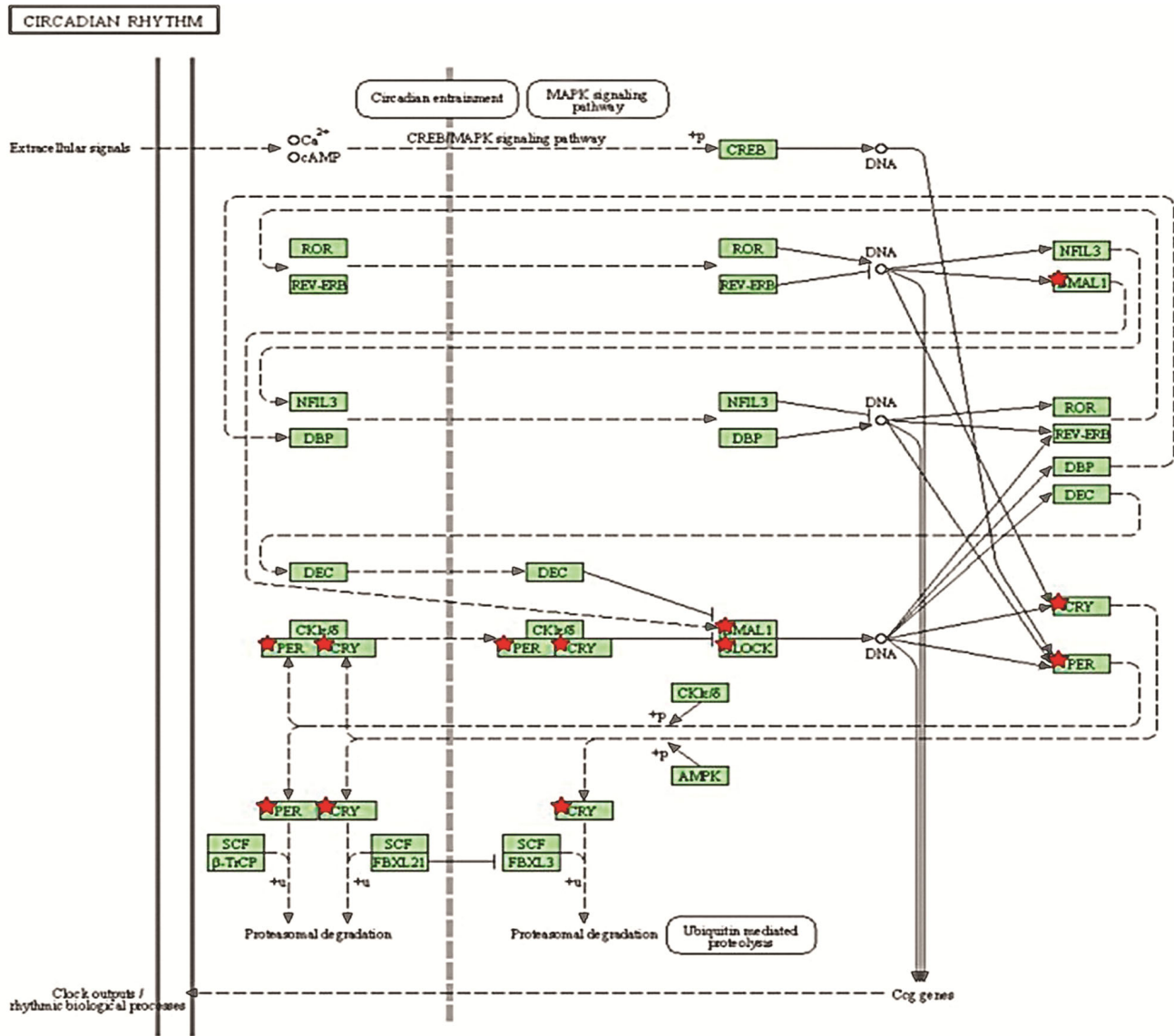


Fig. 2 — Circadian Rhythm Pathway. This figure provides a schematic representation of the core molecular components and their interactions within the mammalian circadian clock. This intricate network of transcriptional-translational feedback loops generates a self-sustained oscillation, resulting in the circadian rhythm.

