

Diurnal variation of fat oxidation rate and energy expenditure in an acute bout of endurance exercise by young healthy males

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ABSTRACT

Background: Few studies have reported that circadian rhythm affects fat oxidation (FOx) during exercise. Time-of-day causing greater FOx and energy expenditure (EE) during exercise would be beneficial in the prevention of physical inactivity-related health disorders such as metabolic syndrome. The study aims to compare EE and FOx rate after an endurance exercise session done in the morning and late-afternoon hours by apparently healthy young male participants. **Material and Methods:** The present crossover quasi-experimental study involved 10 moderately active but physically untrained male participants of age 18–25 years with normal body mass index. Participants did a steady-state exercise on a motorized treadmill for 30 minutes at a moderate-intensity ($50 \pm 2\%$ of their heart rate reserve) on two separate occasions at 9:00–10:00 and 15:00–16:00. A similar meal followed by 2 hours of fasting was done before each trial session. During the last 2 minutes of the exercise session, the respiratory gas analysis estimated volume of oxygen (VO_2) and volume of carbon dioxide (VCO_2) consumed in L/min. Indirect calorimetry equations assessed FOx (mg/min), EE (Kcal/min) and respiratory exchange ratio (RER). Data of one participant were removed to adjust for extreme chronotype. Paired *t*-test was applied, and $P \leq 0.05$ was considered significant. **Results:** Morning versus late-afternoon variations in FOx (269 ± 110 vs 290 ± 110 , $P = 0.016$), RER (0.86 ± 0.05 vs 0.85 ± 0.05 , $P = 0.040$), EE (5.454 ± 0.987 vs 5.494 ± 0.959 , $P = 0.079$) and VO_2 (1.104 ± 0.201 vs 1.113 ± 0.196 , $P = 0.035$) were present after adjustment for chronotype. **Conclusion:** In an acute bout of endurance, exercise done by young and healthy male individuals, FOx was significantly higher, while EE tends to be higher in late-afternoon than in the morning.

Keywords: Circadian, diurnal, endurance exercise, energy expenditure, fat oxidation, respiratory exchange ratio

Introduction

The oxidation of carbohydrates and lipids (fat) majorly contributes to the energy requirement during exercise. The ability to stimulate higher fat oxidation (FOx) during exercise could efficiently prevent metabolic syndrome (MetS).^[1] The excess energy (energy intake – energy expenditure [EE]) stored as fat

increases body mass index (BMI) in an adult. FOx could be a determinant of body composition and body weight or BMI.^[2] Individuals who maintain their BMI are at low risk of developing MetS.^[3] The ‘diseasome of physical inactivity’ hypothesis states that visceral fat accumulation is responsible for MetS that higher FOx could prevent.^[4,5] A higher EE during exercise is also essential for reducing the risk of MetS.^[6]

The frequency, intensity, time, type (FITT) principle by the American College of Sports and Medicine includes frequency (five times a week), intensity (moderate), time (30 minutes per day)

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and type (both endurance and strength) of exercise to improve and maintain the health-related quality of life.^[7] One more aspect that needs to be explored is the time-of-day. The time-of-day resulting in higher FOx and EE should be preferred for exercise, as it might prove more beneficial in preventing the development of physical inactivity-related health disorders. Few studies had documented circadian or diurnal, or time-of-day dependent variations in substrate oxidation during exercise.^[8,9] However, there is a paucity of data, more so from India.

Primary care physician's (PCP) counselling and advice on integrating exercise in daily routine are effective in improving the health-related quality of life of the patients.^[10] Some countries are providing incentives to PCPs for physical activity counselling.^[11] Despite its favourable clinical outcome and cost-effectiveness, the patient's counselling regarding physical activity is not only underutilised by PCPs, but also the time-of-day for exercise that has been shown to affect FOx and EE is not taken into consideration.^[12,13] Hence, the present study was designed to compare FOx rate and EE after a steady-state, moderate-intensity, endurance exercise session in the morning and late-afternoon hours by apparently healthy young males. The results of the present study might encourage PCPs to counsel the patients to take into account the time-of-day for the exercise.

Material and Methods

The present crossover quasi-experimental crossover study was done at the Exercise Physiology laboratory, King George Medical University (KGMU), Lucknow, Uttar Pradesh (UP), India. This pilot study was a part of the leading research proposal approved by the Institutional Ethical Committee (reference code: 81st ECM II-B Thesis/P19). All participants signed the written informed consent.

