

Research

# A comparison of chrononutrition profile, chrononutrition misalignment, dietary intake and obesity indicators in urban and rural adults residing in Delhi-National Capital Region, India

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## Background

Circadian misalignment, when one's internal body clock is out of sync with the external environment, is a known cause of various metabolic and inflammatory diseases. Chrononutrition (timing of meals) has garnered attention in identifying behaviours which lead to circadian misalignment. Urban and rural populations have different lifestyles owing to their nature of work and physical environment.

## Objective

This cross-sectional analytical study aims to compare the urban population of Delhi with the rural population of the National Capital Region of India in their chrononutrition profile, circadian misalignment, dietary intake, obesity indicators and associated variables--chronotype, sleep efficiency, physical activity, and energy intake.

## Methods

Participants (N=151, 75- urban, 76-rural) were recruited by convenience sampling. Data were collected by face-to-face interview using a validated questionnaire.

## Results

There was a significant difference between the urban and rural groups in obesity indicators, chronotype, chrononutrition profile and physical activity status ( $p < 0.001$ ).

## Conclusions

The study reveals differences in the chrononutrition profile between urban and rural groups due to daily routines, habits, physical activity, and meal timings, as well as obesity indicators. We recommend further research to determine if improving chrononutrition behaviour positively impacts health status.

## INTRODUCTION

The human body's master clock, the supra-chiasmatic nucleus, is synchronized with the environment and with the time of day (Moore et al., 1972; Xu and Lu, 2018). Food, like light, acts as a zeitgeber (an environmental agent or event (such as the occurrence of light or dark) that provides the stimulus setting or resetting the biological clock, (Garaulet et al., 2010; Mistlberger, 2011), entraining (synchronizing) the circadian system. In ancient times, sleep was timed based on sunlight and temperature, with hunter-gatherers having lower workloads than the average nowadays. Modern

societies have changed work-life dynamics, with standard work hours being 40 hours a week (Gangwisch et al., 2014), but counting commuting time and overtime, often much more than this.

Circadian misalignment occurs when the behaviour of a person (e.g., eating, sleeping) does not align with the circadian rhythm. The field of chrononutrition developed as a result of negative health impacts seen in shift workers who experienced circadian disruption due to the nature of their work (Mota et al., 2016).

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These workers have constant shifts in their dietary intake and its timings, due to which several metabolic diseases like obesity (Balieiro et al., 2014; Garaulet et al., 2010; Mota et al., 2013; Xu et al., 2018), dyslipidemia (Alefshat et al., 2021; Katamreddy et al., 2022), metabolic syndrome (Milagro et al., 2012), insulin resistance and diabetes mellitus type II (Eckel-Mahan Kristinet et al., 2013; Wang et al., 2014, Rajaratnam, 2001), cardiovascular diseases, cancer, and cognitive impairment (Craig and McDonald, 2008; Reiter et al., 2009) have been observed (Rajaratnam et al., 2001; Wong et al., 2013).

The prevalence of obesity in India is increasing even faster than the world average. The ICMR-INDIAB study (Mohan et al., 2015), found a prevalence rate of obesity ranging from 11.8% to 31.3% in different groups. It has been forecasted that the prevalence of overweight in adults in India will more than double, while obesity will triple between 2010 and 2040 (Luhar et al., 2020).

Erratic eating patterns disrupt the circadian system, with late night eating, reduced overnight fasting, and evening eating associated with increased metabolic disease risk. However, not many studies have delved into this area in India. The aim of the present pilot study was to assess and compare the rural-urban differences in chrononutrition profile, chrononutrition misalignment, chronotype, physical activity, sleep efficiency, dietary intake, and obesity indicators.

## METHODS

### SAMPLE

The present cross-sectional analytical study was conducted in rural and urban areas of Delhi and the National Capital Region (NCR). The NCR was 62.6% urbanized, with a total population of 46,006,900 at the time of the last census (Registrar General of India, 2011), but is estimated to have grown to 64,138,000 by 2021 (National Capital Region Planning Board).

Convenience sampling was used for selection of villages and participants in both urban and rural areas for the ease of access. Due to the exploratory nature of the study, the sample size was based on the maximum number of participants which could be recruited within the time period of the study (August 2022 -April 2023). Selection of participants was done based on the following inclusion criteria: free-living adult (>18 years, <70 years), literate, residing in rural Rewari or urban Delhi.

