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Metabolomic and Proteomic Signatures of Circadian Syndrome by Metabolic Bariatric Surgery Status in QPHI Participants: A cross-sectional analysis

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Abstract

Background

Circadian Syndrome (CircS) combines metabolic risk factors with sleep and depressive symptoms. Metabolic Bariatric surgery (MBS) improves several CircS components, but the molecular features associated with CircS after surgery remain poorly understood. This study examined plasma metabolomic and proteomic signatures of CircS according to MBS status among Qatar Precision Health Institute (QPHI) participants.

Methods

This cross-sectional study included QPHI participants with CircS classification and plasma metabolomic (n = 211; 62 MBS, 149 no-MBS) or proteomic (n = 148; 59 MBS, 89 no-MBS) data. CircS was defined by the presence of ≥ 4 of seven components: central obesity, elevated triglycerides, reduced HDL-cholesterol, elevated blood pressure, elevated blood glucose, short sleep duration, and depressive symptoms. Age- and sex-adjusted linear models were used to assess differential molecular abundance for two contrasts: (1) CircS vs. no-CircS within the MBS group and (2) CircS vs. no-CircS within the no-MBS group. Significance was determined using the Benjamini-Hochberg false discovery rate (FDR).

Results

Within the MBS group, CircS was significantly associated with higher levels of inosine 5'-monophosphate (IMP; $\log_2$FC = 2.94, p_{adj} = 0.01) and keratin 18 (KRT18; $\log_2$FC = 1.25, p_{adj} = 0.048). Within the no-MBS group, 12 metabolites, but no proteins, remained significant after FDR correction, primarily involving amino acid and phospholipid metabolism.

Conclusions

This multi-omics analysis reveals distinct molecular signatures of CircS according to MBS status. In participants with MBS, CircS was associated with alterations in purine metabolism (IMP) and an epithelial cell marker (KRT18). In contrast, the no-MBS group showed broader metabolic associations spanning amino acid and plasmalogen metabolism. These findings suggest that the molecular associations of CircS differ according to MBS status; given the cross-sectional design, independent longitudinal studies are needed to test whether MBS alters these pathways.

1. Introduction

The circadian system is a fundamental biological timing network regulating numerous aspects of human physiology in approximately 24-hour cycles (1). At the center of this system is the suprachiasmatic

nucleus of the hypothalamus, which synchronizes peripheral clocks throughout the body and coordinates essential physiological functions, including the sleep–wake cycle, hormone secretion, body temperature regulation, energy metabolism, and immune responses (1, 2). The circadian system plays a critical role in maintaining metabolic homeostasis through the regulation of gene expression, hormonal release, activity patterns, and energy expenditure (3).

Disruption of normal circadian rhythms contributes to the development of several metabolic disorders. This association has led to the concept of Circadian Syndrome (CircS), which expands the traditional definition of metabolic syndrome by including sleep disturbances, depression, non-alcoholic fatty liver disease, and cognitive dysfunction alongside the conventional metabolic risk factors of central obesity, dyslipidemia, hypertension, and hyperglycemia (1). Rather than viewing these metabolic conditions independently, CircS proposes that circadian disruption is a shared underlying mechanism driving their coexistence (4).

Circadian disruption can impair metabolic health by affecting glucose regulation, lipid metabolism, inflammatory processes, and neuroendocrine function (5, 6). These disturbances are increasingly driven by modern lifestyle factors, including prolonged exposure to artificial light, shift work, chronic psychosocial stress, travel across time zones, continuous food availability, and changes in dietary habits (1). Collectively, these lifestyle-related disruptions to the circadian system are thought to contribute substantially to the growing global burden of obesity, type 2 diabetes, and cardiovascular disease (CVD) (7). Accordingly, CircS has been linked to increased risk of CVD (8), stroke (9), asthma (10), gout (11), kidney stones (12), as well as all-cause mortality (10). Metabolic bariatric surgery (MBS) induces substantial metabolic and hormonal shifts that improve several comorbidities associated with CircS such as obesity (13), insulin resistance (14), hypertension (15), dyslipidemia (16) and sleep disturbances (17). Despite these improvements, the molecular mechanisms underlying CircS following MBS remain poorly understood.

Recent developments in high-throughput omics technologies have enabled more comprehensive investigations into the molecular mechanisms underlying complex metabolic disorders (18). Understanding these molecular mechanisms has important clinical implications, as it may facilitate the identification of biomarkers for early detection and risk stratification, improve disease monitoring, and reveal potential therapeutic targets for more personalized interventions.

While metabolomic and proteomic alterations have been studied in obesity (19), diabetes (20), and metabolic syndrome (21), little is known about biomarker association with CircS. To the best of our knowledge, no previous study has examined metabolomic and proteomic profiles in relation to CircS. Therefore, this study aims to identify metabolic and proteomic biomarkers associated with CircS among Qatar Precision Health Institute (QPHI) participants and to determine how those associations differed by MBS status.

2. Methods

2.1. Study Design and Participants

This cross-sectional secondary analysis used QPHI data. Participants were selected based on documented MBS history, CircS status, and available plasma for the relevant omics assay. Of the 26,028 QPHI participants at baseline, 211 met these criteria for metabolomics, and 148 of these also had proteomic data, as proteomic profiling was performed on a subset of the metabolomics samples (Fig. 1). The metabolomics cohort comprised 62 MBS (6 CircS, 56 no-CircS) and 149 no-MBS (63 CircS, 86 no-CircS) participants; the proteomics subset comprised 59 MBS (6 CircS, 53 no-CircS) and 89 no-MBS (23 CircS, 66 no-CircS) participants. Plasma was collected by QPHI, isolated per standard protocols, and stored at − 80°C.

2.2. Circadian syndrome classification

CircS was defined per our previous study (22) as meeting ≥ 4 of 7 components: (1) central obesity (Waist circumference ≥ 102 cm in men, ≥ 88 cm in women); (2) elevated fasting blood glucose (≥ 100 mg/dL) or using antidiabetic medication; (3) reduced HDL-C (< 40 mg/dL in men, < 50 mg/dL in women), or using lipid-lowering medication; (4) elevated blood pressure (systolic ≥ 130 mmHg, diastolic ≥ 85 mmHg) or using antihypertensive medication; (5) elevated triglycerides (TG) (≥ 150 mg/dL fasting, ≥ 175 mg/dL non-fasting) (23), or using lipid-lowering medication; (6) short sleep duration (< 6 h/day); and (7) depressive symptoms (PHQ-9 score ≥ 5) (24).

2.3. Ethical Approval

The study followed the Declaration of Helsinki and was approved by the Institutional Review Board (QF-QBB-RES-ACC-00254-0340). All participants provided written informed consent before enrollment.

2.4. Metabolomics Protocol

Non-targeted metabolomic profiling was performed by QPHI using the Metabolon global platform (Metabolon, Inc., Morrisville, NC, USA) with UHPLC-MS/MS. Extracts were analysed using four LC-MS/MS methods (two positive, two negative ion modes). Metabolites were identified by matching mass spectra and retention times to Metabolon's proprietary library. Data were normalized for batch and run-day variation and log2-transformed (25).

