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The synergistic impact of sleep duration and obesity on metabolic syndrome risk: exploring the role of microRNAs

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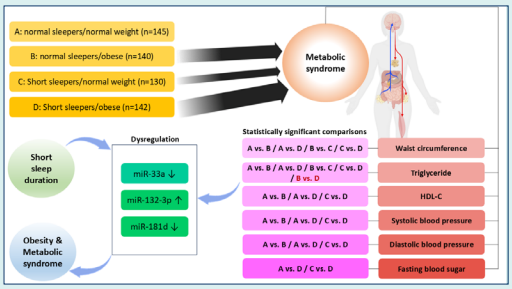
Abstract

Introduction: Given the well-established association between metabolic syndrome (MetS) and obesity, this study elucidates the influences of sleep duration and weight on MetS risk and explores the potential role of miRNAs as underlying mechanisms.



Methods: According to sleep logs and biochemistry tests, this study investigated the association between MetS and its components, sleep duration, and weight in four subgroups: A: normal sleepers with normal weight (N = 145), B: normal sleepers with obesity (N = 140), C: short sleepers with normal weight (N = 130), and D: short sleepers with obesity (N = 142). Chi-square, one-way ANOVA, and Tukey's post hoc tests were used for statistical analysis. Furthermore, following total RNA isolation by TRIzol from blood samples, cDNA was synthesized using stem-loop technique. Quantitative real-time polymerase chain reaction (qRT-PCR) was then employed to evaluate the expression levels of miR-33a, miR-378a, miR-132-3p, and miR-181d. The data were analyzed using one-way ANOVA.

Results: Our findings revealed the strongest association between MetS prevalence and individuals in group D (short sleepers with obesity; Cramer's V = 0.649, $P < 0.001$). This observation underscores the synergistic effect of short sleep and obesity on MetS risk. Furthermore, there was an independent association between short sleep duration and elevated triglyceride levels ($P < 0.05$). MicroRNA expression analysis revealed downregulation of miR-33a and miR-181d in B, C, and D groups compared to the normal group. Conversely, miR-132-3p expression was upregulated in the B, C, and D groups.

Conclusion: Short sleep and obesity synergistically elevate MetS risk, potentially via miR-33a and miR-181d downregulation and miR-132-3p upregulation, impacting triglyceride metabolism.

Introduction

Metabolic syndrome (MetS) is a prevalent condition characterized by a cluster of metabolic abnormalities, including central obesity, high triglycerides (TG), low high-density lipoprotein cholesterol (HDL-C), high blood pressure (BP), and impaired fasting glucose, significantly increasing the risk of cardiovascular diseases, stroke, type 2 diabetes, and mortality.[1] Obesity, a major public health concern, is strongly associated with MetS, and research is ongoing to elucidate the underlying genetic and molecular mechanisms of this association.[2] Despite a known link between obesity and MetS, traditional risk factors like diet and exercise alone cannot explain the high prevalence of both conditions.[3] This highlights the need to investigate other contributing factors. Recent research suggests sleep disorders may be a factor. Studies have shown that sleep disorders like sleep apnea, shift work, insomnia, and abnormal sleep duration are associated with an increased risk of obesity and MetS in various populations.[3,4]

Short sleep duration, particularly less than 7-8

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hours nightly, has been linked to an increased risk of central obesity and high BP.[5,6] The association between short sleep duration and MetS risk has shown mixed results and requires further study. Understanding the combined impact of sleep duration and obesity on MetS risk is crucial for identifying high-risk individuals and developing preventive strategies.[7] While established risk factors and genetic associations provide valuable insights, recent research emphasizes the importance of elucidating the underlying molecular mechanisms that link sleep duration, obesity, and MetS risk.[8] This knowledge is critical for developing targeted preventative and therapeutic strategies.[9] MicroRNAs (miRNAs) have emerged as key regulators of gene expression, influencing diverse physiological processes including metabolism, adipogenesis, and insulin signaling.[10] These small non-coding RNAs function by binding to messenger RNA (mRNA) transcripts, thereby promoting their degradation or inhibiting their translation into protein.[10] Dysregulation of miRNA expression has been implicated in various metabolic disorders, including obesity and type 2 diabetes.[10] Intriguingly, some studies suggest that sleep deprivation can alter miRNA expression profiles in blood and metabolically relevant tissues.[11] However, the precise molecular mechanisms by which sleep duration and obesity co-regulate miRNA expression and contribute to MetS risk remain to be elucidated.