G-power v3.1.9.7 estimated that at 5% alpha error, 20% beta error, 15% SD and 0.95 correlation, 10 participants were required to detect a mean change of 5% if a two-tailed paired *t*-test is to be applied.

Ten healthy male participants, age 18–25 years, having a normal BMI of 18.5–22.9 kg/m² (as per the Asia Pacific classification), were recruited in the study from KGMU after they fulfilled the inclusion and exclusion criteria. BMI was calculated as weight (kg) ÷ height (m²). A locally available stadiometer and an electronic weighing machine were used to measure the weight (nearest to 0.1 kg) and height (nearest to 0.1 cm) of the barefoot participants who wore light clothing during the measurements. Only moderately active individuals (assessed by General Practice Physical Activity Questionnaire) who were fit to perform physical activity (assessed by Physical Activity Readiness Questionnaire for Everyone) were involved in the study. Abnormality in systemic examination or resting electrocardiogram (BPL Cardiart 108T-DIGI), family history of metabolic disease, a recent history of substance abuse and regular strenuous physical activity in the past 6 months, inability to follow exercise protocol were the exclusion criteria.

Two familiarization sessions were done before the actual test. Participants did a steady-state walk on the motorized home-based treadmill (Pro Bodyline Fitness 970) in an air-conditioned laboratory. The exercise was performed at a target heart rate (THR) that corresponds to moderate-intensity exercise. Heart rate reserve (HRR) was assessed by subtracting maximum heart rate (MHR) with resting heart rate (RHR). MHR was calculated by the equation $208 - 0.7 \times \text{age}$.^[14] THR was estimated by adding RHR with $50 \pm 2\%$ of HRR.^[15] During exercise, HR was continuously monitored by the pulse transducer of ADInstruments to ensure a steady-state. Thirty minutes of exercise was done after a steady-state (minimal fluctuation in THR for 2 minutes) was reached.

The exercise session was done either 2 hours after lunch at 15:00–16:00 or 2 hours after breakfast at 9:00–10:00 on different occasions, separated by 3–5 days. The participants were instructed to have a low-fat diet (60–120 g of oatmeal or egg white with 200 mL skimmed milk or juice) in lunch or breakfast and avoid beverages, aerated drinks and energy-laden food-item 24 hours before the exercise session.

Respiratory gas exchange analysis was done during the last 2 minutes of the exercise session by the ADInstruments exercise physiology system with PowerLab v8. Stoichiometric equations based on indirect calorimetry were used to calculate FOx and EE.^[16] $\text{FOx (mg/min)} = [\text{VO}_2 \times 1.695 - \text{VCO}_2 \times 1.701] \times 1000$; $\text{EE (Kcal/min)} = \text{VCO}_2 \times 0.550 + \text{VO}_2 \times 4.471$; where volume of oxygen (VO₂) and volume of carbon dioxide (VCO₂) were in L/min and urinary nitrogen excretion was considered negligible. The ratio of VO₂ to VCO₂ is known as respiratory exchange ratio (RER). RER represents the proportion of carbohydrate and fat oxidized for energy generation during the exercise. Higher the RER value greater the proportion of carbohydrate being oxidized, while lower the RER value greater FOx.^[5]

Microsoft Excel 2019 was used for initial data entry and calculation. Statistical analysis was done in IBM SPSS Statistics (version 26.0 for Windows, IBM Corp., Armonk, NY, USA). The Shapiro Wilk test established the normality of data. Data are presented as mean ± SD. Student (paired) *t*-test was applied, and a *P* value, less than or equal to 0.05, was considered statistically significant. G*Power v3.1.9.7 was used to calculate the effect size.

Results

Table 1 represents the anthropometric characteristics of the participants involved in the study. Table 2 shows the study parameters taken in the morning and late-afternoon exercise sessions. A statistically significant difference was not obtained for all the parameters. Table 2 also shows that the effect size was moderate (>0.5) for FOx, EE, RER and VO₂.