2.5. Proteomics Protocol

Plasma proteomic analysis was performed by QPHI using the SOMAscan® assay (SomaLogic, Inc., Boulder, CO, USA) per the manufacturer's protocols. Samples were incubated with SOMAmer® reagents, unbound proteins removed by bead-based capture, and bound analytes quantified by DNA-based

fluorescence. Data were normalized per SomaLogic's standard procedures; protein levels were reported as relative fluorescent units on a log2 scale (25).

2.6 Functional Analysis

Metabolite pathway enrichment used MetaboAnalyst v6.0. Differentially abundant metabolites were mapped to HMDB identifiers and analysed by Metabolite Set Enrichment Analysis using over-representation analysis (ORA), which queries 15 metabolite set collections (~13,000 sets). Enriched sets were visualized as bubble plots ($p < 0.05$).

Protein pathway predictions used Ingenuity Pathway Analysis (IPA; QIAGEN). Proteins with $p < 0.05$ were uploaded, and canonical pathway z-scores were calculated; pathways with enrichment $p < 0.05$ and $|z| \geq 2$ were considered significantly activated ($z \geq 2$) or inhibited ($z \leq -2$) (26).

2.7. Statistical Analysis

For each omics layer, limma linear models with empirical-Bayes moderation were adjusted for age and sex. Four prespecified contrasts were estimated: (1) CircS vs. no-CircS within MBS group; (2) CircS vs. no-CircS within no-MBS group; (3) MBS versus no-MBS among CircS group; and (4) MBS versus no-MBS among no-CircS group . The first two were primary; the latter two were estimated for completeness and visualized in the overlap analysis (Supplementary File 3, Figure S1). Positive $\log_2$FC reflects increased expression in the first-listed group. Volcano plots were generated with ggplot2. Benjamini-Hochberg FDR (p_{adj}) < 0.05 was the primary threshold; raw $p < 0.05$ findings were retained for exploratory visualization, overlap analysis, hierarchical clustering, and pathway screening. $|\log_2 FC| > 2$ was used to label extreme points. Analyses used R v4.4.1.

3. Results

3.1. Participant characteristics

General characteristics of participants based on the availability of metabolomics and proteomics data are presented in Tables 1 and 2, respectively. Among the MBS group, six participants presented with CircS, and these same participants were included in both the metabolomics and proteomics analysis. In the no-MBS group, 63 participants with CircS underwent metabolomics analysis, and 23 of these also had proteomics data (Figure 1) (Tables 1 and 2). Among 211 participants with metabolomics data, the mean age was 39 (SD: 10) in MBS group and 44 (SD: 11) in no-MBS group, whereas the mean age among those with proteomics data was 38 years (SD: 10) in MBS group and 39 (SD: 9) in no-MBS group. In the MBS group, females with CircS outnumbered males in both omics datasets. In contrast, in the no-MBS group, males were more prevalent. Regarding smoking status, none of the MBS participants with CircS

were current smokers, whereas no-MBS group had few current smokers (10 with metabolomics and 5 with proteomics data).

As expected from the CircS definition, participants with CircS were older than those without CircS in both the metabolomics cohort (MBS: 51 vs 37 years; no-MBS: 50 vs 40 years) and proteomics cohort (MBS: 51 vs 37 years; no-MBS: 43 vs 37 years). In the metabolomics cohort, CircS participants when compared to no-CircS participants in both the MBS and no-MBS groups had a higher prevalence of reduced HDL-C (100% vs 23%; 83% vs 35%), elevated triglycerides (67% vs 13%; 79% vs 14%), elevated blood pressure (33% vs 11%; 70% vs 13%), elevated glucose (50% vs 7%; 73% vs 27%), central obesity (50% vs 27%; 79% vs 47%), and short sleep duration (100% vs 59%; 79% vs 52%). Similar patterns were observed in the proteomics cohort. These descriptive differences were not interpreted as independent associations because many variables contributed directly to CircS classification. Other baseline characteristics including sex distribution, smoking status, physical activity levels, and dietary patterns, were broadly similar between CircS and no-CircS groups.

To provide an exploratory overview of the differential analytes across the four contrasts, we examined the overlap of significantly associated metabolites and proteins (p-value < 0.05) as presented in Supplementary File 3 Figure S1. Majority of significant findings were unique to each contrast, with only a limited number of analytes shared between them. Notably, there was no metabolite or protein which was significantly associated across all four contrasts.

3.2. Molecular Features Associated with CircS Among Participants with MBS

Metabolomics

Metabolomics analysis identified 52 metabolites that were statistically significant (p-value < 0.05), with 27 upregulated and 25 downregulated in the MBS group when comparing CircS with no-CircS (Figure 2A). Of these, only Inosine 5'-monophosphate (IMP) remained significantly upregulated in CircS after FDR correction ($\log_2$FC 2.94, $p = 1.14\times10^{-5}$, $p_{adj} = 0.01$). These findings should be interpreted cautiously given the very small number of participants with CircS in the MBS group (n = 6), which may limit statistical stability and generalizability despite the use of FDR correction.

With respect to effect size, three metabolites, IMP, carboxyibuprofen, and lithocholic acid sulfate exceeded a $\log_2$ fold change threshold of 2, reflecting more than a 4-fold difference between CircS and

no-CircS participants. However, Carboxyibuprofen (log_2FC = 8.08) and lithocholic acid sulfate (log_2FC = 2.73), were only significant and did not survive multiple-testing correction (Figure 2A) (Supplementary File 2, Table S1).

To further explore the overall molecular patterns, hierarchical clustering was performed (Supplementary File 3, Figure S2a). However, this multivariate approach did not distinguish between CircS and no-CircS participants, consistent with these differences being subtle. The high inter-individual heterogeneity within the MBS group appears to exert a stronger influence on the metabolic landscape than CircS status alone.

Of the 52 significant metabolites, 23 were successfully annotated with HMDB identifiers. These annotated metabolites were submitted to over-representation analysis (ORA), which identified four enriched metabolite sets, of which only purine metabolism reached statistical significance (p <0.05). Caffeine metabolism showed the largest enrichment ratio, although it did not reach the significance threshold. Pathway enrichment results are presented in Figure 3A.

Proteomics

Proteomics analysis identified 92 proteins that were statistically significant (p-value < 0.05), with 31 upregulated and 61 downregulated in the MBS group when comparing CircS with no-CircS (Figure 2C). Similar to metabolomics findings, only one protein, KRT18, remained significantly higher in CircS after FDR correction (log_2FC = 1.25, $p = 3.7\times10^{-5}$, p_{adj} = 0.048). However, this interpretation should be made cautiously given the limited CircS-MBS sample size (n = 6), which may increase the influence of individual-level variation on the observed associations. With respect to effect size, two proteins, HAMP (log_2FC = 2.03) and FCGR2A (log_2FC = −2.34), exceeded a log_2 fold change threshold of 2, reflecting more than a 4-fold difference between CircS and no-CircS participants, but neither achieved FDR-adjusted significance (Supplementary File 2, Table S5).

To further explore these findings, hierarchical clustering was performed (Supplementary File 3, Figure S3a). In contrast to the metabolomics data, this analysis revealed distinct groups of co-varying proteins, with clear separation between CircS and no-CircS participants within the MBS group.

