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## EFFECTS OF TIME OF DAY AND THE WINGATE TEST ON APPETITE PERCEPTIONS, FOOD INTAKE AND PLASMA LEVELS OF ADIPOKINES

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It has been demonstrated that several aspects of adipose-related physiology including adipokine release, exhibit daily oscillations. Physical exercise exerts a strong influence on adipokine release and a possible reverse disruption of peripheral circadian clocks. The aim of this study was to establish the effects of time of day and the Wingate test on appetite perception, food intake and plasma levels of adipokines. Twenty-four moderately active non-smoking males (mean  $\pm$  S.D. age:  $27.1 \pm 3.1$  years; height:  $1.79 \pm 0.1$  m; weight:  $76.1 \pm 11.7$  kg) were recruited for this study and divided in two groups; one fed with an *ad libitum* test meal and another one without an *ad libitum* test meal. Each subject participated in the following studies performed at 11:00 and 23:00 hours on separate days: 1) Exercise study (ES): a 30-second Wingate Anaerobic Test (WAnT), and 2) sedentary study (SS). Subjects rated their appetite perceptions (hunger and prospective food consumption) on a 100-milimeter visual analogue scale (VAS) at baseline, after exercise, after test meal and during the postprandial/control period. At those time points blood samples were obtained for the measurement of plasma leptin, visfatin and apelin concentrations. Appetite perception and energy intake results at test meal decreased in response to WAnT in comparison with sedentary subjects. Time of day had no statistically significant effect on energy intake but the appetite perception score after test meal at 24:00 hours was statistically higher than that after test meal at 12:00 hours. No significant differences in the tested plasma adipokine concentrations between the trials existed at baseline, however, all plasma adipokine levels at 24:00 hours were higher than those at 12:00 hours. Plasma apelin concentrations after WAnT were significantly higher than its pre-exercise value at 12:00 hours, unlike those at 24:00 hours. Sedentary experiments showed a modest, yet significant, rise in plasma apelin levels after the test meal at 12:00 hours but not after the one at 24:00 hours. There were no significant changes in plasma leptin concentrations after exercise or test meal but a significant decrease in plasma visfatin concentrations after exercise intervention both at the 12:00 hours test and the 24:00 hours test has been observed. Test meals caused a significant rise in visfatin concentrations in sedentary, but not exercise series, in the daytime and nighttime tests. We conclude that time of day is an important aspect to consider in the relationships between exercise, metabolism and appetite. Further studies are needed to explain the specific mechanisms underlying the effects of acute exercise on postprandial physiology at different times of the day.

Key words: *exercise, appetite, hunger, food intake, adipokines, leptin, visfatin, apelin, circadian rhythm*

### INTRODUCTION

Circadian rhythms (Latin *circa diem* meaning 'around a day') refer to physical and behavioral changes occurring with a periodicity of approximately 24 hours. They are endogenous but synchronized (entrained) with the environment by external time cues called *zeitgebers* (German, meaning 'time givers'). The most important are light/dark cycles, but others, like food intake and physical exercise, can also act as non-photic *zeitgebers*, timing of food intake being the dominant entrainment signal for peripheral circadian clocks (1, 2).

Circadian clocks, present nearly in all cells and tissues including adipose tissue, are organized hierarchically in mammals (3). The master clock is located at the suprachiasmatic

nucleus (SCN) of the anterior hypothalamus, entrained to the 24-hour period by the daily light-dark cycle which in turn communicates with and entrains the peripheral tissue clocks (3). Circadian oscillation of clock genes has been observed in adipose tissues, and an important role of these clocks has been suggested in various aspects of adipose physiology and pathology (4-6). Existing synchrony between the SCN and peripheral clocks is critical to normal physiological functions. It has been demonstrated that not only external circadian disruption but also internal circadian desynchrony could lead to metabolic disorders. Furthermore there is a clear relationship between abnormally timed feeding and metabolic disorders (7-10).

It is widely accepted that shift workers are at increased risk of obesity and other cardio-vascular and metabolic disorders (8,

10-13). The observation that the patients who suffer from night eating syndrome have increased body weight (14) supports the hypothesis that eating outside the endogenous circadian day may be at least in part responsible for metabolic pathologies in shift workers. Another confirmation of that hypothesis comes from rodent studies where sudden changes in feeding time can uncouple peripheral clocks from the SCN clock, which can lead to metabolic disorders (15).