KEGG pathway is enriched with genes exhibiting oscillations in accordance with the circadian rhythm, with peak transcript activity occurring during the active phase. The transcriptional rhythmicity of Period genes (*Per1*, *Per2* and *Per3*) and Cryptochrome genes (*Cry1* and *Cry2*) is evident in the initial negative feedback loop. The interaction between PER and CRY proteins forms a heterodimer, which then inhibits the transcriptional activity of the CLOCK/BMAL1 heterodimer. The pathway diagram in Fig. 2 emphasises the target genes affected by this interaction.

Gene Ontology (GO) Analysis explores into potential biological functions and signaling pathways. The analysis encompasses Biological Process (BP), Cellular Component (CC) and Molecular Function (MF), with statistical significance set at $P < 0.05$. The functional annotation through GO reveals that the differentially entrained genes primarily participate in the circadian regulation of gene expression, as illustrated in Fig. 3.

Genotype tissue expression (GTEx)

GTEx established a data resource and tissue bank to study the relationship between genetic variation

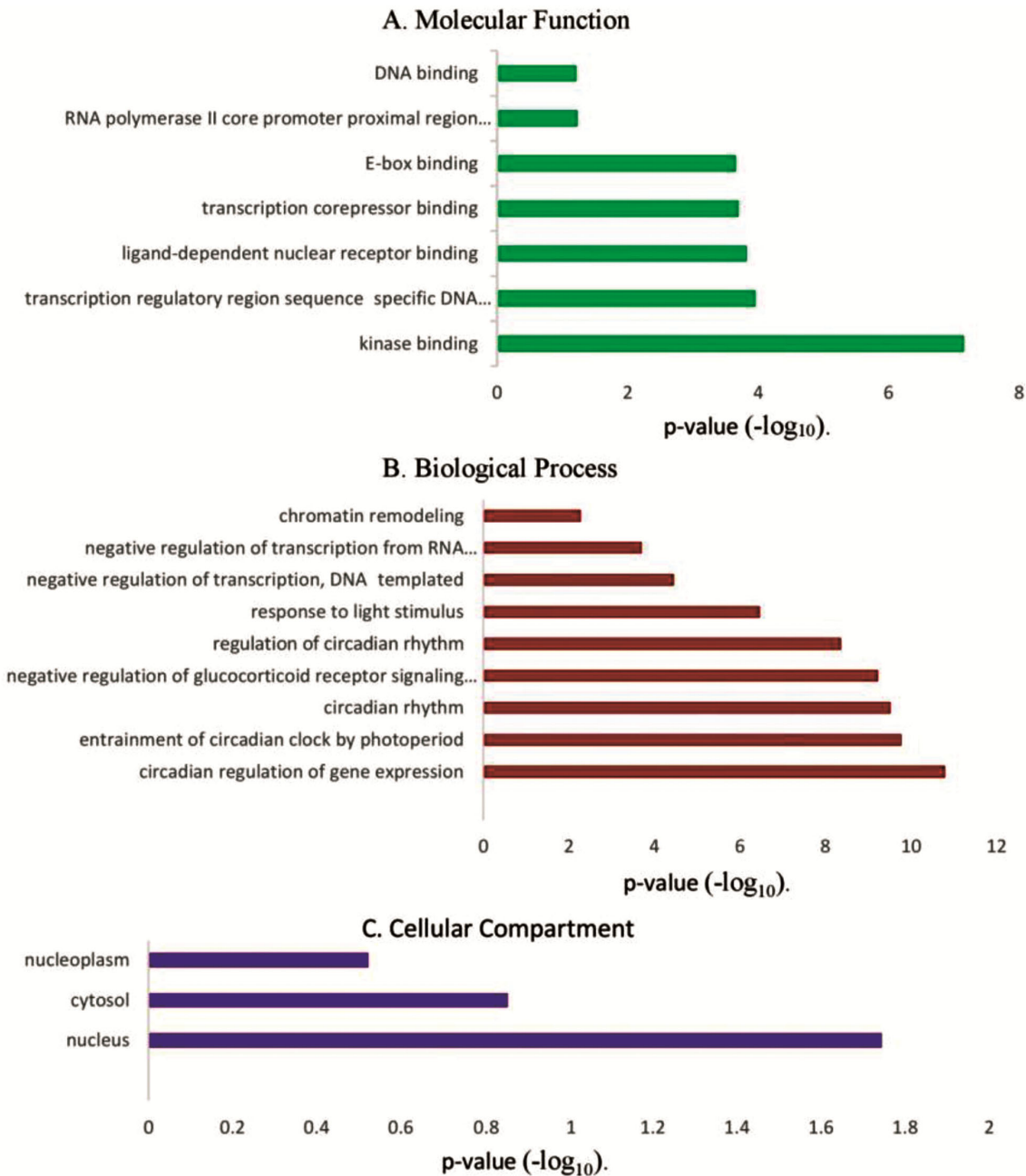


Fig. 3 — Gene Ontology (GO) enrichment analysis of target circadian genes involved in (A) Molecular Function (B) Biological Process and (C) Cellular Compartment. The enrichment scores on the y-axis represent the significance of the GO term enrichment, with higher scores indicating greater significance. Bar charts represent the $-\log_{10}(P\text{-value})$ of each enriched GO term. This analysis provides insights into the potential biological roles and functions of target genes. The analysis categorises genes based on their molecular function, biological process, and cellular component. (A) Molecular Function: This panel shows the enrichment of genes involved in various molecular functions, including DNA binding, transcription factor activity, and protein binding. (B) Biological Process: This panel highlights the enrichment of genes involved in a range of biological processes, such as transcription regulation, cellular response to stimulus, and metabolic processes. (C) Cellular Component: This panel indicates the enrichment of genes localized to specific cellular compartments, primarily the nucleus.

and gene expression in multiple tissues. In this study included gene expression in different central as well as peripheral tissue viz. hypothalamus, blood, adipose and liver (Fig. 4).

Protein-protein interaction (PPI) and its Co-expression

A network illustrating interactions among core clock components and proteins related to the clock has been constructed using the STRING protein-