In contrast to the metabolite ORA, which identifies enriched pathways based on metabolite counts, IPA predicts activation or inhibition of canonical pathways based on the directional patterns of input proteins (Figure 3C). These pathway-level findings are exploratory and hypothesis-generating, as the input was limited to significant proteins at p < 0.05, irrespective of FDR correction, and only KRT18 survived FDR correction in this group (Figure 3C). All pathways exceeding the |z| > 2 threshold were predicted to be inhibited, including Fcγ receptor-mediated phagocytosis (z = −3.46), ERK/MAPK signaling (z = −3.46), and insulin secretion signaling (z = −3.32), with additional pathways related to VEGF, thrombin, mTOR, PI3K/AKT, growth hormone, IGF-1, and insulin receptor signaling also predicted to be inhibited.

PPARα/RXRα activation was the only pathway with a positive z-score (z = 0.71) but did not exceed the threshold of +2. The complete list of predicted pathways is provided in Figure 3C.

3.3. Molecular Features Associated With CircS Among Participants With MBS

Metabolomics

Metabolomics analysis identified 141 metabolites that were statistically significant (*p*-value < 0.05), with 56 upregulated and 85 downregulated in the no-MBS group when comparing CircS with no-CircS (Figure 2B). Of these, Of these, 12 metabolites remained significant after FDR correction (5 upregulated, 7 downregulated; Supplementary File 2, Table S2). The upregulated metabolites included glutamate, aspartate, and two GPE plasmalogen species, while the downregulated metabolites included glycerate, threonate, N-acetylglycine, oxalate, two GPC plasmalogens, and an unidentified metabolite (X-12216). With respect to effect size, three metabolites, cetirizine ($log_2FC = -5.95$), metronidazole ($log_2FC = 5.27$), and norfluoxetine ($log_2FC = -2.03$), exceeded a log_2 fold-change threshold of 2, but none survived FDR correction (Figure 2B).

Hierarchical clustering did not reveal clear separation between CircS and no-CircS participants, which may reflect inter-individual variability within the no-MBS group (Supplementary File 3, Figure S2b). Of the 141 significant metabolites, 76 were annotated with HMDB identifiers and submitted to ORA, which identified 25 enriched pathways, of which 15 reached statistical significance ($p < 0.05$) (Figure 3B). The significant pathways were predominantly related to amino acid and nitrogen metabolism, including arginine and proline metabolism, glycine and serine metabolism, the urea cycle, alanine/aspartate/glutamate metabolism, glutathione metabolism, and phenylalanine and tyrosine metabolism, with additional pathways including phospholipid biosynthesis, purine metabolism, and thyroid hormone synthesis.

Proteomics

Proteomics analysis identified 132 proteins that were statistically significant (*p*-value < 0.05), with 64 upregulated and 68 downregulated in the no-MBS group when comparing CircS with no-CircS (Figure 2D). Unlike metabolomics, no protein remained significant after FDR correction. With respect to effect size, no protein exceeded a log_2 fold change of 2 with FCN3 demonstrating an effect size over 1 ($log_2FC = 1.56$), corresponding to approximately a 3-fold difference (Supplementary File 2, Table S6).

Hierarchical clustering revealed distinct groups of co-varying proteins; however, CircS and no-CircS samples did not separate into distinct clusters, consistent with considerable within-group heterogeneity (Supplementary File 3, Figure S3b).

Pathway-level findings are exploratory and hypothesis-generating, as the input was limited to significant proteins at $p < 0.05$, irrespective of FDR correction. In contrast to the MBS group, the no-MBS group showed predominantly predicted pathway activation (Figure 3D): IL-10 signaling and NAFLD signaling exceeded the $|z| > 2$ threshold, and several additional pathways showed positive z-scores below this threshold, including neutrophil degranulation, cytokine signaling, complement cascade, and hepatic fibrosis signaling. A smaller set of pathways was predicted to be inhibited, most notably IGF transport and uptake by IGFBPs ($z = -1.73$), RAF/MAP kinase cascade, and PI3P-AKT signaling (both $z = -1.34$).

4. Discussion

To our knowledge, this is the first study to identify metabolomic and proteomic markers associated with CircS in participants with and without MBS. In the MBS group, CircS was associated with elevated IMP and KRT18, while in the no-MBS group, 12 metabolites, mainly amino acids and lipids, were associated with CircS. Our findings show that CircS is associated with distinct metabolomic and proteomic markers in participants with and without MBS, suggesting that the biological markers associated with CircS may differ according to MBS status. This study provides insight into the biological changes associated with CircS, which is currently identified primarily based on clinical features such as obesity, elevated blood glucose, abnormal blood lipid levels, poor sleep, hypertension, and depressive symptoms. These observations may support future identification of CircS biomarkers and help explain persistent metabolic and circadian problems after MBS.

One of the most notable findings was the association of IMP with CircS specifically among participants who had undergone MBS. IMP is an intermediate metabolite in the purine metabolism, serving as a precursor for the synthesis of adenosine and guanine nucleotides, which are essential for DNA and RNA production (27). Several recent studies have also reported increased IMP levels in individuals with CircS and following MBS (27-29). This finding may be particularly relevant to CircS, as purine metabolism is closely linked to the circadian clock. IMP dehydrogenase (IMPDH), a key enzyme in purine metabolism that utilizes IMP, is regulated by circadian rhythms. Therefore, disruption of the circadian cycle may alter purine metabolism and contribute to changes in IMP levels (27).

In healthy individuals, IMP is rapidly converted to downstream products such as adenosine 5'-monophosphate (AMP) and guanosine 5'-monophosphate (GMP), maintaining balanced IMP levels (27, 28). In CircS, circadian clock disruption may dysregulate these pathways, resulting in IMP accumulation (27). Following MBS, rapid weight loss and adipose tissue breakdown may further increase circulating purines (27, 29). Our finding that IMP was significantly elevated only in the MBS-CircS group is consistent with this proposed mechanism and suggests that IMP may be a biomarker of the combined effects of circadian disruption and post-surgical metabolic changes.

Elevated KRT18 levels further point to a potential link between CircS and epithelial cell regulation. KRT18 is an epithelial cell protein released during cell death and has been associated with hepatocyte death and cancer progression (30, 31). Although associations between KRT18 levels and CircS or MBS have

not yet been reported in human studies, Krt18 expression shows circadian regulation in the mouse intestinal epithelium (32). In mice, the circadian clock regulates intestinal epithelial cell differentiation and KRT18 expression through BMAL1-mediated regulation of Hes1, a key component of NOTCH signaling (32). Disruption of this circadian regulatory pathway may therefore alter epithelial cell homeostasis and could contribute to the elevated KRT18 levels observed in our study, although this remains speculative and was not directly assessed in our study.

CircS among participants who had not undergone MBS was associated with a distinct metabolomic profile, characterized by increased levels of some amino acid and lipid derivatives and decreased levels of others. Notably, glutamate and aspartate, which were elevated in our study, have been identified as circadian metabolites (27). Li, Li (27) reported that glutamate and aspartate exhibited time-dependent fluctuations in zebrafish, reaching peak levels at 13 and 1 hour after light onset, respectively. This suggests that circadian disruption may alter the timing of these metabolic rhythms, potentially contributing to differences in their circulating levels. Although direct evidence linking these metabolites to CircS in individuals with obesity without MBS remains limited, their associations with individual clinical components of CircS may provide further biological context for our findings.