The worldwide epidemic of obesity has contributed to a better understanding of the biology of adipose tissue. Adipose tissue, which consists not only of adipocytes and preadipocytes but also connective tissue matrix, nerve tissue, stromal vascular cells and immune cells, was traditionally considered as inert energy storage (16). During the last two decades, however, adipose tissue has been shown to be responsible for the secretion of an array of signaling molecules called adipokines (17). Adipose tissue produces and releases a variety of proinflammatory and anti-inflammatory factors including adipokines such as leptin, adiponectin, apelin and visfatin. The dysregulation of adipokines has been implicated in metabolic and cardiovascular diseases (17, 18). Adipokines, like leptin, not only regulate the feeding behavior and energy expenditure but are also involved in the regulation of inflammatory responses (17, 19-21).

Several aspects of adipose-related physiology exhibit daily oscillations. Distinct circadian rhythms in the circulating concentrations of adipokines have been observed in animals and humans. This circadian rhythm which can be entrained by feeding time have been dampened in obese subjects (22-25). Leptin shows circadian oscillations under normal feeding conditions, peaking during the early dark phase in humans and diurnal animals, increasing after food intake and decreasing during fasting (26). As shown in an animal model, the diurnal leptin rhythm is dependent upon an intact SCN (24). These oscillations are essential to its function and have critical implications for the regulation of metabolism (27). Visfatin concentrations also showed a diurnal rhythm peaking in the afternoon, whereas sleep deprivation induced a visfatin rhythm phase shift which was positively related to the impairment of postprandial glucose metabolism due to sleep loss (23). However, most of these studies have been performed with a retained light-dark cycle and the observed rhythms were not necessarily circadian in nature but may depend upon by the presence of a light/dark cycle.

It has been demonstrated that physical activity could also affect the functioning of the SCN and peripheral clocks (28-30), and could reverse disruption of peripheral circadian clocks caused by exposure to constant light (31). Exercise has been also shown to influence the release of adipokines (32). The presence of paraendocrine and endocrine mediators produced by skeletal muscle, called 'myokines', has recently been demonstrated. The myokines activity seems to mimic the function of certain adipokines (produced by adipose tissue) suggesting the functional cross-talk between skeletal muscle and adipose tissue in the anti-inflammatory mechanisms (33-36).

The regulation of hunger and satiety is essential in the control of energy balance. Feeding behavior is a complex interaction of several processes regulating the initiation and termination of meals, their size, composition and frequency, and the long-term control of food intake is adapted to body energy requirements and regulated by the neuroendocrine system (37). However, the question remains how different types of exercise can affect appetite regulation and energy intake (38-40). For instance, the sprint interval training is considered as an alternative to endurance exercise and has recently aroused great attention (41) but its effects on energy intake and hunger sensation have not been fully elucidated. To date, the impact of time of day and time of feeding on the release of adipokines (e.g. leptin, apelin and visfatin) during short-term maximal exercise

has not been investigated. Therefore, the aim of the present study was to evaluate the effects of time of day, high-intensity and short-duration physical activity by a means of a 30-second Wingate test. This test has been proven as a reliable tool for the assessment of physiological responses to supra-maximal exercise (42) on subjective perception of hunger sensations, *ad libitum* food intake and plasma levels of adipokines (leptin, apelin, visfatin) in experiments with and without a test meal.

## MATERIALS AND METHODS

### Participants

To participate in the study, twenty-four moderately active non-smoking males (mean  $\pm$  S.D. age:  $27.1 \pm 3.1$ ; height:  $1.79 \pm 0.1$  meters; weight:  $76.1 \pm 11.7$  kilograms) were recruited from among trainee firefighters. The trainees were billeted in a firefighters' training center for three months, which allowed the investigators to control the subjects' sleep time, the time and composition of their meals and their physical activities.

Age to the nearest month, height to the nearest 0.25 cm, and weight to the nearest 0.10 kg were determined for each subject. None of the subjects had signs or symptoms of acute or chronic disease nor were any of them taking any medications or undertaking regular sprint-type training. The volunteers were fully informed of the study details and gave their written consent prior to participation.

The study protocol was approved by the Jagiellonian University Bioethics Committee and all procedures complied with the Declaration of Helsinki.