To address this knowledge gap, this observational cross-sectional study aims to investigate the association between MetS, its components, sleep duration, weight status, and the expression levels of four key miRNAs: miR-33a, miR-378a, miR-132-3p, and miR-181d. These miRNAs have been implicated in regulating metabolic processes relevant to MetS development, such as insulin signaling, adipogenesis, and lipid metabolism.[12-14] By employing a novel participant classification system encompassing normal sleepers with normal weight, normal sleepers with obesity, short sleepers with normal weight, and short sleepers with obesity, this comprehensive approach will provide valuable insights into the complex interplay between sleep duration, weight status, MetS, and potential underlying miRNA regulatory pathways. The results of this study may be applicable in designing targeted preventive and interventional strategies to address this growing public health issue, potentially by identifying individuals at high risk based on sleep patterns, weight status, and miRNA expression profiles.

Materials and Methods

Study participants

The 557 participants were categorized into four groups based on their sleep duration and BMI: Group A: normal sleepers with normal weight (145 participants), Group B: normal sleepers with obese weight (140 participants), Group C: short sleepers with normal weight (130 participants), and Group D: short sleepers with obese weight (142 participants). Of each group 24 participants were selected for miRNA expression study and blood samples were collected. The sample size was calculated based on expected miRNA expression levels from previous studies, considering a power of 80% and a significance level of 5%. Written informed consent was obtained from all participants.

Assessment of sleep duration, anthropometrics, and blood parameters

Sleep duration was assessed using sleep logs provided to all participants. The sleep logs consisted of a two-week table where participants recorded their sleep awake times. Average sleep hours were calculated based on these entries. Also, anthropometric parameters were measured using standardized instruments. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Waist circumference (WC) was measured to the nearest 0.1 cm. BP was measured using the Riester Exacta R-1350 sphygmomanometer (Jungingen, Germany). Furthermore, fasting blood samples were collected from participants after a 10-12 hour fasting period. Fasting blood sugar (FBS), TG, and HDL-C levels were measured at Danesh Laboratory (Tabriz, Iran), which adheres to strict quality control measures and is accredited by the National Reference Laboratory.

Participant classification

This study investigated the association between MetS, short sleep duration, and obesity. Participants with an average sleep duration of less than 7 hours per day were categorized as short sleepers, while those with sleep duration between 7 and 8 hours per day were classified as normal sleepers. For BMI classification, participants were categorized into two groups: normal weight (BMI 18.5–24.9 kg/m^2) and obese (BMI ≥ 30 kg/m^2).[15] Subsequently, all participants were divided into four groups: A. normal sleepers with normal weight (N = 145), B. normal sleepers with obese weight (N = 140), C. short sleepers with normal weight (N = 130), and D. short sleepers with obese weight (N = 142). The MetS diagnosis criteria were based on the American Heart Association and the National Heart, Lung, and Blood Institute (AHA/NHLBI).[16]

RNA extraction

A commercial RiboEx reagent (Gene All Biotechnology, Seoul, Korea) was employed for the isolation of total RNA from 250 µL blood samples. Briefly, the blood samples were lysed and homogenized by thoroughly mixing them with 750 µL of RiboEx reagent according to the manufacturer's instructions. The lysates were incubated at room temperature for 5 minutes to facilitate efficient cell lysis and RNA extraction. Subsequently, 200 µL of chloroform was added to each tube to induce

phase separation. The mixture was vigorously shaken for 2 minutes to ensure complete disruption of cellular components and efficient partitioning of RNA. Following this, centrifugation was performed at 12 000 × g for 20 minutes at 4 °C to separate the lysate into distinct aqueous and organic phases. The upper aqueous phase, containing the isolated RNA, was carefully transferred to clean RNase-free tubes. To precipitate the RNA, an equal volume of absolute ethanol was added to the collected aqueous phase. The samples were then stored at -20 °C overnight to allow for efficient RNA precipitation. Following overnight incubation, centrifugation was again performed at 12 000 × g for 15 minutes at 4 °C to pellet the precipitated RNA. The supernatants, containing cellular debris and contaminants, were discarded. To remove residual salts and improve RNA purity, the RNA pellets were washed with 1 mL of 70% ethanol and centrifuged at 12 000 × g for 20 minutes at 4 °C. The wash step was repeated to ensure thorough cleaning of the RNA. After discarding the final supernatant, the RNA pellets were air-dried at room temperature to eliminate residual ethanol. The isolated RNA pellets were then resuspended in a known volume of RNase-free water. To eliminate any contaminating genomic DNA, 5 units of RNase-free DNase I enzyme (Sigma-Aldrich, Germany) were added to each sample and incubated for 15 minutes at room temperature. The DNase I treatment efficiently digests any residual genomic DNA that might co-purify with the RNA. Following DNase I digestion, the enzyme was inactivated by incubation at 70 °C for 10 minutes, thereby halting its enzymatic activity. Following RNA isolation, RNA quantity and quality were assessed by a Nanodrop OneC Spectrophotometer (Thermo Scientific™, USA) and gel electrophoresis respectively. The purified RNA was stored at -80°C until further downstream applications.