Pregnant and lactating women, shift workers, people with known medical conditions or on medications, appetite suppressant drugs, restrictive diets, people diagnosed with mental illness, or recently migrated to urban/rural areas (within 1 year) were excluded. For rural areas, various villages from Rewari were selected-- Mayan, Ramgarh, Jatuwas, Bhagwanpur, Chihad, Siha. For urban areas, adults were selected from districts of Delhi (North Delhi, South Delhi). Ethical clearance was obtained for the study protocol from the Institutional Ethics Committee of Lady Irwin College, University of Delhi. All ethical considerations were followed while conducting the research study. An informed consent form and a study information sheet were provided to all participants. The study was conducted on a sample of 151

participants--75 from urban areas and 76 from rural areas.

The data were collected using questionnaires that asked for information on sociodemographic profile, including name, age, place of stay, occupation, gender and contact information. Anthropometric measurements were taken using standard protocols from WHO/UNICEF. Height, weight, and waist circumference were measured using a stadiometer, TANITA-digital weighing scale, and SECA-non stretch elastic tape respectively. Participants were classified into the categories for BMI and waist circumference recommended for the Asian Indians (Misra et al., 2009). A validated 5-item version of the morningness-eveningness questionnaire was used to assess the chronotype of the participants (rMEQ) (Danielsson et al., 2019). Participants were categorized based on their Total rMEQ score: 4 to 9, definitely the evening type; 9 to 12, mainly an evening type; 13 to 16, intermediate; 17 to 20, mainly a morning type; 21 to 25, definitely a morning type.

A modified chrononutrition profile questionnaire (CP-Q) was used to assess six chrononutrition variables (breakfast skipping, largest meal, evening eating, evening latency, night eating, and eating window). It measures: i) Chrononutrition preference i.e. aspects of 'preference' of a participant to wake up, eat, and fall asleep and ii) Actual behaviours, i.e., aspects of when participant 'actually' performs the above-mentioned activities (Veronda et al., 2019). Eating window refers to the time interval between the first eating event of the day and the last eating event. Evening latency is the duration of time interval between the last eating event of the day and onset of sleep while morning latency refers to the duration of time interval between waking up and first eating event of the day. Misalignment is the difference between actual and preferred timings which is known to cause ill effects on the health (Veronda et al., 2019). In this study, the actual window being  $\geq 30$  minutes prior or past the preferred window is considered to be misalignment.

A weighted average of work days and work-free days was used for eating windows and misalignment calculations. The questionnaire was modified in format for ease of administration after pretesting.

Three questions were used to evaluate sleep efficiency using sleep patterns and based on the Pittsburgh Sleep Quality Index (Buysse et al., 1989). The three questions were 'What time do you usually go to bed at night?' (AM/PM), 'What time do you usually get up in the morning?' (AM/PM), 'How many hours of actual sleep do you usually get at night?' (in hours:minutes). Sleep Efficiency was calculated by averaging the sleep hours for work and work free days throughout the week using the ratio of time spent asleep (total sleep time) to the amount of time spent in bed, expressed as a percentage. Participants were categorised into 3 groups based on their sleep efficiency: a) <50% sleep efficiency b) 50-75% sleep efficiency c) 75-100% sleep efficiency (Carvalho et al., 2014).

To assess physical activity, a short version of the International Physical Activity Questionnaire (IPAQ) was used, in order to reduce respondent burden. The IPAQ criteria were used to classify participants based on their MET-Minutes values--which are derived from the duration

and intensity of their reported typical pattern of exercise over the last 7 days (Craig et al., 2003).

Two 24-hr recalls were conducted, for specific foods and amounts consumed. Participants were asked about quantities of ingredients of all food recipes they consumed, as well as any further ingredients added, including oil (Gibson, 2005). This information was used to calculate the energy intake of the individual for the entire day using the Indian Food Composition Tables (NIN, 2017). DietCal version 13.0 (ProfoundTech Solutions, 2024) was used to simplify calculations. The energy obtained from both days was averaged to get the final figure of energy. The energy intake of each half-day was reported in categories such as 500-800 kcal, 800-1100 kcal, and 1100-1400 kcal. The timing of meals was used to create each participant’s chrononutrition profile. The morning:evening ratio of energy intake was calculated by dividing the total calories consumed during the morning (early morning, breakfast, mid-morning, lunch before 3 pm) by the total calories consumed in evening (meals after 3 pm-evening snack, dinner, post dinner meals).