Following an initial session of familiarization with the test procedure, the subjects completed two experimental trials using a randomized counterbalanced design: one at 12:00 hours (D) and the other at 24:00 hours (N). The two test sessions were performed on two separate days, with a minimal period of 14 days between trials. The subjects were requested to consume their last standard isocaloric meals at least 12 hours before each test session. Water was allowed *ad libitum*. Subjects adhered to their usual sleeping habits as closely as possible and maintained their habitual physical activity but avoided strenuous activities, alcohol and caffeine for 24 hours prior to each test. Throughout the testing period, mean ambient temperature and relative humidity of the laboratory were kept stable ( $21.7 \pm 1.1^\circ\text{C}$  and  $48.2 \pm 4.1\%$ , respectively).

To investigate the effect of exercise on subjective appetite sensations, food intake and hormonal and metabolic parameters, the subjects were divided in two groups: one with an *ad libitum* test meal (M) and one without an *ad libitum* test meal (WM). Each participant took part in the following studies performed at 11:00 and 23:00 hours on separate days: 1) exercise study (ES): a 30-second Wingate Anaerobic Test (WAnT); 2) sedentary study (SS): participants rested for the entire duration of the trial.

The Wingate test was conducted on a friction-loaded cycle ergometer (Monark Exercise AB 894E Peak Bike Anaerobic Testing Ergometer, Varberg, Sweden) equipped with Windows-based software and interfaced with a computer. The seat height and handlebars were adjusted appropriately for each subject. The Wingate test consisted of a 30-second maximal sprint against constant resistance related to body mass (7.5% of body mass). The WAnT began from a rolling start at 60 rpm against minimal resistance (weight basket supported). When a constant pedal rate of 60 rpm was achieved, a countdown was started. With less than 1 second left in the countdown test, resistance was instantaneously applied. All subjects were verbally encouraged to continue to pedal as fast as they could for the entire period of 30 seconds. Every second, power output was calculated and stored by the computer. The highest power output over 1 second (corresponding

to the ratio between the total work done and the time to do it, i.e. 30 seconds) was recorded at the end of the test. Peak power ( $P_{\text{peak}}$ ), mean power ( $P_{\text{mean}}$ ) and minimum power were calculated by the computer and recorded in watts (W) and watts per kilogram of body weight ( $\text{W}/\text{kg}^{-1}$ ). Peak power was the highest mechanical power elicited during the test. The index was used as the highest average power during any given 5-second period. Mean power was the average power sustained throughout the 30-second period. Minimum power was determined from the lowest power output during the test averaged over 5 consecutive seconds. Minimum power was used to determine the fatigue index (decrease in power). The fatigue index was calculated as a percentage of peak power minus minimum power divided by peak power and multiplied by 100 (43).

After the end of 15 minutes of each period exercise or in the corresponding time in sedentary group, a test meal was introduced within another 30 minutes each followed by postprandial observation on fed individuals or rest time in the control group. In resting condition the participants remained seated and were allowed to read/write quietly. For the test meal, the subjects were served sandwiches made with bread, butter and ham (2.73 kcal/g, energy percentage: 44.4% carbohydrate, 16.2% protein and 39.4% fat) to be eaten until satiety has been reached. The total amount of food was assessed by weighing food items to the nearest 0.1 gram and the energy content (kcal) was determined as described previously (38).

Appetite perceptions were assessed using the visual analogue scale (VAS) in accordance with current recommendations (44). The subjects rated their subjective feelings of 'hunger' ('how hungry do you feel?') and 'prospective consumption' ('how much do you think you could eat right now?') on 100 mm scales at (1) baseline, (2) immediately post exercise (or at the same time in sedentary series), (3) immediately post standard meal (or at the same time in the without-meal series), and (4) 30 minutes later. Furthermore, Borg's Rating of Perceived Exertion (RPE) Scale was used to assess subjective perception of effort directly after each bout of exercise (45).

In all the experiments, venous blood samples were obtained from the subjects' antecubital veins 30 minutes before exercise, immediately after exercise, immediately after meals, and 30 minutes following meals (or within a corresponding time in control tests). The samples were collected in test tubes containing ethylenediaminetetraacetic acid (EDTA) and aprotinin, and in tubes without anticoagulants. The test tubes were immediately delivered to a laboratory and centrifuged for 15 minutes at 3,000 rpm (horizontal rotor). Plasma hormone levels were measured using the commercially available EIA kits for leptin (R&D Systems, Inc., USA), apelin and visfatin (Phoenix Pharmaceuticals Q7, Inc., USA) in a laboratory at the Department of Clinical Biochemistry, Jagiellonian University Medical College, as described previously (46, 47). At each sampling point, duplicate 20  $\mu\text{L}$  blood samples were collected into micropipettes for the determination of hemoglobin, and 20  $\mu\text{L}$  blood samples were collected in triplicates into heparinized micro-hematocrit tubes for the determination of blood hematocrit concentration. Plasma lactate concentration ( $[\text{La}]\text{p}$ ) was measured using an automatic analyzer (Ektachem XR 700, Kodak, USA), as described previously (38).

### Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate normal distribution of continuous data. The paired Student's t-test (for normally distributed variables) or the Wilcoxon matched paired ranked-signs test (for skewed distribution) were used to compare changes between measurements.  $P < 0.05$  was considered as statistically significant. All statistical analyses were performed using SPSS version 20 for Windows.

## RESULTS

### Exercise responses

The results of the WAnT variables and RPE results calculated at the two time-of-day conditions are displayed in *Table 1*. There were no significant effects of the time of day (TOD) on the  $P_{\text{peak}}$ ,  $P_{\text{mean}}$ , FI or RPE, although  $P_{\text{peak}}$  and  $P_{\text{mean}}$  values were slightly lower at 24:00 hours as compared with respective values recorded with 12 h.

The single WAnT bout caused pronounced increases in plasma lactate from the baseline of  $1.02 \pm 0.02$  mmol/L to  $13.1 \pm 0.05$  mmol/L. In all the experiments heart rate at baseline was reaches the value  $72.4 \pm 8.5$  bpm. The exercise caused an increase to  $181.7 \pm 14.1$  bpm and there were no significant effects of the time of day or test meal on the results. No significant changes in either hemoglobin or hematocrit were observed over time during exercise or the resting sessions. Hemoconcentration was thus unlikely to have occurred during the exercise sessions performed in the present study.

### Blood analyses

No significant differences between trials existed in the plasma concentrations of apelin, leptin, or visfatin at baseline (*Figs. 1-6*). Plasma apelin concentrations are presented in *Figs. 1 and 4*. The plasma apelin concentrations after WAnT at 12:00 hours were significantly higher ( $P < 0.05$ ) than pre-exercise values and the apelin values tended to decrease in the subsequent part of the experiment for both groups, with or without the test meal yet they still remained significantly higher than basal values. In sedentary experiments, we observed a modest, yet significant ( $P < 0.05$ ), rise in plasma apelin level after the test meal at 12:00 hours; however, this increase in plasma apelin was not observed at 24:00 hours. The plasma apelin levels at 24:00 hours were however higher than those at 12:00 hours in all types of experiments. The small increase in plasma apelin levels observed at 24:00 hours after WAnT failed to reach a statistical significance.

The leptin concentrations are presented in *Fig. 2 and Fig. 5*. The plasma leptin concentrations remained unchanged after exercise at 12:00 hours but after the test meal small but statistically insignificant increase in leptin concentrations was observed in both sedentary and exercise experiments. As expected, leptin plasma levels at 24:00 hours were much higher than at 12:00 hours throughout the entire duration of the experiment. Leptin values after WAnT at 24:00 hours tended to decrease but this change failed to reach statistical significance.

*Table 1.* Time-of-day effects on peak power ( $P_{\text{peak}}$ ), mean power ( $P_{\text{mean}}$ ), fatigue index (FI) and RPE ( $n = 24$ ).

Time (h)	$P_{\text{peak}}$ (W)	$P_{\text{mean}}$ (W)	FI (%)	$P_{\text{peak}}$ ( $\text{W}/\text{kg}$ )	$P_{\text{mean}}$ ( $\text{W}/\text{kg}$ )	RPE
12:00	772.55 $\pm$ 128.25	584.40 $\pm$ 81.28	55.21 $\pm$ 9.72	9.74 $\pm$ 1.33	7.38 $\pm$ 0.88	17.1 $\pm$ 1.03
24:00	732.32 $\pm$ 110.04	561.54 $\pm$ 75.37	55.65 $\pm$ 17.39	9.26 $\pm$ 1.20	7.10 $\pm$ 0.77	17.5 $\pm$ 1.96