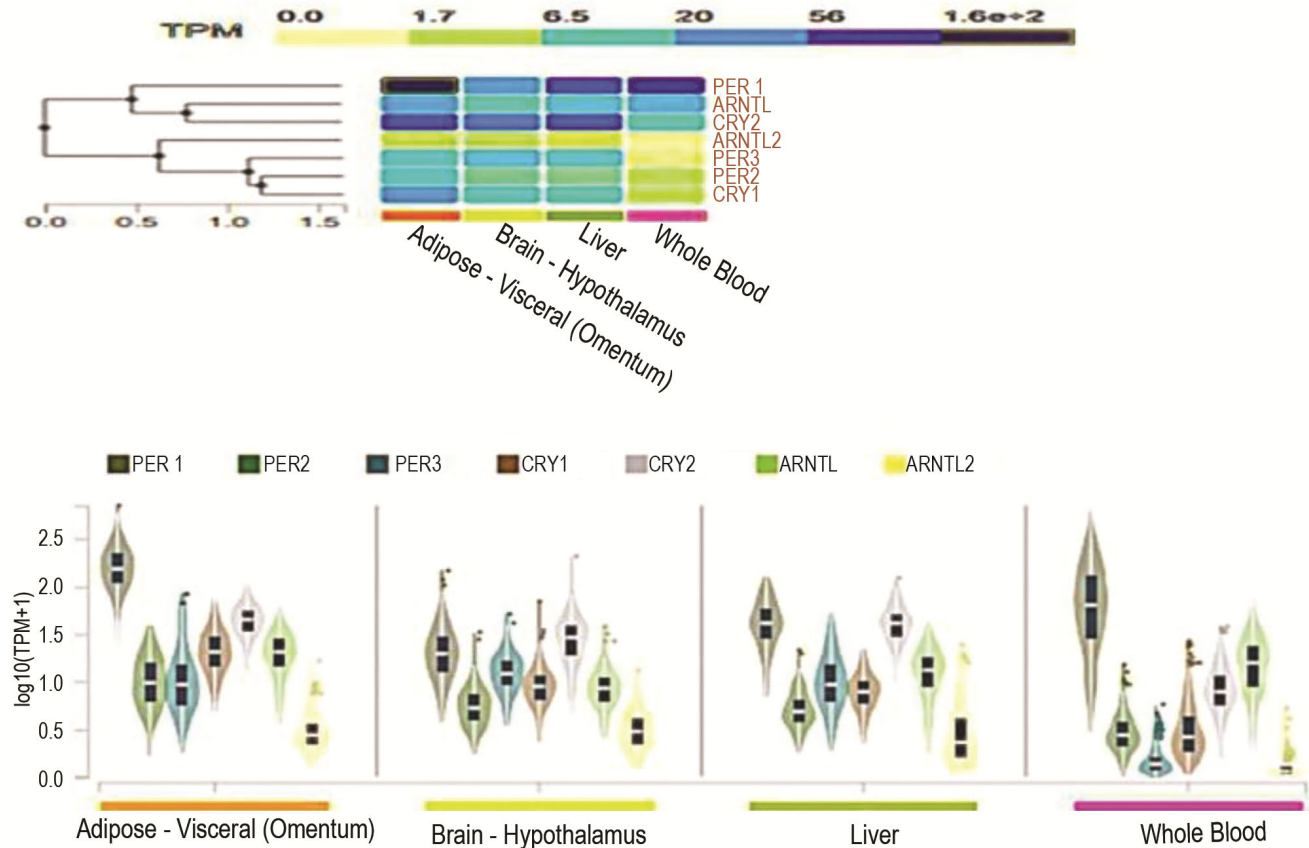


Fig. 4 — Tissue specific expression of core clock genes across various tissues. The top panel shows the heatmap illustrating the relative expression levels of core clock genes in different tissues. Each row represents a gene, and each column represents a tissue. The colour intensity corresponds to the gene expression level. A hierarchical clustering dendrogram shows the similarity between genes based on their expression patterns across tissues. The bottom panel shows the violin plots visualising the distribution of gene expression levels across different tissues. The wider parts of the violin indicate higher density of data points. The black dots represent individual data points.

protein interaction database. The thickness of the lines in the network corresponds to the level of support from various sources, such as databases, experiments, or computational predictions, indicating the strength of protein-protein interactions. An in-depth examination of the network depicted in Fig. 4 reveals a protein-protein interaction (PPI) network connecting core clock components and co-expressed genes associated with circadian regulation within intracellular pathways. The clock-related proteins, originating from diverse pathways, exhibit robust interactions, markedly elevating the intricacy of the PPI network (Fig. 5).

Effect of TRF on HFD feeding regimen

The rate of weight gain per month was markedly higher in the HA group (79.83 g) and HAT group (80 g) compared to the CA (22.04 g) and HT (21.97 g) groups. Notably, HAT animals exhibited the highest rate of weight gain among all groups (Fig. 6A). HA

and HAT groups showed a steeper increase during the initial months, with HAT maintaining the highest body weight from month 3 onwards. In contrast, CA and HT groups demonstrated a more gradual weight gain trajectory (Fig. 6B).

TRF effect on glucose metabolism in different diet composition

The HA group exhibited an elevated blood glucose level (BGL) compared to the control groups ($P=0.0089$). Following TRF intervention in the high-fat diet group (HT), there was a decrease in blood glucose levels, although it did not reach statistical significance. Upon returning these rats to *ad libitum* feeding, there was an increase in BGL. Blood glucose levels were insignificantly reduced in TRF with chow-fed rats, whereas there was a significant increase ($P=0.0094$) in glucose levels in chow-fed rats under *ad libitum* conditions followed by TRF (Fig. 6C).