### STATISTICAL ANALYSIS

Statistical analysis was carried out using IBM Statistical Package for Social Sciences (version 21) and Microsoft Excel. Before applying any parametric statistical test, the normality and homogeneity of data was tested using Shapiro and Levene’s test. The independent sample t-test was used for normally distributed, homogeneous variables and the Mann Whitney U test for skewed variables. Chi square test was used for nominal variables.

### RESULTS

The general profile of the participants is depicted in Table 1.

**Table 1 General profile of the participants (N=151)**

General profile	Urban (n=75)	Rural (n=76)
<b>Age</b>		
<25	21 (28%)	1 (1.3%)
25-34	10 (13.3%)	24 (31.6%)
35-44	16 (21.3%)	28 (36.9%)
45+	28 (37.4%)	23 (30.3)
<b>Occupation of male participants</b>	(n=34)	(n=45)
Business owner/Self employed	8 (25.8%)	0 (0%)
Professional	14 (35.5%)	19 (42.2%)
Government/ Civil service	6 (19.4%)	7 (15.6%)
Manager/Supervisor	6 (19.4%)	0 (0%)
Sales/Service worker	0 (0%)	16 (35.6%)
Agriculture and Fishery worker	0 (0%)	2 (4.4%)
Home maker	0 (0%)	1 (2.2%)
<b>Occupation of female participants</b>	(n=45)	(n=31)
Business owner/Self employed	2 (4.4%)	0 (0%)
Professional	13 (37.8%)	1 (3.2%)
Government/ Civil service	3 (6.7%)	3 (9.7%)
Manager/Supervisor	1 (2.2%)	0 (0%)
Sales/Service worker	0 (0%)	3 (9.7%)
Agriculture and Fishery worker	0 (0%)	3 (9.7%)
Home maker	22 (48.9%)	21 (67.7%)

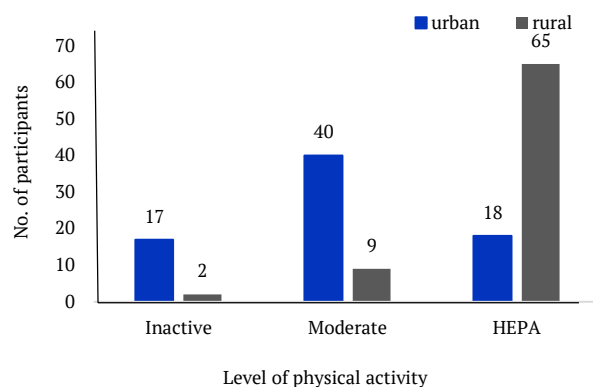
### ANTHROPOMETRY

**BODY MASS INDEX:** The mean BMI of urban participants was 25.5±4.6 kg/m<sup>2</sup>, while for the rural it was 23.2±5. 56% of urban participants were obese and 24% were overweight. In the rural sample, 32% were obese and 19, overweight.

**WAIST CIRCUMFERENCE:** The mean waist circumference of urban participants was 33.6±3.5 inches, while for rural participants it was 27.1±6.1 inches. 42.3% of the urban participants and 22.3% of the rural participants had a waist circumference above normal.

### PHYSICAL ACTIVITY

Figure 1 shows the distribution of participants’ physical activity levels. Rural participants were involved in more strenuous work such as agriculture, animal husbandry, travelled long distances on bicycles and walked for short distances. Therefore, they were in the active HEPA category. The 8.8±2 hour sitting time for the urban sample was higher than the 5.9±2.1-hour rural mean.



**Figure 1. Physical activity levels of urban and rural participants**

(International Physical Activity Questionnaire cut offs, N=151) HEPA-Health-enhancing physical activity

**CHRONOTYPE:** The mean total reduced Morningness-Eveningness Questionnaire (rMEQ) score of the urban participants was 16.5±3.7, and for the rural it was 20.6±2.8. While 57.9% of the rural participants had a “definitely morning chronotype,” only 10% of the urban participants belonged to this category. None of the rural participants had the definitely evening type and more evening type chronotype; while 2% (2) and 7% (7) urban participants had definitely evening type and more evening type chronotype respectively.