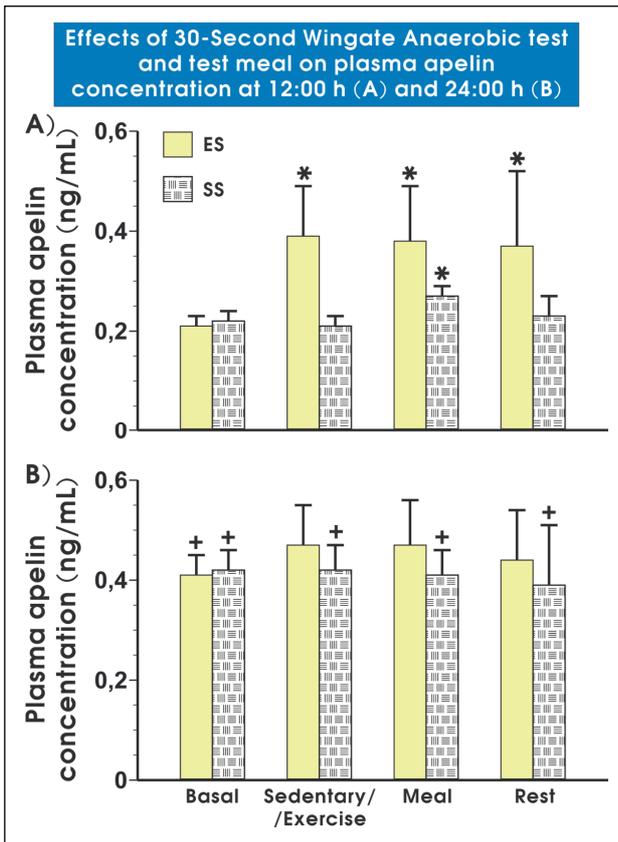


Fig. 1. Effects of the 30-second Wingate Anaerobic Test and test meal on plasma apelin concentrations at 12:00 hours (A) and 24:00 hours (B). Basal: represents values at baseline, Exercise/Sedentary: immediately post exercise (or at the same time in sedentary series), Meal: immediately post standard meal, Rest: 30 minutes later. ES, exercise series; SS, Sedentary series. Values shown are mean  $\pm$  standard deviations,  $n = 24$ . \* indicates results significantly different from the basal ( $P < 0.05$ ), † indicates results significantly different from 12:00 hours ( $P < 0.05$ ).

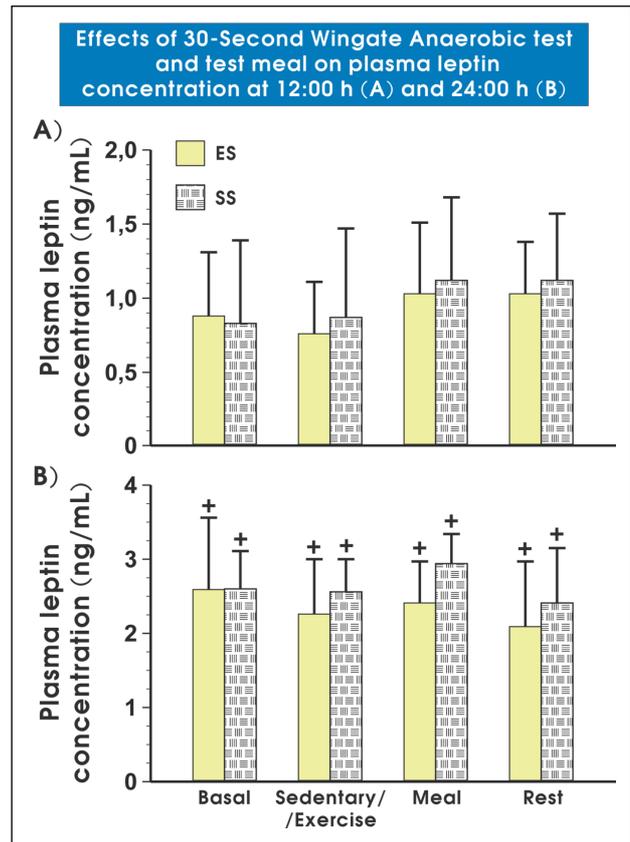


Fig. 2. Effects of the 30-second Wingate Anaerobic Test and test meal on plasma leptin concentrations at 12:00 hours (A) and 24:00 hours (B). Basal: represents values at baseline, Exercise/Sedentary: immediately post exercise (or at the same time in sedentary series), Meal: immediately post standard meal, Rest: 30 minutes later. ES, exercise series; SS, Sedentary series. Values shown are mean  $\pm$  standard deviations,  $n = 24$ . † indicates results significantly different from 12:00 hours ( $P < 0.05$ ).