Elevated blood glucose in our study, a clinical component of CircS, may further explain the higher glutamate levels observed in our study. Glutamate and aspartate have been reported to be elevated in type 2 diabetes (33), while a systematic review identified an association between elevated glutamate and diabetes risk (34). Mechanistically, glutamate is involved in glucose-stimulated insulin secretion in pancreatic β-cells, including signaling through mGluR5 receptors (35). However, prolonged hyperglycemia may disrupt glutamate homeostasis and contribute to β-cell dysfunction through excessive NMDA receptor activation (35). Hypertension, another component of CircS, may also be relevant, as circulating glutamate has been positively associated with both systolic and diastolic blood pressure (36). **Importantly, glutamate has also been identified as a circadian metabolite** (27)**, providing a potential link between the circadian and metabolic disturbances underlying CircS.** Thus, elevated glutamate in CircS may reflect the interplay between circadian disruption and metabolic abnormalities, including hyperglycemia, hypertension, and obesity.

In addition to purine and amino acid metabolism discussed above, our findings also point to changes in lipid metabolism in CircS. Several plasmalogen species, including 1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1) and 1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1), were reduced in participants with CircS in the no-MBS group. Plasmalogens are a unique class of glycerophospholipids characterized by a vinyl ether bond at the sn-1 position and play important roles in maintaining cell membrane structure and function, and this bond also makes them particularly susceptible to oxidative degradation (37, 38).

Previous studies have reported reduced plasmalogen levels under conditions of oxidative stress (37, 38). Notably, Chua, Shui (38) observed significant reductions in several choline plasmalogens following 40 hours of total sleep deprivation. Sleep deprivation can increase reactive oxygen species, while plasmalogens act as endogenous antioxidants by scavenging these molecules, leading to their oxidation

and depletion (37, 38). Given that sleep disturbance is a component of CircS, the lower plasmalogen levels observed in our no-MBS CircS group may reflect increased oxidative stress. Together with the alterations in purine, amino acid, and other lipid metabolites, these findings suggest that CircS is associated with broader metabolic disturbances involving oxidative stress and metabolic dysfunction.

Taken together, these findings suggest distinct molecular patterns of CircS according to MBS status. In the MBS group, elevated IMP and KRT18 point to potential alterations in purine metabolism and epithelial cell processes. In contrast, the no-MBS group showed broader alterations in amino acid and lipid metabolism, including glutamate, aspartate, and plasmalogens. Overall, these molecular changes suggest that CircS involves interconnected metabolic function, and oxidative disturbances, with patterns that may differ according to MBS status.

Several limitations should be considered. First, only six participants with CircS were available in the MBS subgroup for both metabolomic and proteomic analyses. This substantially limits statistical power, increases uncertainty around effect estimates, and reduces the generalizability of the findings. Although IMP and KRT18 remained significant after FDR correction, the possibility that these associations were influenced by individual-level variability cannot be excluded. Therefore, the MBS-related findings should be considered exploratory and require confirmation in larger independent cohorts. Second, although models were adjusted for age and sex, residual confounding cannot be excluded. Factors such as adiposity, medication use, diabetes status, smoking, dietary habits, and time since surgery may influence circulating metabolomic and proteomic profiles, and the small number of MBS participants with CircS precluded broader covariate adjustment without risking model overfitting. Third, the interval between MBS and baseline assessment varied considerably (approximately 1–13 years), meaning participants were at different post-surgical stages and may have experienced changes in weight and metabolic status. Finally, biomarkers were measured at a single time point. Given the dynamic nature of circadian rhythms, a single measurement cannot determine altered metabolite rhythmicity or distinguish the effects of circadian disruption from other contributing factors.

Despite these limitations, this study has several strengths. A key strength is the integrated analysis of metabolomic and proteomic data to investigate CircS separately in individuals with and without MBS. This approach identified distinct molecular patterns that may have been masked if both groups were analyzed together. Several findings were also biologically consistent with previous evidence linking purine metabolism, glutamate, aspartate, and plasmalogens with circadian and metabolic processes. In contrast, the association of circulating KRT18 with CircS has limited previous evidence and provides a hypothesis for investigating epithelial pathways in CircS following MBS.

Clinically, CircS is largely characterized by using clinical and behavioral features. Molecular markers may complement these measures by providing insight into the underlying biological processes and heterogeneity of CircS, particularly according to MBS status. However, the markers identified here require further validation before their potential clinical utility can be established.

Future studies should include larger MBS and no-MBS cohorts and validate these findings in independent populations. Standardizing the interval between MBS and sample collection would improve comparability, while longitudinal sampling at predefined post-surgical intervals could determine how molecular profiles and CircS status change over time.

Conclusion

In conclusion, this study provides multi-omics investigation of Circadian Syndrome (CircS) according to metabolic bariatric surgery (MBS) status. CircS was associated with distinct molecular profiles in the MBS and no-MBS groups with elevated inosine 5'-monophosphate (IMP) and keratin 18 (KRT18) in the MBS group vs broader amino acid and lipid alterations in the no-MBS group. These findings suggest that similar clinical features of CircS may be underpinned by different molecular alterations depending on MBS status. Integrating molecular markers with clinical features may improve the biological characterization of CircS and help identify subgroups not captured by clinical assessment alone, although these markers warrant validation in larger, independent cohorts before their clinical utility can be established.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of Qatar Precision Health Institute (QPHI) (approval number: QF-QBB-RES-ACC-00254-0340). All participants provided written informed consent before enrollment.

Authors' contributions

S.Z. performed the data analysis and contributed to data interpretation. S.L. performed the descriptive analyses and contributed to writing. Z.S and A.N. conceptualized the underlying work and contributed to the study design. S.Z. and A.N. drafted the initial manuscript. All authors critically reviewed and revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

References

1. Zimmet P, Alberti K, Stern N, Bilu C, El-Osta A, Einat H, et al. The Circadian Syndrome: is the Metabolic Syndrome and much more! J Intern Med. 2019;286(2):181–91.