**SLEEP EFFICIENCY:** The urban and rural population had a mean sleep efficiency of 83.2%, and 85.0% respectively (Table 3). Based on the three questions of the Pittsburgh Sleep Quality Index, 2.7% urban and 6.6% rural participants had <50% sleep efficiency; 22.7% urban and 18.4% rural participants had 50-75% sleep efficiency and 75% of both urban and rural participants had >75% sleep efficiency.

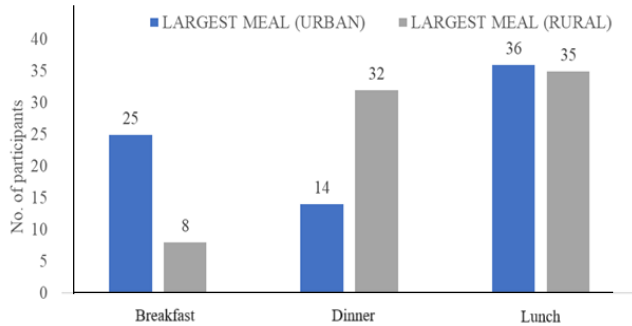
### CHRONONUTRITION PROFILE

**BREAKFAST SKIPPING:** 32% urban and 30% rural participants missed breakfast for at least 1 day in a week. 2.7% of the urban and 1% of the rural participants did not consume

breakfast any day of the week.

**LARGEST MEAL:** Figure 2 shows the largest meals consumed during the day by the participants.

Urban participants had lunch as their largest meal, followed by breakfast (33.4%) and dinner as their smallest meal. Often, they skipped breakfast and consumed a "brunch" instead of separate breakfast and lunch.



**Figure 2. Largest meal of urban and rural participants (N=151)**

#### EATING WINDOW

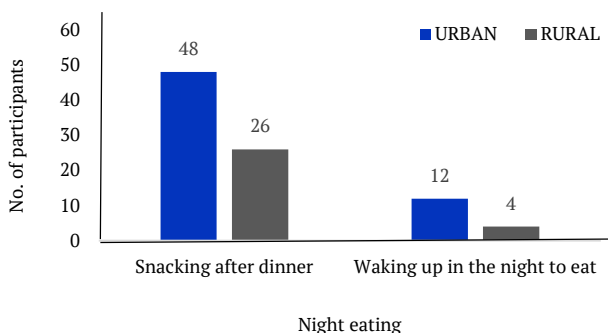
Urban participants had a mean preferred eating window of  $11.9 \pm 1.8$  hours, while rural participants preferred a slightly longer window of  $12.9 \pm 2.2$  hours (Table 3). The mean actual eating window of the participants in the urban sample was  $12 \pm 1.5$  hours while in the rural participants it was  $13.4 \pm 1.4$  hours. The actual eating window was longer in rural participants. Urban participants had similar durations of preferred and actual eating windows, but rural participants had a longer actual eating window of 13.4 hours.

#### EVENING LATENCY

The mean preferred evening latency was  $1.7 \pm 2.5$  hours and  $2 \pm 0.9$  hours in the rural and urban population respectively (Table 3). The actual evening latency in the urban sample was  $2.2 \pm 1.3$  hour while in the rural it was  $1.5 \pm 0.8$  hours (Table 4).

#### NIGHT EATING

Figure 3 shows the number of urban and rural participants who engage in night eating.



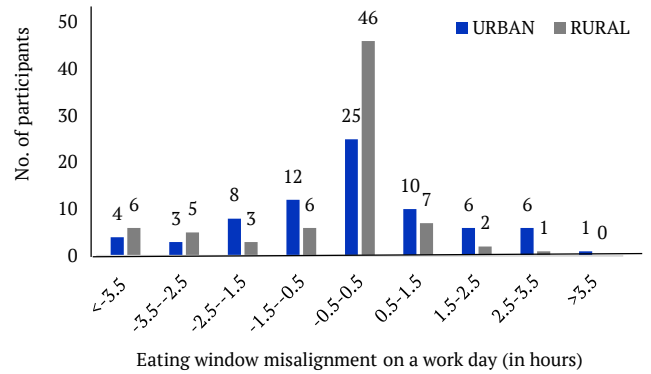
**Figure 3. Number of urban and rural participants who engage in night eating (N=151)**

#### CHRONONUTRITION MISALIGNMENT

It is to note that 30 minutes prior or past preferred window is considered to be misalignment.