On examining the data, participants were retrospectively asked to answer an 'Automated Horne-Ostberg Morningness-Eveningness

Questionnaire'. It was found that five participants were of intermediate chronotype; three were moderate evening type; one was moderate morning type; one was definite morning type. After removing the 'definite morning type' participant data, diurnal variations in FOx, RER and VO₂ became statistically significant, while diurnal variation in EE tended to be significant [Table 3].

Discussion

The present crossover, quasi-experimental, pilot study was done on healthy young males and found that late-afternoon hours lead to higher EE, RER, VO₂ and FOx than morning hours. To the best of the author's knowledge, the present study is the first Indian study to report the effect of time-of-day on FOx and EE during exercise.

The present study neutralized many factors that affect FOx^[17,18] – gender (all males), age (narrow-range young age-group), body composition (narrow-range normal BMI), environmental temperature (air-conditioned laboratory), meal/nutritional status (similar meal before both exercise session and 2 hours fast), exercise intensity (moderate-intensity), exercise modality (only treadmill walk), exercise duration (30 minutes) and training status (untrained moderately active).

Moderate-intensity exercise was chosen for the study because it induces greater FOx and is well tolerated by untrained individuals.^[17,18] A long-duration steady-state moderate-intensity exercise is better termed as 'endurance' exercise.^[19] Treadmill causes a higher FOx and EE than stationary cycling at a similar intensity of exercise; hence, treadmill walk was chosen as exercise modality in the present study.^[19,20]

The present study results are similar to few previous studies regarding FOx and RER (less RER indicates high FOx). Mohebbi *et al.*^[9] involved 12 untrained, young, normal BMI males in a treadmill exercise and reported that FOx was significantly higher in the evening (18:00) than in the morning (8:00) at moderate-intensity. The morning session was done after 8–12 hours of fasting, while the evening session was done after 5–6 hours of fasting. Participant's chronotypes were not considered. Kim *et al.*^[21] involved 14 untrained males with normal BMI in 60 minutes of treadmill walk at moderate intensity. The participants ate the same diet 3 hours before the exercise trial; their chronotype was not considered. It was observed that interleukin-6 (IL-6) was significantly higher immediately after the evening exercise session (17:00–18:00) compared to the morning (9:00–10:00). Also, a significant correlation was found between IL-6 and fatty acids in an evening exercise session, leading to the conclusion that the evening hours are more effective for exercise-induced lipolysis than the morning hours. IL-6 during exercise is anti-inflammatory that assists in the clearance of glucose and lipoproteins from circulation and improves insulin sensitivity, thereby reducing the risk of MetS.^[22]

Souissi *et al.*^[23] involved 12 young, normal BMI and physically trained individuals in self-paced endurance cycling and reported that the RER was insignificantly lower during the evening (17:00–18:00) than in the morning (7:00–8:00). The timing of the meal and the gender of the subjects were not specified. All participants were of intermediate chronotype. Amaro-Gahete *et al.*^[24] involved 12 endurance-trained male athletes with normal BMI in an incremental treadmill walk until exhaustion and reported that FOx in the evening (17:00–20:00) was significantly higher than in the morning (8:00–11:00) exercise session. Similar diet and fasting time (approximately 8 hours) were provided in the morning and evening exercise trials. One participant was the definitive evening type; two were moderate evening type; six were intermediate type; three were moderate morning type.

In contrast to our study result, Ezagouri *et al.*^[8] involved 12, normal BMI male participants, age 20–50 years, in a 60-minute steady-state moderate-intensity cycling and reported that a higher RER and hence lesser FOx occurs in the evening (18:00) than the morning exercise session (8.00). A standardized meal was provided to the participants 2 hours before the tests. The chronotype of the participants was not considered. Further, it was reported that the time-of-day effect on RER is exercise-dependent and was not observed at rest. Kim *et al.*^[25] involved nine untrained young males with normal BMI in 30 minutes of treadmill walk at moderate intensity and reported that the time-of-day (9:00–10:00 and 17:00–18:00) does not affect FOx in endurance exercise. The

Table 1: Anthropometrical characteristics of the Participants (n=10)

Parameters	Mean±SD
Height (cm)	170.1±4.3
Weight (kg)	59.3±7.4
BMI (kg/m ²)	20.0±1.6
Age (years)	20.5±1.8