Reverse transcription

Following RNA isolation, complementary DNA (cDNA) specific to each miRNA of interest was synthesized using a reverse transcription (RT) reaction. A commercially available RT-PCR Pre-Mix Universal cDNA synthesis kit (BioFACT™, Seoul, South Korea) was employed for this purpose, following the manufacturer's protocol. The kit utilizes miRNA-specific stem-loop primers for efficient cDNA synthesis. The specific sequences of the stem-loop primers used for each miRNA are detailed in Table 1. Each RT reaction contained 5 µg of purified RNA, 1 µL of the corresponding stem-loop RT primer (4 pmol), and nuclease-free water to achieve a final reaction volume of 20 µL. The thermal cycling conditions for the RT reaction were as follows: incubation at 25°C for 5 minutes for primer annealing, followed by cDNA synthesis at 50 °C for 30 minutes. The reaction was then terminated by enzyme inactivation at 95 °C for 5 minutes. The synthesized cDNA was stored at -20 °C until further use.

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was employed to quantify the expression levels of the target miRNAs. The reactions were performed on a StepOne™ qRT-PCR system (Applied Biosystems, Foster City, CA, USA) using Real Q Plus Master Mix Green (Ampliqon, Denmark) according to the manufacturer's instructions. Each reaction mixture contained a final volume of 20 µL, consisting of 10 µL of Real Q Plus Master Mix Green, 2 µL of cDNA, 1 µL of a forward and reverse primer mix (at a final concentration of 4 pmol/µL each), and 7 µL of nuclease-free water. The primer sequences are presented in Table 1. The housekeeping gene U6 was used for normalization purposes. The thermal cycling conditions for the qRT-PCR comprised an initial denaturation step at 94 °C for 10 minutes, followed by 40 cycles of amplification, each consisting of denaturation at 94 °C for 10 seconds and annealing at an optimized temperature (provided in Table 1) for 50 seconds.

Statistical analysis

All statistical analyses were conducted using SPSS software version 24. The values for skewness and kurtosis were assessed to ensure they were within the range of -2 and + 2, verifying the normality of data distribution.[17] Descriptive statistics were presented as mean ± standard deviation (SD) for the studied variables. Chi-square tests were employed to examine the association of MetS across four classifications. Additionally, chi-square analysis was utilized to evaluate the presence and strength of associations in pairwise groups. Subsequently, the association of MetS components within the four classifications was assessed using one-way ANOVA tests. These tests were then performed with adjustments for age and sex. Also, to assess the statistical significance of differential miRNA expression levels between groups, the $2^{-\Delta\Delta CT}$ method was first employed to quantify the relative expression levels. Subsequently, a one-way ANOVA test was performed to evaluate these differences. Post hoc tests were subsequently conducted to identify the exact points of difference and a *P* value less than 0.05 was considered statistically significant.

Results

Descriptive characteristics of the study population

A total of 557 participants completed the study. The average age of the participants was 35.7 ± 6.9 years. Of the total participants, 284 (51.0%) were male and 273 (49.0%) were female. The baseline characteristics of the participants are presented in Table 2.

Association of MetS with four classifications based on sleep duration and obesity status

The prevalence of MetS in each group is presented in Table 3. The chi-square test results revealed a

Table 1. RT stem-loops and primers used for transcription and qRT-PCR of miRNAs

miRNA	Specific forward primer (5′ → 3′)	RT-specific stem-loop primer (5′ → 3′)	Annealing temperature
hsa-miR-33a	CATCCGCAGTGCATTGTAGTTG	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGCAATG	57°C
hsa-miR-132-3p	TGCGCTAACAGTCTACAGCCA	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGACCA	58°C
hsa-miR-378a	CCGCACTGGACTTGGAGTC	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCCTTC	58°C
hsa-miR-181d	CCGACCGAACATTCATTGTTGTC	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCCAC	57°C
U6-housekeeping gene	GCTTCGGCAGCACATATACTAAA	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAAATAT	57°C

Universal reverse primer: GTGCAGGGTCCGAGGT
U6 reverse primer: CGCTTCACGAATTTGCGTGTCAT