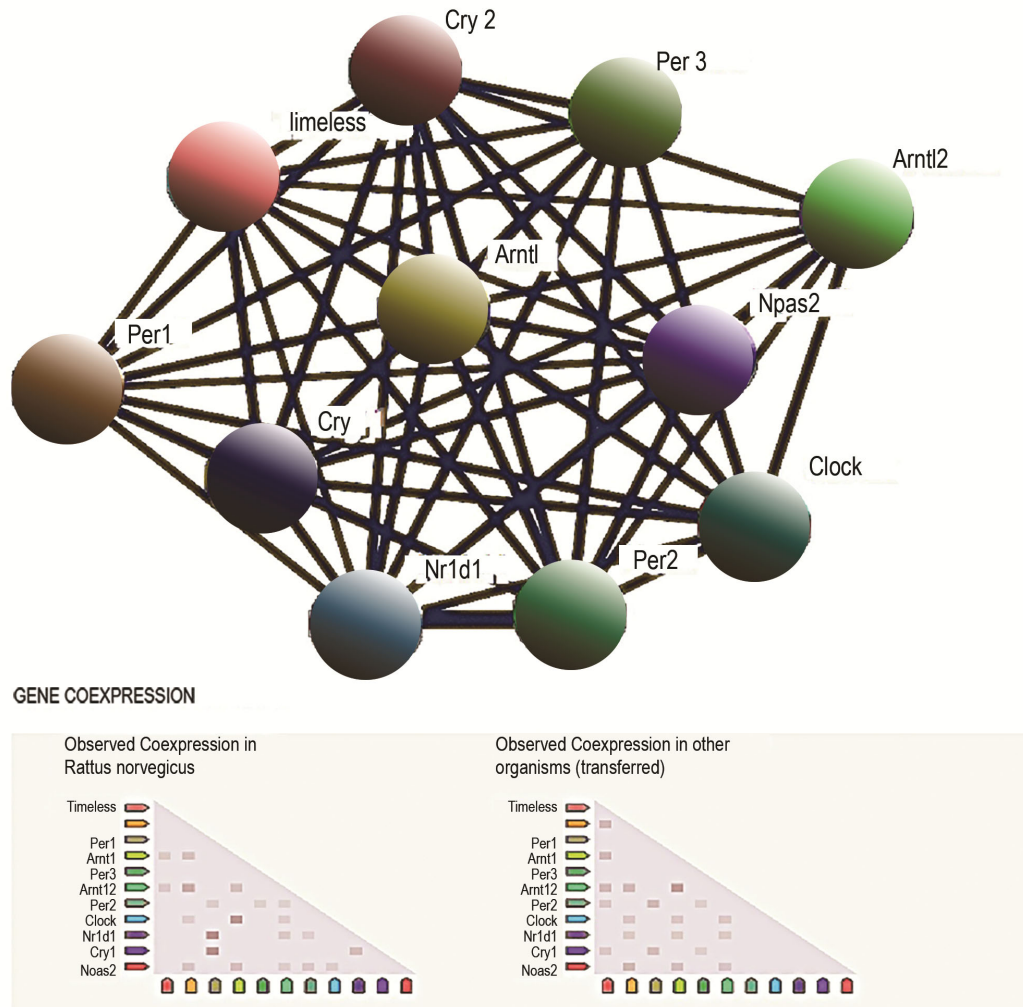


Fig. 5 — Gene co-expression Network of Core Clock Genes. This figure depicts a gene co-expression network illustrating the relationships between core clock genes. Nodes represent individual genes (*Cry1*, *Per1*, *Per2*, *Per3*, *Cry2*, *Bmal1/Arntl*, *Npas2*, *Clock*, *Timeless*, *Nrl*, *Nrd1*), and edges represent significant co-expression relationships between genes. Lines connecting nodes represent significant co-expression relationships.

Insulin homeostasis affected by the TRF on HFD

The group of rats fed a HFD exhibited a reduction in serum insulin levels, and the insulin levels improved when these rats underwent TRF. After TRF intervention in the HT group, when returned to *ad libitum* feeding, the insulin levels decreased, and the data were not statistically significant (Fig. 6D).

Effect of TRF on melatonin on HFD induced obesity

Serum melatonin concentration was significantly higher in the HT group compared to CA, HA, and HAT groups, indicating a potential influence of TRF on circadian hormone regulation. The HA group exhibited the lowest melatonin levels, suggesting that continuous HFD may suppress pineal melatonin synthesis or secretion. CA and HAT groups showed

intermediate values, with HAT displaying slightly higher levels than HA, though still markedly lower than HT. This pattern suggests that TRF, when applied from the start of the experiment (HT), is more effective in maintaining physiological melatonin rhythms compared to TRF initiated after a prolonged period of high-fat feeding (HAT) (Fig. 6E).

Diet and feeding pattern effect on the lipid metabolism-

In the lipid profile analysis, the intake of a HA led to increased levels of total cholesterol, triglycerides (TG) and low-density lipoprotein (LDL), while high-density lipoprotein (HDL) levels decreased compared to the control group. In the HT group, TG and LDL levels decreased, and total cholesterol and HDL levels increased, although the changes were not statistically