#### EATING WINDOW MISALIGNMENT

Figure 4 compares eating window misalignment between rural and urban participants.

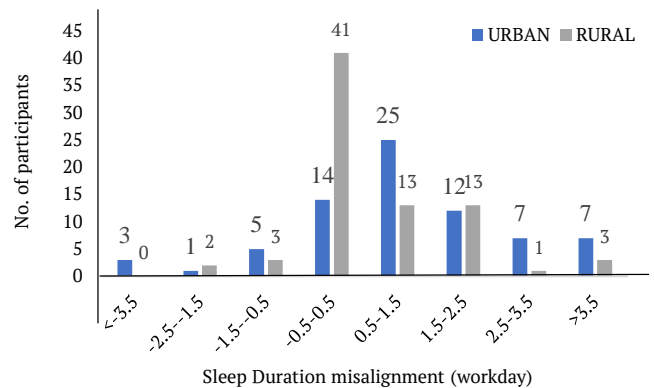


**Figure 4. Eating window misalignment in urban and rural participants on a work day (N=151)**

*Eating window: time interval between the first and the last eating event, Misalignment: difference between actual and preferred timings; Actual window if 30 minutes prior or past the preferred window is considered to be misalignment.*

#### SLEEP DURATION MISALIGNMENT

Figure 5 compares sleep duration misalignment for urban and rural participants. Urban participants had maximum misalignment greater than 3.5 hours, while rural participants had maximum misalignment up to 1.5 hours.



**Figure 5. Sleep duration misalignment in urban and rural participants (N=151)**

*Sleep duration misalignment: difference between actual and preferred sleep duration; Actual duration if 30 minutes prior or past the preferred duration is considered to be misalignment.*

#### MORNING LATENCY MISALIGNMENT

Mean morning latency misalignment in urban and rural participants was  $0.8 \pm 0.9$  hours and  $0.6 \pm 1.0$  hours respectively (Table 4). 53.4% of the urban participants and 68.4% of the rural participants did not show morning latency misalignment. 23.9% of the urban sample had their breakfast/first eating event of the day later than their preferred timing. On the other hand, 14.4% of the rural sample had their first meal later than the preferred timing– Mean evening latency misalignment in urban and rural participants was  $0.9 \pm 0.8$  hours and  $0.6 \pm 2.5$  hours respectively (Table 4). The study revealed that while 40% of urban and 75% of rural participants did not show misalignment in their evening latency, 22.7% of urban participants showed up to 1.5 hours discrepancy. Additionally, 30.5% of urban and 9.2%

of rural participants had their actual timings after their preferred timings, while 29.4% and 15.7% had their actual timings before their preferred timings.

**ENERGY INTAKE**

Mean morning energy intake in urban and rural participants was 814±197 kcals and 815±161 kcals respectively; while the mean evening energy intake was 737±132 kcals and 717±105 kcals respectively (Table 5). The study found that rural participants consume 500-800 kcals in the morning and evening, followed by 28 participants who consume 800-1100 kcals. A small number consumes less than 1100 kcals in the morning, while only 4 consume less than 500 kcals in the evening. Urban participants consume 500-800 kcals in the morning, with more than half consuming it in the evening. Urban participants consume 1100-1400 kcals in the evening and 1 participant consumes 1500-1800 kcals in the evening.

**URBAN-RURAL COMPARISON IN CHRONONUTRITION PROFILE, OBESITY INDICATORS, CHRONOTYPE, SLEEP EFFICIENCY, APPETITIVE TRAITS, PHYSICAL ACTIVITY, AND DIETARY INTAKE**

Tables 3 compares obesity indicators, chronotype, sleep efficiency and energy intake between the rural and urban sample. Table 4 compares chrononutrition between the rural and urban sample.