Table 2: Study parameters recorded/calculated during steady-state moderate-intensity 30 minutes of exercise in the morning (9:00-10:00) and late afternoon (15:00-16:00) session

n=10	9:00-10:00	15:00-16:00	P*	Effect size
RHR (bpm)	74.7±8.4	74.9±8.6	0.901	0.04
THR (bpm)	134.4±4.7	134.3±5.0	0.906	0.04
VO ₂ (L/min)	1.147±0.233	1.154±0.225	0.089	0.56
FOx (mg/min)	273.8±104.4	289.1±103.9	0.095	0.59
EE (kcal/min)	5.667±1.148	5.699±1.112	0.135	0.52
RER	0.86±0.05	0.85±0.05	0.087	0.61

*Paired t-test was applied

Table 3: Study parameters recorded/calculated after chronotype adjustment

n=9	9:00-10:00	15:00-16:00	P*	Effect size
VO ₂ (L/min)	1.104±0.201	1.113±0.196	0.035	0.88
FOx (mg/min)	269.3±109.7	290.1±110.1	0.016	1.03
EE (kcal/min)	5.454±0.987	5.494±0.959	0.079	0.77
RER	0.86±0.05	0.85±0.05	0.040	0.85

*Paired t-test was applied

participants ate the same diet 3 hours before the exercise trial; their chronotype was not considered.

A slightly higher VO_2 during late-afternoon exercise was obtained in our study, indicating more significant aerobic metabolism and increased exercise capacity. Souissi *et al.*^[23] reported that the evening session caused significantly higher oxygen consumption than the morning exercise session. Ezagouri *et al.*^[8] concluded that improved exercise efficiency occurs in the evening. In contrast to our study result, Kim *et al.*^[25] reported that VO_2 was not affected by time-of-day during moderate-intensity exercise. Amaro-Gahete *et al.*^[24] reported that $\text{VO}_{2\text{max}}$ was not altered by the time-of-day. Movaseghi *et al.*^[26] explained that lung function rather than VO_2 improves in the evening compared to the morning.

Energy deficit is vital for fat loss.^[27] Our study results indicate that EE tends to be higher during the late afternoon.

A complex interaction of circadian rhythm with body temperature, hormones, cytokines and neuromuscular adaptations during exercise results in diurnal variation of FOx, EE and VO_2 .^[28-30] Rodent studies indicate that few genes and circadian clock proteins affect metabolism.^[8,29,30] Most studies indicate that the early evening exercise minimizes physical-inactivity-related health disorders by increasing FOx and exercise capacity. A recent study concluded that performing exercise in the afternoon or early evening hours leads to the most significant metabolic health benefits even in subjects with MetS.^[31] Additional studies are required to clarify the effect of time-of-day on the FOx and EE during exercise training and its application in public health.

The present study had several limitations. Individual chronotype ('morning larks', 'night owls' or 'in between') was not considered initially; instead, later on, we had to exclude the data of one participant based on his chronotype. Different chronotypes respond differently to similar exercises at the identical time-of-day.^[28] Knaier *et al.*^[32] reported that though there are statistically significant diurnal variations in VO_2 , the variation gets masked as different people achieve $\text{VO}_{2\text{max}}$ at a different time-of-day. The late-afternoon time-of-day was taken in our study due to resource and time limits. Most previous studies have taken evening hours (17:00–18:00) for comparison with morning hours (9:00–10:00). Basti *et al.*^[33] reported that the exercise performance correlates with daily fluctuation in core-clock genes and peaks in the late-afternoon (15:00–18:00) hours irrespective of gender, but it is always better to keep individual chronotype in perspective for achieving better results of the exercise. An acute bout, participant characteristics and sample size limit the application of our study results to the general population. We acknowledge that our study results might not be precise and accurate due to reliance on HRR for exercise intensity and stoichiometric equation for indirect estimation of EE and FOx.

Conclusion

Late-afternoon hours induce significantly greater FOx, RER and VO_2 in an acute bout of endurance exercise by young and

healthy males. Statistical significance was not established for EE, though the effect size was moderate. PCPs could consider the time-of-day while advising and counselling the patient for initiating or enhancing their physical activity.

Caution is necessary to interpret our study results that involve indirect and equation-based estimation of FOx and EE. Individual chronotype should be considered while advising the exercise protocol for best performance and FOx. Further studies involving different population characteristics and exercise protocols are required for the external validity of our data.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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