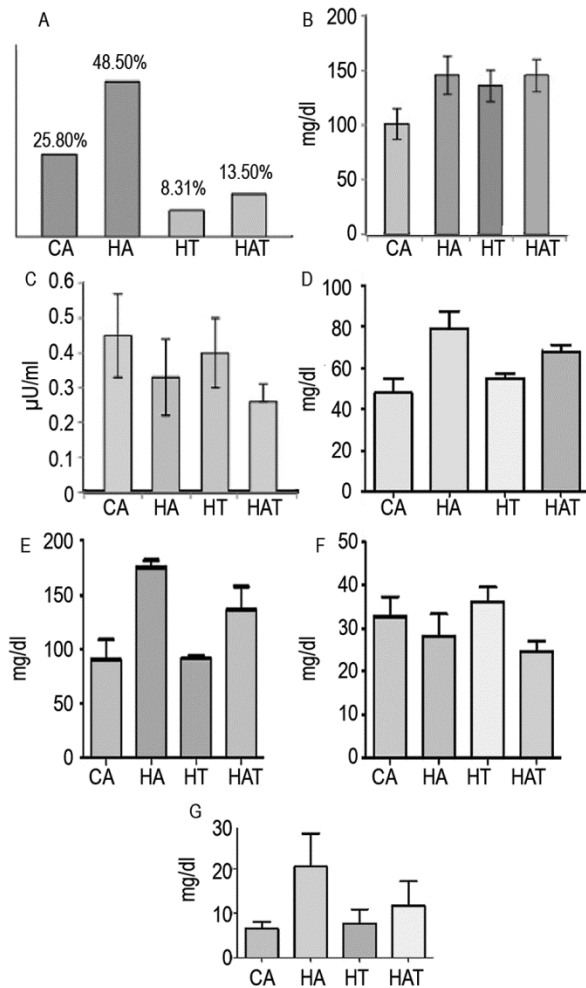


Fig. 6 — Effects of dietary interventions and time-restricted feeding on anthropometric, biochemical, and hormonal parameters in experimental groups. (A) Rate of weight gain per month in control *ad libitum* chow-fed (CA), high-fat diet *ad libitum* (HA), high-fat diet with time-restricted feeding for the entire duration (HT), and high-fat diet with time-restricted feeding introduced after initial *ad libitum* feeding (HAT) groups. (B) Kinetics of body weight changes recorded monthly over six months. (C) Blood glucose levels (BGL) at the end of the experimental period. (D) Serum insulin concentrations. (E) Serum melatonin concentrations, showing the impact of high-fat diet and feeding schedule on circadian hormone regulation. (F&G) Impact of HFD on lipid profile. [Values are presented as mean \pm standard deviation (SD). Group abbreviations: CA – Chow diet *ad libitum*; HA – High-fat diet *ad libitum*; HT – High-fat diet with time-restricted feeding; HAT – High-fat diet with time-restricted feeding after initial *ad libitum* feeding].

significant. In the HAT group, there was a significant increase in both total cholesterol ($P=0.0154$) and HDL ($P=0.0181$), while TG and LDL levels decreased (Fig. 6F & 6G).

Effect of TRF on circadian gene expression in blood

Our study reveals that dietary challenges impact gene expression, with *Per1*, *Cry1*, and *Bmal1* genes

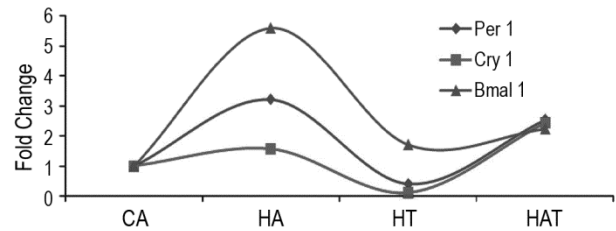


Fig. 7 — Effect of TRF on mRNA Expression of Core Clock Genes (*Per1*, *Cry1*, and *Bmal1*) in blood under different dietary conditions - CA: Chow diet (control), HA: High-fat diet *ad libitum*, HT: High-fat diet with time-restricted feeding, HAT: High-fat diet *ad libitum* after time-restricted feeding. [The data are presented as fold change relative to the CA group. Error bars represent standard error of the mean (SEM)].

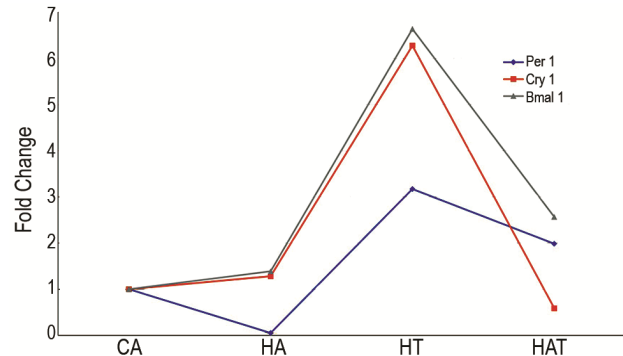


Fig. 8 — Effect of TRF on mRNA Expression of Core Clock Genes in the Hypothalamus. The figure depicts the relative mRNA expression levels of three core clock genes (*Per1*, *Cry1*, and *Bmal1*) in the Hypothalamus under different dietary conditions - CA: Chow diet (control), HA: High-fat diet *ad libitum*, HT: High-fat diet with time-restricted feeding and HAT: High-fat diet *ad libitum* after time-restricted feeding. [The data are presented as fold change relative to the CA group. Error bars represent standard error of the mean (SEM)].