**Table 3. Comparison between urban and rural participants on obesity indicators, chronotype, sleep efficiency and energy intake**

	Urban (n=75)	Rural (n=76)	p value
<b>Obesity indicators</b>			
BMI <sup>ab</sup> kg/m <sup>2</sup>	25.5±4.6	23.2±5.0	0.002*
Waist circumference (in inches) <sup>b</sup>	33.6±3.5	27.1±6.1	0.000**
<b>Chronotype</b>			
Total rMEQ score <sup>b</sup>	16.5±3.6	20.6±2.8	0.000**
Chronotype <sup>c</sup>			0.000**
Sleep efficiency % <sup>a</sup>	81.9±14.5	82.6±18.6	0.815
<b>Energy intake (kcal)</b>			
Morning energy intake <sup>a</sup>	814±197	815±161	0.872
Evening energy intake <sup>a</sup>	737±132	717±105	0.384

\* p value (<0.05) \*\*p value (<0.001), <sup>a</sup>Independent sample t-test. <sup>b</sup>Mann Whitney U Test, <sup>c</sup>Chi Square test. Chronotype measured through reduced morningness-eveningness questionnaire (rMEQ)

**Table 4. Comparison between urban and rural participants on chrononutrition profile variables**

	Urban (n=75)	Rural (n=76)	p value
<b>Chrononutrition Profile</b>			
Largest meal <sup>c</sup>			0.000**
<b>Preferred timings</b>			
Preferred sleeping midpoint <sup>b</sup> (hh:mm)	02:30	01:47	0.000**
Preferred sleep duration <sup>a</sup> (h)	8.2±1.3	7.9±1.4	0.18
Preferred eating window <sup>a</sup> (h)	11.9±1.7	12.8±2.3	0.007*
Preferred morning latency <sup>a</sup> (h)	0.5±3.6	1.5±1.5	0.025*
Preferred evening Latency <sup>a</sup> (h)	1.9±0.8	1.7±2.5	0.40
<b>Actual timings</b>			
Actual sleep duration <sup>b</sup> (h)	7.8±0.96	7.5±1.1	0.16

**Table 4. continued**

Actual sleep midpoint <sup>b</sup> (hh:mm)	02:30	01:50	0.00**	
Actual morning latency <sup>b</sup> (h)	2±10 h	1.5±1.1	0.003*	
Actual lunch latency <sup>b</sup> (h)	4.8±1.2	5.7±1.5	0.00**	
Actual afternoon latency <sup>b</sup> (h)	7.2±1.4	7.9±1.06	0.001*	
Actual evening latency <sup>b</sup> (h)	2.3±1.3	1.5±0.8	0.00**	
Evening eating <sup>b</sup> (hh:mm)	21:00	20:30	0.000**	
Breakfast skipping <sup>a</sup>	0.9±1.8	0.8±1.6	0.64	
Night eating <sup>b</sup> (h)	0.4±1.3	0.1±0.7	0.038*	
<b>Misalignment (h)</b>				
Sleep misalignment <sup>b</sup>	duration	2.4±3.6	0.7±1.0	0.012*
Morning misalignment	latency	0.8±0.9	0.6±1	0.39
Evening misalignment <sup>a</sup>	latency	0.9±0.8	0.6±2.5	0.00**
Eating Misalignment <sup>a</sup>	window	1.4±1.4	1.01±2	0.17

\* p value (<0.05) \*\* p value (<0.001), <sup>a</sup>Independent sample t-test, <sup>b</sup>Mann Whitney U Test. <sup>c</sup>Chi Square test

Chrononutrition preference: aspects of 'preference' of a participant to wake up, eat, and fall asleep. Actual behaviours: aspects of when participant 'actually' wake ups, eats, and falls asleep. Eating window: time interval between the first and the last eating event. Evening latency: duration of time interval between the last eating event of the day and onset of sleep Morning LATENCY: the duration of time interval between waking up and first eating event of the day. Misalignment: difference between actual and preferred timings which is known to cause ill effects on health

**DISCUSSION**

To our knowledge, this study is possibly the first comparing chrononutrition profile, chrononutrition misalignment, obesity indicators and other variables like sleep, chronotype, physical activity, sedentary behaviour, and energy intake) in urban and rural participants of Delhi- National Capital Region of India. Descriptive statistics showed that a wide range of chrononutrition preferences were reported by participants. Some individuals in the urban population reported a discrepancy of over 3 h between their preferred timing of food intake and their actual timing of food intake. These results are consistent with previous research on sleep/wake timing, a related chronobiological component. Research indicates that people's social and biological sleep/wake schedules (also known as chronotypes) can diverge significantly (Carvalho et al. 2014; Roenneberg et al. 2012; Wittmann et al. 2006). In fact, rural populations may have better sleep efficiency than the urban (Carvalho et al. 2014). However, there was no such difference found in our study.