Table 2. Basic characteristics of study participants

	Total (N = 557)	Normal sleepers (n = 285)	Short sleepers (n = 272)	*P* value*
Age (y), Mean (SD)	35.7 (6.9)	35.6 (6.8)	35.8 (7.0)	0.740
BMI (kg/m^2), Mean (SD)	27.9 (5.4)	27.5 (5.4)	28.4 (5.3)	0.044
Waist circumference (cm), Mean (SD)	100.4 (12.1)	100.1 (12.3)	100.8 (12.0)	0.481
Triglycerides (mg/dL), Mean (SD)	124.2 (62.1)	114.8 (56.1)	134.1 (66.6)	< 0.001
HDL cholesterol (mg/dL), Mean (SD)	48.6 (7.7)	49.6 (7.4)	47.6 (7.9)	0.002
Systolic blood pressure (mm Hg), Mean (SD)	121.5 (5.1)	121.1 (5.2)	121.9 (5.0)	0.061
Diastolic blood pressure (mm Hg), Mean (SD)	81.2 (4.7)	80.6 (4.7)	81.8 (4.7)	0.004
Fasting blood sugar (mg/dL), Mean (SD)	91.7 (9.5)	91.4 (9.0)	92.0 (10.0)	0.449
Sleep duration (h), Mean (SD)	6.6 (1.0)	7.6 (0.2)	5.7 (0.5)	< 0.001
Sleep duration history (y), Mean (SD)	6.4 (3.0)	7.0 (2.3)	5.8 (3.5)	< 0.001
Sex, No. (%)				
Male	284 (51.0)	142 (49.8)	142 (52.2)	0.574
Female	273 (49.0)	143 (50.2)	130 (47.8)	
Metabolic syndrome, No. (%)				
Negative	380 (68.2)	216 (75.8)	164 (60.3)	< 0.001
Positive	177 (31.8)	69 (24.2)	108 (39.7)	

*The *P* value, derived from either the t-test or the chi-square test, serves as a measure of the statistical significance of the observed difference in sleep duration. *P* values < 0.05 were considered statistically significant.

statistically significant difference in the association of MetS among the four classifications ($\chi 2$ (3, 557) = 154.4, *P* value < 0.001). Additionally, chi-square test results of pairwise comparisons of MetS prevalence in different groups (A vs. B, A vs. C, and A vs. D) demonstrated a significant difference in the association of MetS between groups B, C, and D compared to group A, which was considered the normal control group. Cramer's V values were also calculated to determine the strength of the association in each pairwise analysis. The results are presented in Table 3 and indicate that the association of MetS with group D is the strongest (Cramer's V = 0.649, *P* value < 0.001), followed by the association with group B (Cramer's V = 0.526, *P* value < 0.001) and group C (Cramer's V = 0.231, *P* value < 0.001).

MetS component association across four classifications based on sleep duration and obesity status

The one-way ANOVA tests indicated significant differences across all MetS components for the four classifications. Following adjustment for age and sex, these differences persisted for all components, as detailed in Table S1 (Supplementary file 1). Subsequent Tukey's post hoc tests, conducted after the ANOVA revealed significant outcomes, pinpointed the exact significant differences between groups for each component. Tukey's post hoc test results revealed significant differences between groups B and D compared to group A in all MetS components except for FBS in group B. This indicates that groups B and D exhibit a higher prevalence of abnormal MetS components compared to group A. Additionally, group B demonstrated significant differences from group C in WC and TG. This implies that group B has a higher prevalence of abnormal WC and TG levels compared to group C. Notably, group B also showed significant differences from group D in TG, suggesting that group D has a higher prevalence of abnormal TG levels compared to group B. Furthermore, group C exhibited significant differences from group D in all MetS components, implying that group D has a higher prevalence of abnormal

Table 3. Pairwise differences in metabolic syndrome association

Classification	Metabolic syndrome frequency (%)	Chi-Square test			Cramer's V	
		Chi-Square Statistic	df	*P* value*	Cramer's V coefficient	*P* value*
A. Normal sleepers with normal weight	3 (2.1)	Reference				
B. Normal sleepers with obese weight	66 (47.1)	78.8	1	< 0.001	0.526	< 0.001
A. Normal sleepers with normal weight		Reference				
C. Short sleepers with normal weight	19 (14.6)	14.6	1	< 0.001	0.231	< 0.001
A. Normal sleepers with normal weight		Reference				
D. Short sleepers with obese weight	89 (62.7)	120.9	1	< 0.001	0.649	< 0.001

*A *P* value < 0.05 was considered statistically significant.

MetS components compared to group C. Overall, these findings suggest that groups B and D are at the highest risk of developing MetS, followed by group C, and then group A.

miRNA expression analysis by qRT-PCR

RNA quality was assessed using a Nanodrop OneC spectrophotometer (Table S2, Supplementary file 1) and agarose gel electrophoresis (Fig. 1). The relative expression levels of four miRNAs (miR-33a, miR-132-3p, miR-378a, and miR-181d) were analyzed using qRT-PCR. The results are presented in Fig. 2. One-way ANOVA identified significant changes in expression for miR-33a, miR-132-3p, and miR-181d ($P < 0.05$ for all). miR-33a expression was downregulated in groups B, C, and D compared to group A (Fig. 2). Additionally, miR-33a levels were lower in group D compared to group C. Also, miR-181d expression was downregulated in groups B, C, and D compared to group A (Fig. 2). Interestingly, miR-181d levels were also lower in group C compared to group B. Conversely, miR-132-3p expression was significantly increased in groups B, C, and D compared to group A (Fig. 2). Notably, miR-132-3p levels were also higher in group D compared to groups B and C. Finally, no significant changes were observed in miR-378a expression across all groups (Fig. 2).