showing upregulation in the HA group. However, rats subjected to a three-month TRF intervention exhibited downregulation of these genes. Upon removal of the TRF intervention, there was a reoccurrence of upregulation in the expression of *Per1*, *Cry1*, and *Bmal1*. Specifically, *Bmal1* expression was significantly increased ($P=0.0003$) in the blood of the HA group (Fig. 7).

Effect of TRF on Hypothalamus of HFD induced obese rats

The examination of tissue-specific expression of circadian rhythm genes unveiled notable changes. In the hypothalamus, *Per1* expression significantly decreased ($P=0.0118$) compared to the control group, while *Cry1* and *Bmal1* were upregulated in rats fed HFD. The introduction of TRF led to an upregulation in the expression of *Per1*, *Cry1*, and *Bmal1* genes, with the *Bmal1* gene displaying significant expression ($P=0.0309$) (Fig. 8).

Discussion

This study provides compelling evidence that implementing a 12-hour feeding period during the active phase is an effective and easily adoptable strategy to prevent obesity and its associated metabolic disturbances. Unlike intensive dietary restriction protocols, TRF does not require caloric counting or food-type limitations, making it more practical for sustained adoption. Consistent with previous reports, our findings suggest that TRF not only limits caloric intake through time gating but also exerts direct metabolic benefits by aligning nutrient availability with endogenous circadian rhythms. Similar benefits have been observed with an 8-hour feeding window, which has been shown to attenuate weight gain and reduce the risk of obesity-associated complications such as insulin resistance, dyslipidemia, and hepatic steatosis¹¹. The protective effects of TRF appear to be relatively robust across feeding window durations, as studies comparing 8, 9, and 12 hours reported comparable benefits in high-fat diet (HFD)-fed animals. Even when TRF adherence is relaxed such as allowing *ad libitum* access on weekends the metabolic improvements remain superior to continuous unrestricted feeding¹¹⁻¹². In our experiment, the magnitude of weight gain suppression was particularly striking: 12-hour TRF limited weight gain to 8.31% in HFD-fed mice, compared to 25.80% in chow-fed *ad libitum* controls and a dramatic 65% in HFD-fed *ad libitum* animals. This gradation underscores a dose response relationship between feeding window length and metabolic benefit, with shorter daily eating windows generally conferring greater protection. Similar effects have been demonstrated in other rodent models, including a 6-week TRF regimen that significantly reduced body weight despite equivalent caloric availability¹³, suggesting that TRF's benefits extend beyond simple energy restriction. The interaction between diet composition and feeding schedule has profound implications for insulin regulation. HFD-induced obesity is well known to impair insulin sensitivity, often via the accumulation of ectopic fat in pancreatic tissue¹⁴. Lipid infiltration into metabolically active organs disrupts insulin receptor signaling and glucose transporter function, leading to systemic hyperglycemia¹⁵. Consistent with this, our HFD-fed rats displayed elevated fasting glucose compared to chow-fed controls¹⁶, a finding that aligns with literature showing that chronic HFD impairs both insulin secretion and insulin action,