2. Liu C, Tang X, Gong Z, Zeng W, Hou Q, Lu R. Circadian Rhythm Sleep Disorders: Genetics, Mechanisms, and Adverse Effects on Health. Frontiers in Genetics. 2022;Volume 13–2022.
3. Kunze KN, Hanlon EC, Prachand VN, Brady MJ. Peripheral circadian misalignment: contributor to systemic insulin resistance and potential intervention to improve bariatric surgical outcomes. Am J Physiol Regul Integr Comp Physiol. 2016;311(3):R558-63.
4. Zheng K, Wu M, Cao Y, Wang J, Zhu H, Li B, et al. Circadian syndrome and mortality risk in adults aged ≥ 40 years: a prospective cohort analysis of CHARLS and NHANES. Scientific Reports. 2025;15(1):14791.
5. Hou Y, Liu L, Chen X, Li Q, Li J. Association between circadian disruption and diseases: A narrative review. Life Sci. 2020;262:118512.
6. Fishbein AB, Knutson KL, Zee PC. Circadian disruption and human health. J Clin Invest. 2021;131(19).
7. Crnko S, Du Pre BC, Sluijter JPG, Van Laake LW. Circadian rhythms and the molecular clock in cardiovascular biology and disease. Nat Rev Cardiol. 2019;16(7):437–47.
8. Shi Z, Tuomilehto J, Kronfeld-Schor N, Alberti G, Stern N, El-Osta A, et al. The Circadian Syndrome Is a Significant and Stronger Predictor for Cardiovascular Disease than the Metabolic Syndrome-The NHANES Survey during 2005–2016. Nutrients. 2022;14(24).
9. Wang Y, Yang L, Zhang Y, Liu J. Relationship between circadian syndrome and stroke: A cross-sectional study of the national health and nutrition examination survey. Front Neurol. 2022;13:946172.
10. Zhang S, Lin J. Association of circadian syndrome and lung health: A population-based cohort study. Respir Med. 2025:108031.
11. Ren JW, Wang JH, Xiao YF, Bai YJ, Han P. Associations of circadian syndrome with gout and hyperuricemia: a cross–sectional analysis of NHANES 2007–2018. BMC Public Health. 2025;25(1):2152.
12. Xiao Y, Yin S, Bai Y, Yang Z, Wang J, Cui J, et al. Association between circadian syndrome and the prevalence of kidney stones in overweight adults: a cross-sectional analysis of NHANES 2007–2018. BMC Public Health. 2023;23(1):960.
13. Abdul Wahab R, le Roux CW. A review on the beneficial effects of bariatric surgery in the management of obesity. Expert Rev Endocrinol Metab. 2022;17(5):435–46.
14. Imtiaz R, Doumouras AG, Hong D, Anvari M, Shah BR. Long-term impact of bariatric surgery on glycemic control and glucose-lowering therapy for people with type 2 diabetes: population-based cohort study. Surg Obes Relat Dis. 2021;17(6):1049–56.
15. Wang L, Lin M, Yu J, Fan Z, Zhang S, Lin Y, et al. The Impact of Bariatric Surgery Versus Non-Surgical Treatment on Blood Pressure: Systematic Review and Meta-Analysis. Obes Surg. 2021;31(11):4970–84.
16. de Souza Bett G, Schuelter-Trevisol F, do Nascimento RR, Fernandes BB, da Silva LE, da Silva MR, et al. Bariatric Surgery Reduces Lipid Profile and Oxidative Stress in Patients With Obesity: A

Prospective Cohort Study. J Metab Bariatr Surg. 2025;14(1):32–42.
17. Mutlu V, Karaci R, Yuksek MA, Sahin S, Domac FM, Ozbalci GS, et al. Effects of Bariatric Surgery on Sleep Disorder Symptoms and Sleep Quality. Obes Surg. 2025;35(12):5471–9.
18. Gonzalez-Covarrubias V, Martinez-Martinez E, Del Bosque-Plata L. The Potential of Metabolomics in Biomedical Applications. Metabolites. 2022;12(2).
19. Abdullah A, Haron N, Mohamed E, Yusof MIM, Shahril MR. Metabolites alterations associated with obesity: A scoping review. Med J Malaysia. 2024;79(Suppl 1):158–67.
20. Kalita B, Bano S, Vavachan VM, Taunk K, Seshadri V, Rapole S. Application of mass spectrometry based proteomics to understand diabetes: A special focus on interactomics. Biochim Biophys Acta Proteins Proteom. 2020;1868(10):140469.
21. Roberts JA, Varma VR, Huang CW, An Y, Oommen A, Tanaka T, et al. Blood Metabolite Signature of Metabolic Syndrome Implicates Alterations in Amino Acid Metabolism: Findings from the Baltimore Longitudinal Study of Aging (BLSA) and the Tsuruoka Metabolomics Cohort Study (TMCS). Int J Mol Sci. 2020;21(4).
22. Shi Z, Tuomilehto J, Kronfeld-Schor N, Alberti GK, Stern N, El-Osta A, et al. The circadian syndrome predicts cardiovascular disease better than metabolic syndrome in Chinese adults. J Intern Med. 2021;289(6):851–60.
23. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Eur Heart J. 2016;37(25):1944–58.
24. Zuithoff NP, Vergouwe Y, King M, Nazareth I, van Wezep MJ, Moons KG, et al. The Patient Health Questionnaire-9 for detection of major depressive disorder in primary care: consequences of current thresholds in a crosssectional study. BMC Fam Pract. 2010;11:98.
25. QPHI. Data Catalog. In: Institute QPH, editor. 2026.
26. Krämer A, Green J, Pollard J, Jr, Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics. 2014;30(4):523–30.
27. Li Y, Li G, Görling B, Luy B, Du J, Yan J. Integrative analysis of circadian transcriptome and metabolic network reveals the role of de novo purine synthesis in circadian control of cell cycle. PLoS Comput Biol. 2015;11(2):e1004086.
28. Li F, Zhang J, Kang J, Tong X, Zhang P, Zhu Y. Inosine: biofunctions and the roles in human diseases. Frontiers in Endocrinology. 2025;Volume 16–2025.
29. Bashyal S, Qu S, Karki M. Bariatric Surgery and Its Metabolic Echo Effect on Serum Uric Acid Levels. Cureus. 2024;16(4):e58103.
30. Vatsalya V, Cave MC, Kong M, Gobejishvili L, Falkner KC, Craycroft J, et al. Keratin 18 Is a Diagnostic and Prognostic Factor for Acute Alcoholic Hepatitis. Clin Gastroenterol Hepatol. 2020;18(9):2046–54.

31. Wang P-B, Chen Y, Ding G-R, Du H-W, Fan H-Y. Keratin 18 induces proliferation, migration, and invasion in gastric cancer via the MAPK signalling pathway. Clinical and Experimental Pharmacology and Physiology. 2021;48(1):147–56.
32. Goker S, Lee S, Gaizer B, Matsu-Ura T, Tsoi S, Wu G, et al. Circadian clock regulates intestinal epithelial cell differentiation via NOTCH/Hes1 oscillations. bioRxiv. 2026:2026.07.30.741736.
33. Li J, Hu J, Yun H, Mei Z, Wang X, Luo K, et al. Circulating metabolites, genetics and lifestyle factors in relation to future risk of type 2 diabetes. Nature Medicine. 2026;32(2):660–70.
34. Morze J, Wittenbecher C, Schwingshackl L, Danielewicz A, Rynkiewicz A, Hu FB, et al. Metabolomics and Type 2 Diabetes Risk: An Updated Systematic Review and Meta-analysis of Prospective Cohort Studies. Diabetes Care. 2022;45(4):1013–24.
35. Palazzo E, Marabese I, Ricciardi F, Guida F, Luongo L, Maione S. The influence of glutamate receptors on insulin release and diabetic neuropathy. Pharmacology & Therapeutics. 2024;263:108724.
36. Graça ICR, Martins C, Ribeiro F, Nunes A. Understanding Hypertension: A Metabolomic Perspective. Biology (Basel). 2025;14(4).
37. Chen Z, Dong C, Chen L, Song M, Zhou X, Lv D, et al. The Changes in Plasmalogens: Chemical Diversity and Nutritional Implications-A Narrative Review. Nutrients. 2025;17(22).
38. Chua EC, Shui G, Cazenave-Gassiot A, Wenk MR, Gooley JJ. Changes in Plasma Lipids during Exposure to Total Sleep Deprivation. Sleep. 2015;38(11):1683–91.