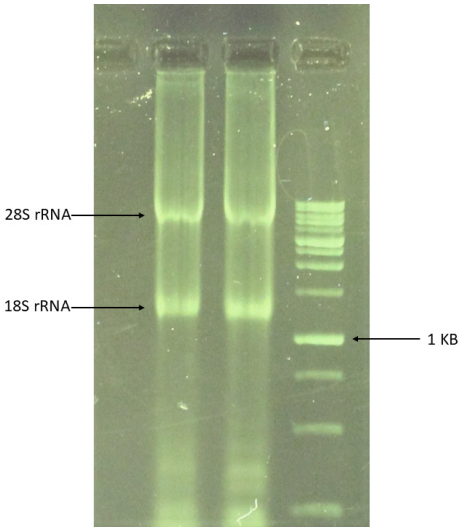

Fig. 1. Agarose gel electrophoresis of total RNA. RNA integrity was assessed by electrophoresis on a 1% agarose gel. Two micrograms of total RNA from samples were loaded. The 28S and 18S ribosomal RNA (rRNA) bands are indicated. A 1 kb DNA ladder was used as a molecular weight marker.

Discussion

The interactive effect of short sleep duration and obesity on MetS risk is complex and the underlying molecular mechanisms are under investigation. While most individual studies have separately linked both factors to an increased risk of MetS, the combined effect of these two conditions and molecular aspects remains understudied. To address this knowledge gap, the present study investigated the expression levels of four key miRNAs and their association with MetS prevalence across four distinct participant classifications: normal sleepers with normal weight (Group A), normal sleepers with obese weight (Group B), short sleepers with normal weight (Group C), and short sleepers with obese weight (Group D).

Our results showed a statistically significant difference in the prevalence of MetS among the four groups. This finding was consistent across pairwise comparisons (A vs. B, A vs. C, and A vs. D). Furthermore, an analysis of Cramer's V values from the pairwise comparisons, presented in Table 3, demonstrated that the strength of the association was strongest when participants were both short-sleepers and obese (Cramer's V = 0.649). This is in comparison to participants who were only obese (Cramer's V = 0.526) or only short-sleepers (Cramer's V = 0.231). These findings suggest that both obesity and short sleep duration independently increase the prevalence of MetS. However, the presence of both conditions simultaneously amplifies the strength of this association. There is a consistent association between obesity and MetS prevalence, observed across both pediatric and adult populations.[18,19] This association is further underscored by the identification of reduced lung function and enhanced levels of biomarkers that effectively predict future cardiovascular risk, highlighting the significant implications for long-term health outcomes.[20] Also, insufficient sleep duration is associated