There were significant differences in BMI, waist circumference, chronotype, chrononutrition profile parameters (largest meal, preferred sleeping midpoint, preferred eating window, preferred morning latency, actual sleep midpoint, actual morning latency, actual afternoon latency, actual evening latency, evening eating, night eating), and physical activity between urban and rural participants. Additionally, there was a significant difference in sleep duration and eating window misalignment between the two groups.

The urban participants had greater adiposity which might be due in part to lower levels of physical activity, greater sitting time, greater eating misalignment, and more night eating. The rural participants were involved more intense HEPA. In addition, the urban participants had more misalignments in their eating patterns, and often consumed

meals later than their reported preferred time to eat.

According to ICMR (2009), prevalence of obesity in the urban population was higher than the rural population ( $p < 0.001$ ) which is in consistency with our study. The study also showed that mean waist circumference values was higher in men than the women ( $p < 0.001$ ). Our study also shows similar trend where more men have greater than normal waist circumference than women. Jayamani et al. (2013) found that rural women were more active than urban women, with 50.7% being HEPA active. However, this study was limited to women. An Indian study reports that 60% people were skipping their breakfast in the urban areas of Chennai and Bangalore (Iyer and Kumar, 2014), this is higher as compared to our data. A study in China found that breakfast skipping was higher in rural Mongolia than urban areas, with a prevalence of 27.9% in rural areas and 30.22% in urban areas (Ba et al., 2013). Though the study set up is in China, it provides similar results to our study.

Our rural participants were inclined more towards the morning time (had morning chronotype) for their daily activities, showed lesser misalignment in timings of food and sleep than the urban participants. Night time eating was more common in the urban participants. Lunch was found to be the largest meal for both groups of participants. Many studies have pointed out towards more prevalence of morning chronotype in the rural population, which aligns with our study result that suggests 57.9% of the rural participants have a morning chronotype as compared to 10% of the urban population (Ba et al., 2013; Carvalho et al., 2014; Nag and Pradhan, 2012; Evans et al., 2011; Louzada et al., 2004)

Knowledge of individuals' preferred timing of food intake may be vital for the creation of targeted eating schedule interventions and to maximize patients' potential for adherence to prescribed eating schedules. Given the high prevalence of eating misalignment in the present sample, this construct may also be important for future health and weight-related intervention efforts. Future research should work to identify underlying factors that contribute to eating misalignment (e.g., hormonal signals, work/school schedules, food availability, personality factors, sociodemographic characteristics) and attempt to uncover why we may see such a large individual variation in eating

misalignment.

The field of chrononutrition is still in its initial stage and is unexplored and therefore there was limited data available to support the results. However, to address these limitations, it was made sure that the data collected is of good quality and checks within the questionnaires were administered properly to exclude participants who were giving erroneous responses.

## CONCLUSION

The study reveals differences in the chrononutrition profile between urban and rural groups due to daily routines, habits, physical activity, and meal timings, as well as obesity indicators.

We recommend further research to determine if improving chrononutrition behaviour positively impacts health status. Target groups can be identified using the chrononutrition profile questionnaire results. Guidelines for chrononutrition should be developed to promote counselling and behaviour change to prevent malnutrition and non-communicable diseases in India.

## AUTHOR CONTRIBUTIONS

SG and SJ participated in the conceptualization and design of the study. SG collected and analysed the data, administered the project and prepared the first draft of the manuscript. SJ did the supervision. SJ reviewed and edited the final draft of the manuscript. Both the authors have read and approved the final manuscript and gave approval for the publication.

## CONFLICT OF INTEREST

Authors declare no competing interests

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