Tables

Table 1 Baseline Characteristics of Participants with Metabolomics Data in QPHI / QBB Who underwent Metabolic Bariatric Surgery and without Bariatric Surgery, According to Circadian Syndrome Status

Status of Circadian Syndrome in participants with Metabolic Bariatric Surgery				
Characteristics	**No (n= 56)**	**Yes (n= 6)**	**Total (n =62)[1]**	***p*-Value**
Current age (years)	37(9)	51(13)	39 (10)	0.011
Sex				0.2
Male	27 (48%)	1 (17%)	28 (45%)	
Female	29 (52%)	5 (83 %)	34 (55%)	
Education				>0.9
Low	5 (8.9 %)	0 (0%)	5 (8.2 %)	
Medium	0 (0%)	0 (0%)	0 (0%)	
High	51 (91 %)	5 (100 %)	56 (92 %)	
Smoking status				0.4
Non-smoker	33 (59 %)	5 (83%)	38 (61%)	
Current Smoker	14 (25 %)	0 (0 %)	14 (23%)	
Ex-smoker	9 (16 %)	1 (17 %)	10 (16 %)	
Physical activity levels (METs minutes/week)				>0.9
Low (<600 MET/week)	42 (75%)	5 (83 %)	47 (76 %)	
Moderate (600-1200 MET/week)	11 (20%)	1 (17 %)	12 (19 %)	
High (>=1200 MET/week)	3 (5.4%)	0 (0%)	3 (4.8 %)	
Western Pattern	-0.11 (0.83)	0.08 (1.41)	-0.09 (0.89)	>0.9
Prudent pattern	0.01 (0.97)	-0.19 (0.48)	-0.01 (0.93)	0.7
Reduced HDL-C	13 (23 %)	6 (100%)	19 (31%)	<0.001
Elevated blood pressure	6 (11%)	2 (33%)	8 (13%)	0.2

Elevated triglycerides	7 (13%)	4 (67 %)	11 (18%)	0.007
Elevated blood glucose	4 (7.1%)	3 (50%)	7 (11 %)	0.016
Central Obesity	15 (27 %)	3 (50%)	18 (29%)	0.3
Short sleep duration	33 (59%)	6 (100%)	39 (63%)	0.076
Depression symptoms	8 (14 %)	3 (50 %)	11 (18%)	0.063
Metabolic Syndrome	5 (8.9%)	5 (83%)	10 (16%)	<0.001
Status of Circadian syndrome in participants without Metabolic Bariatric Surgery				
	No (n=86) (n= 23)	**Yes (n= 63)**	**Total (n =149)[2]**	***p*-Value**
Current age (years)	40(10)	50(10)	44 (11)	<0.001
Sex				0.2
Male	40 (47%)	36 (57%)	76 (51%)	
Female	46 (53%)	27 (43%)	73 (49%)	
Education				<0.001
Low	15 (17 %)	23 (37%)	38 (26 %)	
Medium	2 (2.3 %)	8 (13%)	10 (6.7%)	
High	69 (80%)	32 (51 %)	101 (68 %)	
Smoking status				0.6
Non-smoker	61 (71 %)	42 (67%)	103 (69%)	
Current Smoker	9 (10%)	10 (16 %)	19 (13%)	
Ex-smoker	16 (19%)	11 (17 %)	27 (18 %)	
Physical activity levels (METs minutes/week)				>0.9
Low (<600 MET/week)	69 (80 %)	51 (81 %)	120 (81 %)	
Moderate (600-1200 MET/week)	15 (17 %)	10 (16 %)	25 (17 %)	
High (>=1200 MET/week)	2 (2.3 %)	2 (3.2%)	4 (2.7 %)	

Western Pattern	-0.15 (0.67)	-0.35 (0.74)	-0.23 (0.71)	0.031
Prudent pattern	-0.11 (0.88)	0.11 (0.75)	-0.02 (0.83)	0.12
Reduced HDL-C	30 (35 %)	52 (83%)	82 (55%)	<0.001
Elevated blood pressure	11 (13%)	44 (70%)	55 (37%)	<0.001
Elevated triglycerides	12 (14%)	50 (79 %)	62 (42%)	<0.001
Elevated blood glucose	23 (27 %)	46 (73%)	69 (46 %)	<0.001
Central Obesity	40 (47 %)	50 (79%)	90 (60%)	<0.001
Short sleep duration	45 (52%)	50 (79%)	95 (64%)	<0.001
Depression symptoms	17 (20 %)	12 (19 %)	29 (19%)	>0.9
Metabolic Syndrome	10 (12 %)	57 (90%)	67 (45 %)	<0.001

Data presented are Mean (SD) for continuous measures and n (%) for categorical measures. *p*-values are based on Wilcoxon rank sum test for continuous measures and Pearson's Chi-squared test for categorical measures. *p*-value <0.05 is set as significant. Abbreviations: HDL-C, high-density lipoprotein-cholesterol; METs, Metabolic equivalent of tasks. [1] n=1 had missing data on circadian syndrome and was excluded. [2] n=5 had missing data on circadian syndrome and was excluded.

Table 2 Baseline Characteristics of Participants with Proteomics Data in QPHI / QBB Who underwent Metabolic Bariatric Surgery and without Metabolic Bariatric Surgery, according to Circadian Syndrome Status

Status of Circadian Syndrome in participants with Metabolic Bariatric Surgery				
Characteristics	**No (n= 53)**	**Yes (n= 6)**	**Total (n =59)[1]**	***p*-Value**
Current age (years)	37(9)	51 (13)	38 (10)	0.012
Sex				0.2
Male	25 (47%)	1 (17%)	26 (44%)	
Female	28 (53%)	5 (83%)	33 (56%)	
Education				>0.9
Low	4 (7.5 %)	0 (0 %)	4 (6.9 %)	
High	49 (92 %)	5 (100 %)	54 (93 %)	
Smoking status				0.4
Non-smoker	31 (58 %)	5 (83 %)	36 (61%)	
Current Smoker	13 (25 %)	0 (0 %)	13 (22%)	
Ex-smoker	9 (17%)	1 (17%)	10 (17 %)	
Physical activity levels (METs minutes/week)				>0.9
Low (<600 MET/week)	40 (75%)	5 (83 %)	45 (76 %)	
Moderate (600-1200 MET/week)	11 (21 %)	1 (17 %)	12 (20 %)	
High (>=1200 MET/week)	2 (3.8 %)	0 (0%)	2 (3.4 %)	
Western Pattern	-0.13 (0.82)	0.08 (1.41)	-0.11 (0.89)	>0.9
Prudent pattern	0.00 (0.99)	-0.19 (0.48)	-0.02 (0.95)	0.8
Reduced HDL-C	13 (25%)	6 (100 %)	19 (32%)	<0.001
Elevated blood pressure	5 (9.4%)	2 (33%)	7 (12%)	0.14
Elevated triglycerides	7 (13%)	4 (67%)	11 (19%)	0.009
Elevated blood glucose	3 (5.7 %)	3 (50%)	6 (10 %)	0.011
Central Obesity	13 (25%)	3 (50%)	16 (27 %)	0.3