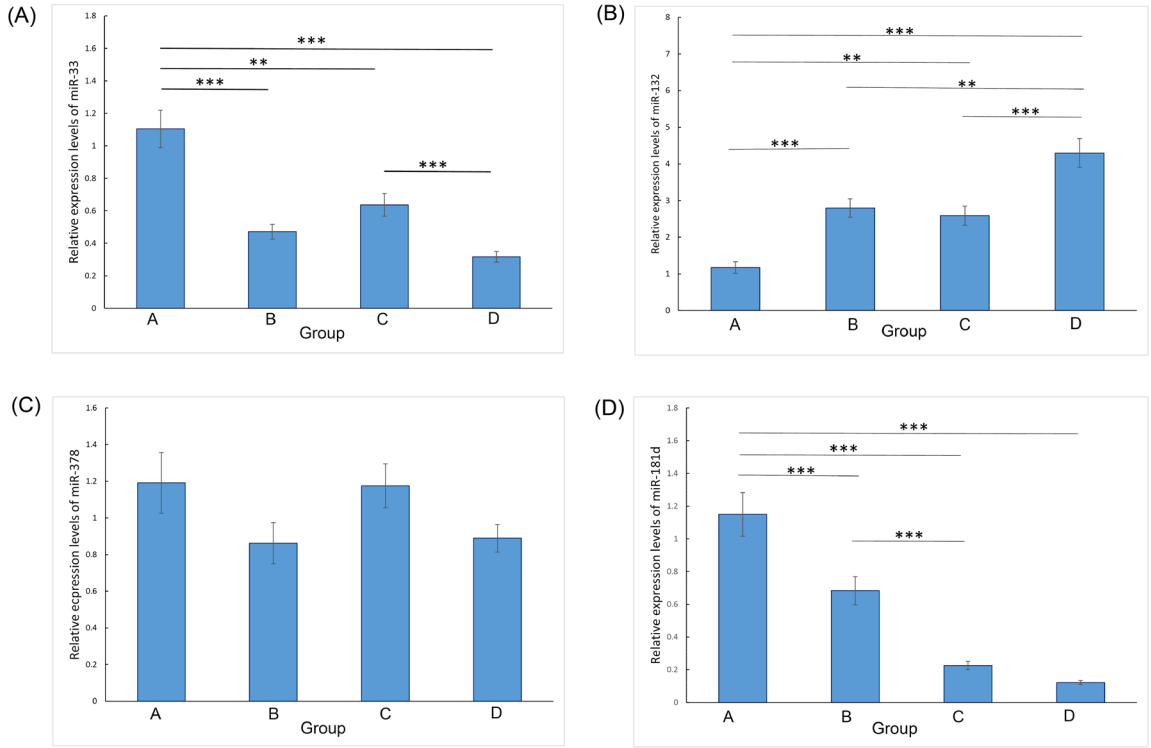

Fig. 2. Relative expression levels of four miRNAs were analyzed by qRT-PCR. The figure shows the relative expression levels of four miRNAs (miR-33a, miR-132-3p, miR-378a, and miR-181d) normalized to a reference gene (U6) in groups A, B, C, and D. One-way ANOVA identified significant differences in expression for miR-33a, miR-132-3p, and miR-181d (*$P<0.05$, **$P<0.01$, ***$P<0.001$). (A) miR-33a expression was downregulated in groups B, C, and D compared to group A (**$P<0.01$, ***$P<0.001$). Additionally, miR-33a levels were lower in group D compared to group C (***$P<0.001$). (B) Conversely, miR-132-3p expression was significantly increased in groups B, C, and D compared to group A (**$P<0.01$, ***$P<0.001$). Notably, miR-132-3p levels were also higher in group D compared to groups B and C (**$P<0.01$, ***$P<0.001$ respectively). (C) No significant changes were observed in miR-378a expression across all groups ($P>0.05$). (D) miR-181d expression was downregulated in groups B, C, and D compared to group A (***$P<0.001$), with levels being lower in group C compared to group B (***$P<0.001$).

with an increased risk of developing MetS in both sexes[21] and enhances the risk of obesity, glucose and insulin dysregulation.[22] Studies highlight the complexity of this relationship, revealing a U-shaped curve where both short and long sleep durations contribute to increased MetS risk, with optimal sleep duration of around 7 hours per night.[23] The severity of MetS also appears to be influenced by sleep duration, with shorter and longer sleep leading to higher symptom scores.[24] Notably, Individuals with MetS experiencing insufficient sleep faced a heightened mortality risk. This association could be attributed to the potential detrimental effects of short sleep on central autonomic and metabolic functions in individuals with MetS.[25] While most studies show a positive association between short sleep duration and MetS prevalence, some studies show the association only in men or only in women.[26,27] Also, a study found no association between sleep duration and MetS in adolescents but did report an association between short sleep duration and elevated BP and long sleep duration and hypertriglyceridemia.[28] These studies underscore the importance of prioritizing healthy sleep habits for reducing the risk of developing MetS and its associated health complications.[29]

Obesity and short sleep duration synergistically increase the risk of developing MetS through intertwined mechanisms. Obesity directly contributes to MetS via ectopic fat deposition, chronic inflammation, oxidative stress, gut dysbiosis, and hormonal dysregulation (e.g. leptin and adiponectin), all impacting insulin sensitivity and metabolic function.[30,31] Short sleep duration amplifies these pathways by disrupting hormonal balance (leptin, ghrelin, cortisol, melatonin), exacerbating inflammation and oxidative stress, and impairing the autonomic nervous system, particularly through sympathetic overactivity.[32-34] Additionally, disrupted circadian rhythms due to short sleep influence appetite regulation and metabolism, potentially leading to weight gain and further amplifying the obesity-MetS link.[35] Moreover, sleep deprivation enhances reward system activity and cravings for unhealthy foods, promoting overeating and weight gain.[36,37] Together, these mechanisms act in a cascading fashion, highlighting the complex interplay between obesity and short sleep duration in their detrimental effect on metabolic health and MetS development. This interconnectedness underscores the vital importance of addressing both sleep and weight management as crucial preventive strategies for MetS.

Our results also revealed statistically significant differences in every MetS component across the four classifications, as detailed in Table S1 (Supplementary

file 1). Post hoc analysis using Tukey's test revealed that despite sharing normal sleep duration, groups A (normal weight) and B (obese) exhibited statistically significant differences in all MetS components except for FBS. This finding suggests that independent of sleep duration, obesity significantly impacts WC, TG, HDL-C, and BP, further strengthening its association with the development of MetS. Comparing groups A (normal sleep, normal weight) and D (short sleep, obese) indicated statistically significant differences in all MetS components. This finding suggests that the combined influence of obesity and short sleep duration significantly impacts multiple metabolic parameters.