thereby increasing the risk of type 2 diabetes mellitus (T2DM)¹⁷. TRF appears to counteract these effects, potentially by promoting periods of low insulin demand during fasting intervals, which may help restore insulin receptor sensitivity and beta-cell responsiveness. In addition to its glycemic benefits, TRF exerts favourable effects on lipid homeostasis. HFD feeding typically elevates total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) while reducing high-density lipoprotein (HDL). In contrast, our TRF regimen improved lipid profiles, lowering TG and LDL while increasing TC and HDL. This is in line with prior studies showing that 9-12 hour TRF reduces TG in obese mice¹² and that a 4-hour TRF can lower TC and raise HDL¹⁸. Mechanistically, TRF has been shown to downregulate hepatic lipogenic transcription factors, notably PPAR γ ¹⁹, while sustaining normal lipid oxidation and storage patterns²⁰. However, the literature also reveals variability in TRF's effects on lipid parameters, likely due to differences in macronutrient composition, feeding duration, and species studied. This variability underscores the need for mechanistic studies dissecting how TRF influences lipid metabolism at the transcriptional and post-transcriptional levels. Our results also highlight a novel link between TRF, melatonin secretion, and circadian regulation. HFD-fed rats exhibited significantly reduced melatonin levels compared to chow-fed controls, consistent with reports that dietary fat intake suppresses pineal melatonin synthesis²¹ and alters pituitary clock gene expression²². Given melatonin's role in lipid metabolism where pinealectomy increases fat deposition by enhancing lipogenesis²³, this reduction may contribute to HFD-induced adiposity. Importantly, our 12-hour TRF regimen restored melatonin secretion, suggesting that feeding fasting cycles may resynchronize pineal function. This is supported by previous mouse studies reporting a twofold melatonin increase with TRF, attributed to enhanced pineal receptor activity during the restricted feeding phase.

At the molecular level, we found that HFD disrupted the expression of core circadian genes *Per1*, *Cry1*, and *Bmal1* in a tissue-specific manner, with TRF partially reversing these changes. Jamshed *et al.*²⁴ similarly observed time-of-day-dependent modulation of *Bmal1* and *Per1* under TRF, indicating that feeding windows act as potent zeitgebers (time cues) for peripheral clocks. In our study, blood

(a proxy for peripheral clocks) in the HA group displayed marked upregulation of all three genes compared to chow-fed controls, suggesting systemic circadian activation under sustained HFD. TRF (HT group) attenuated this activation, consistent with the hypothesis that restricted feeding realigns peripheral clocks through altered nutrient and hormonal cues¹²⁻²⁴. Upon returning to *ad libitum* HFD (HAT), *Per1* and *Cry1* expression rebounded, highlighting the reversibility of TRF's effects in peripheral tissues. Interestingly, the hypothalamus central circadian pacemaker responded differently. HFD selectively increased *Cry1* and *Bmal1* expression without altering *Per1*. TRF further elevated all three genes, particularly *Bmal1*, suggesting reinforcement of SCN-driven transcriptional rhythms and improved central peripheral synchrony. Even after TRF withdrawal, hypothalamic expression remained above control levels, implying a memory effect of TRF entrainment, as described in earlier rodent studies¹¹. This differential regulation supports the concept that peripheral clocks are more acutely sensitive to feeding cues, while central clocks are more resistant to rapid changes and retain entrainment signals for longer²⁵. Nutrient-sensitive signaling pathways may explain the observed gene expression changes. During fasting, AMP-activated protein kinase (AMPK) is activated, promoting degradation of CRY proteins and thereby preventing repression of CLOCK:BMAL1 targets such as *Rev-erba*, *Per*, and *Cry*²⁶. Conversely, feeding activates the mechanistic target of rapamycin (mTOR) pathway, which influences *Per* gene transcription²⁷. Perturbations in these pathways, as seen in maternal HFD models, can downregulate CLOCK, BMAL1, REV-ERB, PER, and CRY in offspring tissues²⁸, predisposing them to metabolic disorders such as non-alcoholic fatty liver disease²⁹. Moreover, genetic disruption of BMAL1 or CLOCK has been shown to lower glucose and TG levels³⁰, underscoring the tight interplay between circadian regulation and metabolic homeostasis.

Conclusion

Our research revealed that a 12-hour TRF protocol significantly alleviates the detrimental metabolic and circadian impacts of HFD in obese Wistar rats. TRF markedly diminished body weight increase, blood glucose, triglycerides, and LDL cholesterol, while elevating insulin, melatonin, total cholesterol, and HDL levels. At the molecular level, TRF regulated the

expression of essential circadian clock genes *Per1*, *Cry1*, and *Bmal1* in both peripheral (blood) and central (hypothalamus) tissues, partially ameliorating HFD-induced disturbances and improving central/peripheral clock synchrony. The effects were most significant when TRF was initiated early and were partially preserved even after reverting to *ad libitum* feeding, indicating a legacy effect. Our findings endorse TRF as an effective, non-pharmacological strategy that simultaneously enhances metabolic health and realigns circadian rhythms in diet-induced obesity.

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Conflict of interest

The authors have no conflict of interest to declare.

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