Short sleep duration	31 (58%)	6 (100 %)	37 (63%)	0.075
Depression symptoms	7 (13 %)	3 (50 %)	10 (17%)	0.055
Metabolic Syndrome	4 (7.5 %)	5 (83 %)	9 (15 %)	<0.001
Status of Circadian Syndrome in participants without Metabolic Bariatric Surgery				
	No (n=66) (n= 23)	**Yes (n= 23)**	**Total (n =89)[2]**	***p*-Value**
Current age (years)	37(8)	43 (10)	39 (9)	0.013
Sex				0.12
Male	25 (38%)	13 (57%)	38 (43%)	
Female	41 (62%)	10 (43%)	51 (57%)	
Education				0.4
Low	5 (7.6 %)	3 (13 %)	8 (9.0 %)	
High	61 (92 %)	20 (87 %)	81 (91 %)	
Smoking status				0.2
Non-smoker	48 (73 %)	16 (70 %)	64 (72%)	
Current Smoker	6 (9.1 %)	5 (22 %)	11 (12%)	
Ex-smoker	12 (18 %)	2 (8.7%)	14 (16 %)	
Physical activity levels (METs minutes/week)				0.7
Low (<600 MET/week)	57 (86%)	21 (91%)	78 (88 %)	
Moderate (600-1200 MET/week)	9 (14 %)	2 (8.7 %)	11 (12 %)	
High (>=1200 MET/week)	0 (0%)	0 (0%)	0 (0 %)	
Western Pattern	-0.12 (0.57)	-0.16 (0.74)	-0.13 (0.61)	0.6
Prudent pattern	-0.26 (0.81)	0.17 (1.01)	-0.15 (0.89)	0.071
Reduced HDL-C	23 (35%)	17 (74 %)	40 (45%)	0.001
Elevated blood pressure	6 (9.1%)	18 (78%)	24 (27%)	<0.001
Elevated triglycerides	7 (11%)	18 (78%)	25 (28%)	<0.001

Elevated blood glucose	6 (9.1 %)	8(35%)	14 (16 %)	0.007
Central Obesity	34 (52 %)	21 (91%)	55 (62 %)	<0.001
Short sleep duration	36 (55%)	16 (70 %)	52 (58%)	0.2
Depression symptoms	17(26 %)	8 (35 %)	25 (28%)	0.4
Metabolic Syndrome	4 (6.1 %)	19 (83 %)	23 (26 %)	<0.001

Data presented are Mean (SD) for continuous measures and n (%) for categorical measures. *p*-values are based on Wilcoxon rank sum test for continuous measures and Pearson's Chi-squared test for categorical measures. *p*-value <0.05 is set as significant. Abbreviations: HDL-C, high-density lipoprotein-cholesterol; METs, Metabolic equivalent of tasks. [1] n=1 had missing data on circadian syndrome and was excluded.[2] n=5 had missing data on circadian syndrome and was excluded.