Furthermore, statistically significant elevations in WC and TG levels were observed in group B (normal sleep, obese) compared to group C (short sleep, normal weight). This finding underscores the importance of considering both sleep duration and weight status when assessing metabolic health, as even normal sleep may not compensate for the detrimental effects of obesity on specific metabolic parameters and obesity, while contributing to metabolic dysregulation, may have a greater impact than short sleep duration alone. Comparing groups B (normal sleep, obese) and D (short sleep, obese) revealed statistically significant increased TG levels in group D. This observation indicates that short sleep duration within the context of obesity may further exacerbate TG dysregulation, suggesting potential mechanisms beyond weight gain associated with sleep deprivation that contribute to metabolic health complications. Comparison of groups C (short sleep, normal weight) and D (short sleep, obese) showed statistically significant elevations in all MetS components. These findings suggest that even amongst individuals with short sleep duration, obesity has an additional and stronger impact on all MetS components compared to sleep duration.

Our qRT-PCR findings demonstrate a significant downregulation of miR-33a expression in all groups with abnormal metabolic profiles compared to the normal sleep/normal weight control group (A). This downregulation was further pronounced in participants with both short sleep duration and obesity (group D) compared to those with only one factor (groups B and C). Additionally, group D exhibited elevated TG levels compared to group C, suggesting an additive effect of sleep deprivation and obesity on the dysregulation of lipid metabolism.

These findings align with previous research highlighting the role of miR-33a in regulating cholesterol and fatty acid synthesis.[38] Ortega et al suggest that low miR-33 expression promotes fat accumulation and inflammation in adipose tissue.[39] miR-33 directly targets the Sterol regulatory element-binding protein 1 (SREBP-1) transcription factor, a master regulator of lipogenic genes.[38] Interestingly, research by Mariano et al. suggests a link between SREBP-1 and sleep regulation in fruit flies. They observed that flies with overactive SREBP-1 displayed sleep deficits, while those with reduced SREBP-1 activity slept more.[40]

Our data, alongside existing literature, suggests a potential mechanism linking sleep, miR-33a expression, and metabolic health. Downregulation of miR-33a, observed in participants with sleep abnormalities and/or obesity, may lead to increased SREBP-1 activity, consequently promoting lipogenesis and potentially contributing to dysregulated lipid metabolism, as evidenced by elevated TG levels in group D. By elucidating the interplay between sleep, miR-33a, and SREBP-1, we can gain valuable insights into the development of metabolic disorders and potentially identify novel therapeutic targets for improving overall health. However, miR-33a deficiency can cause metabolic issues, complete miR-33 antagonism might not be a suitable therapeutic approach.[38] Nevertheless, Goedeke et al suggest that targeted silencing of specific miR-33 isoforms might be a viable strategy.[41] Future studies investigating the impact of sleep disruption on miR-33 isoform expression and its downstream effects on SREBP-1 and other metabolic targets could guide the development of more precise therapeutic interventions for individuals with sleep disorders and obesity.

Our study also revealed a significant upregulation of miR-132-3p expression in all participant groups compared to the control group with normal sleep duration and weight (group A). This upregulation was further amplified in the group with both short sleep duration and obesity (group D) compared to those with only one factor (groups B and C). Notably, the observed differences in miR-132-3p expression between groups B/D and C/D suggest independent contributions of both sleep duration and obesity to its regulation. Intriguingly, group D (short sleep, obese) displayed significantly higher TG levels compared to group B (normal sleep, obese). This observation suggests that short sleep duration may exacerbate the negative effects of obesity on TG production. Collectively, these findings highlight a potential interplay between sleep duration, obesity status, miR-132-3p expression, and dysregulation of lipid metabolism.

Previous studies suggest a role for miR-132 in sleep regulation and synaptic plasticity within the brain.[42] miR-132 expression appears to be responsive to neurotrophic factors and cAMP Response Element-Binding Protein (CREB) signaling, both crucial for neuronal health and memory consolidation. Sleep deprivation has been shown to decrease miR-132 levels in brain regions,[43] potentially contributing to the cognitive impairments observed in sleep-deprived individuals.

Our findings of elevated miR-132-3p in groups with sleep abnormalities (C and D) seem to contradict these earlier reports. However, it is important to consider

potential tissue-specific regulation. While sleep deprivation might decrease miR-132-3p in sleep centers of the brain, it could be simultaneously upregulated in other tissues, such as adipose tissue, in response to the metabolic stress associated with sleep disruption.

A growing body of research highlights the involvement of miR-132 in regulating adipocyte function and lipid metabolism.[44,45] MiR-132-3p has been shown to target Sirtuin-1 (SIRT1), a protein involved in fatty acid metabolism and inflammation.[45] SIRT1 also plays a critical role in regulating sleep and clock genes.[46] SIRT1 activity peaks during the resting phase and promotes sleep by deacetylating and activating key clock proteins (e.g., BMAL1).[46] Therefore, miR-132-3p upregulation by potentially inhibiting SIRT1 could contribute to sleep disturbances and further disrupt the healthy regulation of metabolism. Additionally, miR-132 expression is elevated in adipose tissue of obese individuals and correlates with markers of metabolic dysfunction potentially through mechanisms involving SIRT1 or other targets.[14,44] Interestingly, Kariba et al. identified exosomal miR-132-3p derived from brown adipose tissue (BAT) as a regulator of hepatic lipogenesis. This suggests a potential inter-organ communication pathway mediated by miR-132.[47]