Figures

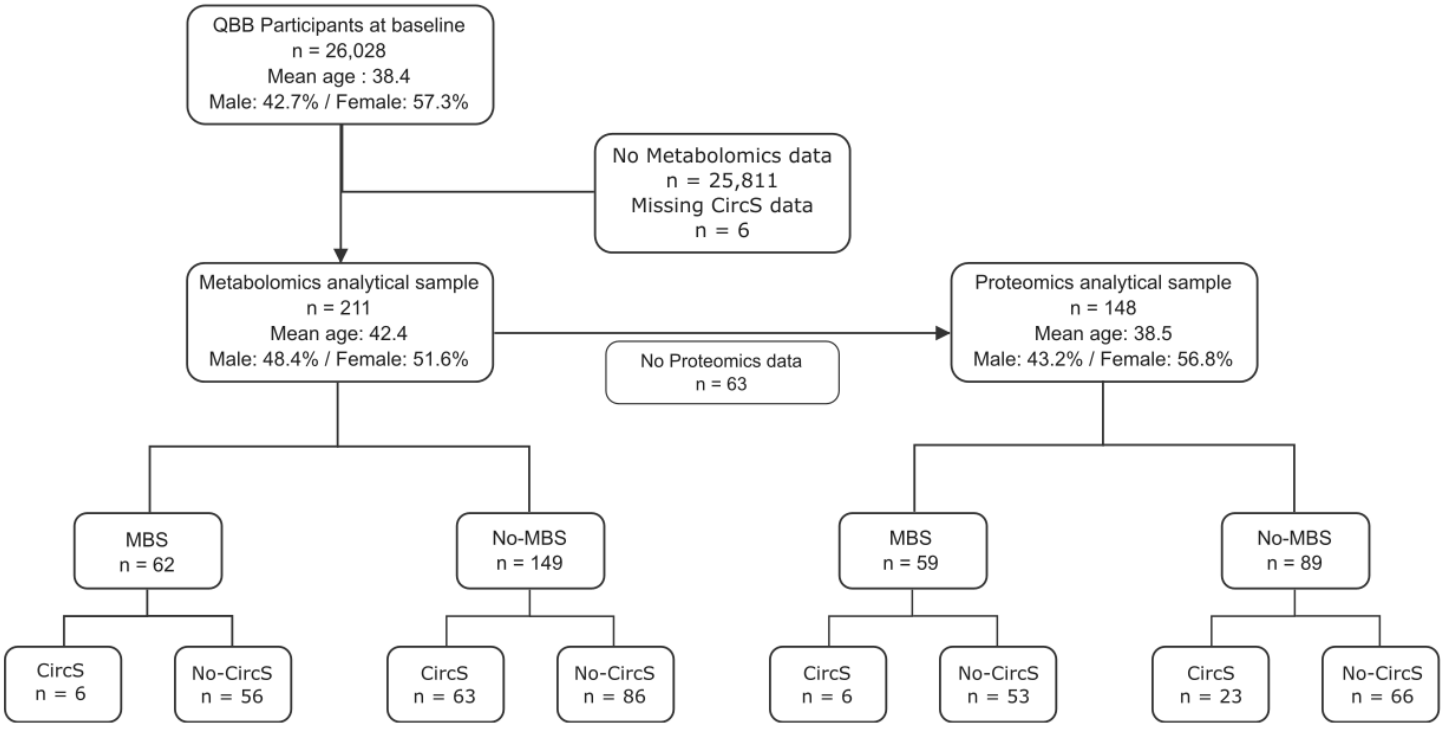


Figure 1

Flow chart for sample derivation

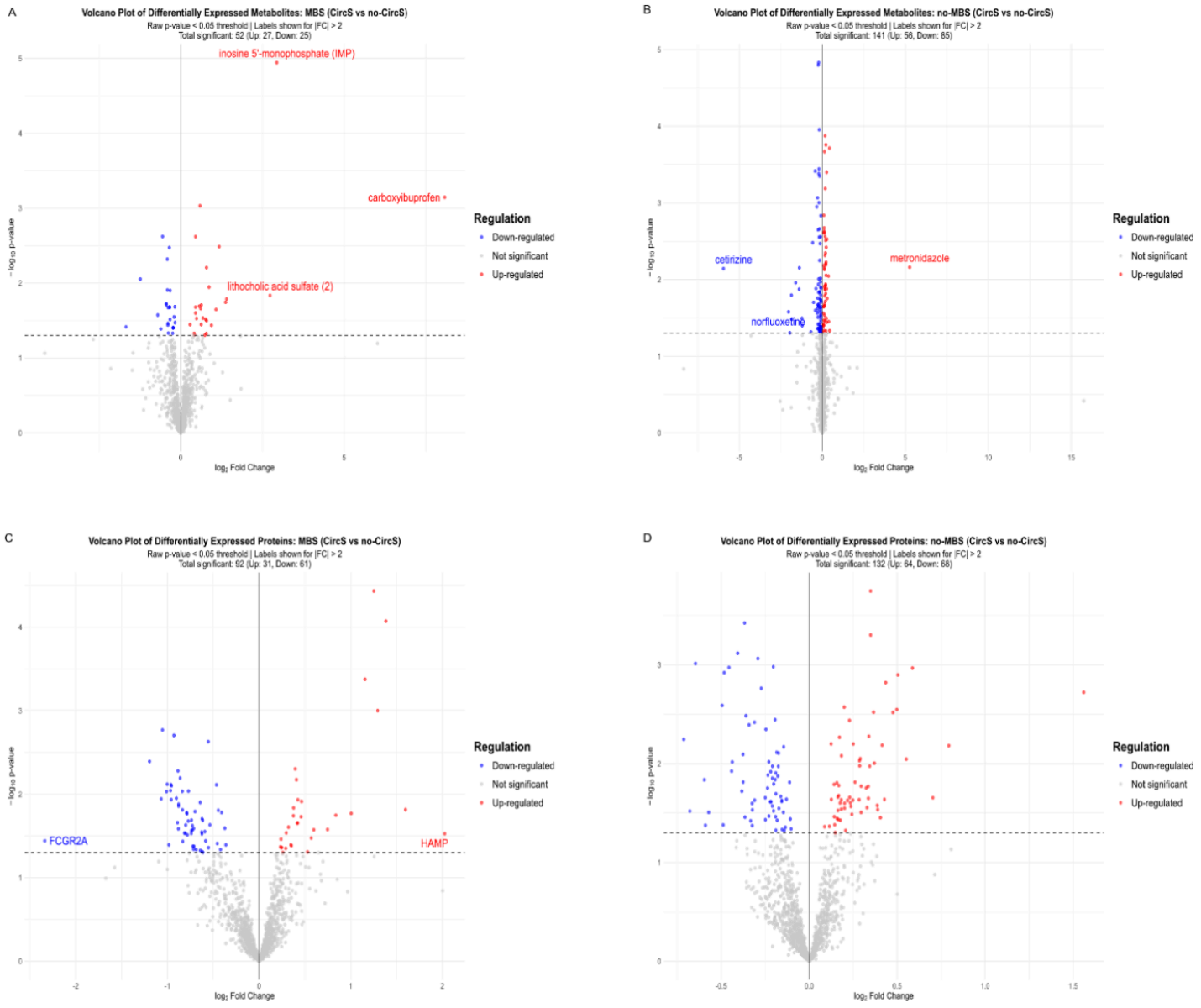


Figure 2

Volcano Plots of Differentially Expressed Metabolites and Proteins by CircS Status in MBS and no-MBS Groups.

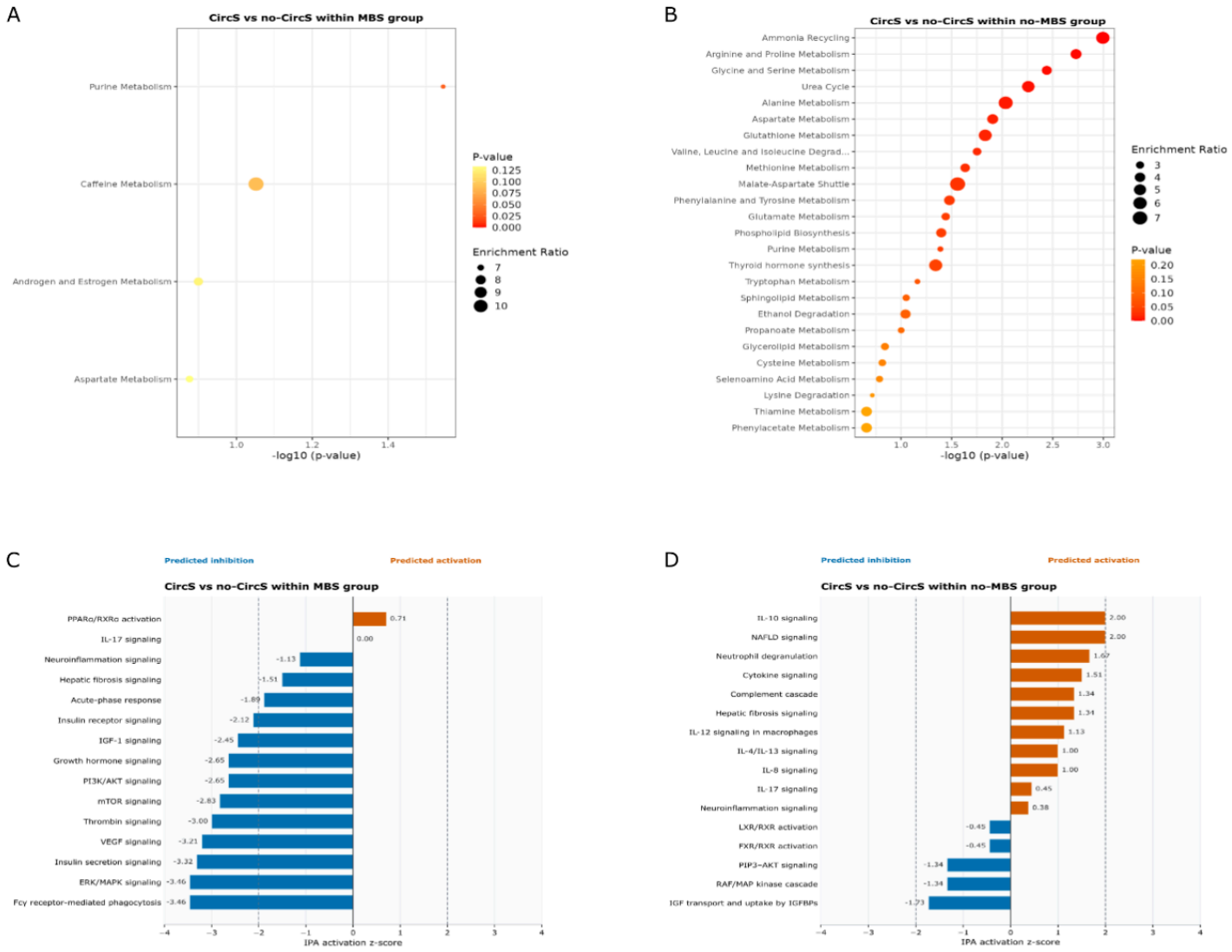


Figure 3

Metabolomic Pathway Enrichment and Proteomic IPA-Predicted Activation States by CircS Status in MBS and no-MBS Groups.

Supplementary Files

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