Furthermore, our findings showed significant downregulation of miR-181d expression in all groups with abnormal metabolic profiles (obesity and/or short sleep) compared to the normal sleep/normal weight control group (A). Notably, the degree of downregulation appeared more pronounced in groups with short sleep (C and D) compared to normal sleep with obesity (B). Interestingly, group D (short sleep with obesity) exhibited the most severe metabolic disruption, as evidenced by elevated TG levels compared to group B. Thus, insufficient sleep duration potentiates the detrimental health consequences associated with obesity and TG metabolism, potentially through mechanisms involving miR-181d dysregulation.

Recent studies have shown that miR-181d expression is reduced in obese individuals compared to lean controls.[12,48] Mechanistically, miR-181d has been shown to target Angiopoietin-like protein 3 (ANGPTL3), a protein involved in regulating TG levels.[12] Downregulation of miR-181d, as observed in our study, could lead to increased ANGPTL3 expression and subsequently contribute to dysregulated lipid metabolism, potentially explaining the elevated TG levels observed in group D.

Also, miR-181d expression appears to be positively correlated with clock genes, including PER and CRY.[49,50] These clock genes play a critical role in regulating the circadian rhythm, which is known to be disrupted by sleep deprivation.[49] While the exact regulatory mechanisms remain unclear, the observed downregulation of miR-181d in our sleep-disrupted groups (C and D) could be indicative of a disrupted sleep-wake cycle, potentially contributing to the observed metabolic disturbances.

Our investigation did not reveal significant alterations in miR-378a expression across any participant groups. This finding may deviate from previous studies that reported correlations between miR-378 and conditions like obesity and sleep disorders.[13,40] One potential explanation for this discrepancy could be the duration of obesity in prior studies. Future research efforts are warranted to explore the influence of obesity chronicity on miR-378a expression.

All these findings suggest potential roles for these microRNAs in the complex relationship between sleep, obesity, and metabolic dysfunction. Future investigations are needed to explore downstream targets, and decipher the functional significance of these microRNAs in the context of MetS development. By unraveling these intricate pathways, we can pave the way for the development of novel therapeutic interventions that target not only obesity and its associated metabolic disturbances but also the detrimental effects of sleep disruption on metabolic health. Ultimately, a comprehensive approach that addresses both sleep and weight management holds significant promise for reducing the growing burden of MetS and its associated health complications.

Conclusion

This study investigated the combined effects of obesity and short sleep duration on metabolic health and miRNA expression. Our findings demonstrate a significant synergistic impact of these factors on the prevalence of MetS and its associated components. We observed a statistically significant increase in MetS prevalence across groups, with the highest risk in individuals with both short sleep and obesity. All MetS components were significantly elevated in participants with both short sleep and obesity compared to those with only one factor. Moreover, we identified differential expression patterns of miR-33a, miR-132-3p, and miR-181d in relation to sleep duration, obesity status, and MetS risk. miR-33a downregulated in participants with abnormal metabolic profiles, potentially contributing to dysregulated lipid metabolism. miR-132-3p upregulated in all groups compared to controls, suggesting a potential role in sleep-metabolism interplay, and miR-181d downregulated in participants with abnormal metabolic profiles, potentially impacting TG metabolism through ANGPTL3 regulation. Overall, our study highlights the detrimental synergy between short sleep duration and obesity on metabolic health. The identified miRNA expression patterns provide valuable insights into potential underlying mechanisms and offer promising avenues for future research.

Authors' Contribution

Conceptualization: Atefeh Ansarin, Khalil Ansarin.
Data curation: Habib Zarredar, Atefeh Ansarin, Neda Gilani.

Research Highlights

What is the current knowledge?

- Obesity is a well-established risk factor for short sleep duration and MetS.
- The association between short sleep duration and MetS risk is inconsistent across studies.
- Molecular mechanisms connecting short sleep duration to obesity and MetS are unclear, especially in humans.

What is new here?

- Obesity and short sleep duration independently and synergistically increase MetS prevalence and its components.
- This study revealed distinct miRNA expression profiles associated with sleep duration, obesity, and MetS risk.
- miR-33a and miR-181d were downregulated, while miR-132-3p was upregulated, in individuals with abnormal metabolic profiles and short sleep duration.
- This study suggests that all three miRNAs may impact TG metabolism through distinct regulatory pathways.

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Competing Interests

The authors declare no conflict of interests

Ethical Statement

This study was conducted with ethical approval from the Tabriz University of Medical Sciences (reference number IR.TBZMED.REC.1399.958). Written informed consent was obtained from all participants.

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Supplementary files

Supplementary file 1 contains Tables S1 and S2.

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