The test meal caused a small increase in plasma leptin levels in the sedentary series but this effect was not statistically significant.

As depicted in Fig. 3 and Fig. 6, the basal plasma visfatin levels were significantly higher at 24:00 hours than at 12:00 hours ( $P < 0.05$ ). There was a significant decrease in plasma concentrations after exercise intervention, both at the 12:00 hours and 24:00 hours test ( $P < 0.05$ ). The test meal caused a significant rise in visfatin concentrations in the sedentary series ( $P < 0.05$ ) but not in the exercise series, in the daytime and nighttime tests.

#### Post-exercise ad-libitum energy intake

The effects of exercise and the time of day on energy intake are presented in Fig. 7. Energy intake following WAnT was lower than energy intake in the sedentary series. Time of the day had no statistically significant effect on energy intake.

#### Perception of appetite

Fasting appetite (hunger and prospective consumption) did not differ between trials prior to exercise, as shown in Tables 1 and 2. Hunger and prospective consumption scores decreased significantly ( $P < 0.05$ ) immediately upon the cessation of sprint

exercise and that tendency was maintained at 30 minutes after exercise. Following the test meal, hunger and motivation-to-eat ratings significantly decreased ( $P < 0.05$ ) as compared to the respective values in the exercising subjects without meals. Hunger and prospective consumption ratings after the test meal were significantly higher at 24:00 hours than at 12:00 hours ( $P < 0.05$ ).

## DISCUSSION

The present study investigated the time-of-day effects on adipokine profiles, appetite perceptions (hunger and prospective consumption) and subsequent food intake in non-obese young men performing the Wingate test. The WAnT decreased the energy intake at the *ad libitum* meal compared to sedentary series. We also observed that hunger and prospective consumption ratings were significantly reduced after exercise. The observation that appetite perceptions were suppressed after intensive exercise is consistent with previous studies showing that intensive exercise induced a transient suppression of appetite called 'exercise-induced anorexia' (38, 48, 49). On the other hand, in the present study the time of day had no statistically significant effect on energy intake. However, the hunger and prospective consumption ratings in the sedentary

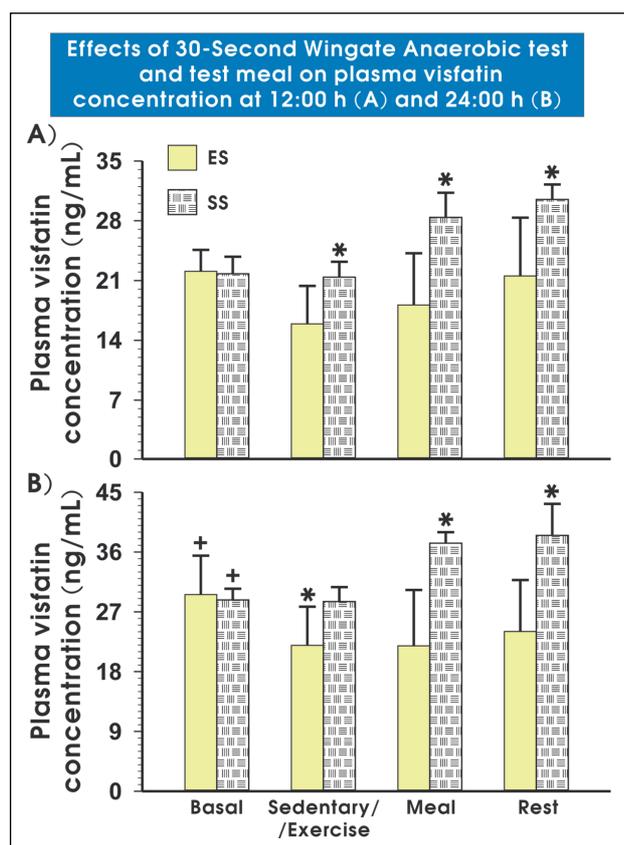


Fig. 3. Effects of the 30-second Wingate Anaerobic Test and test meal on plasma visfatin concentrations at 12:00 hours (A) and 24:00 hours (B). Basal: represents values at baseline, Exercise/Sedentary: immediately post exercise (or at the same time in sedentary series), Meal: immediately post standard meal, Rest: 30 minutes later. ES, exercise series; SS, Sedentary series. Values shown are mean  $\pm$  standard deviations,  $n = 24$ . \* indicates results significantly different from the basal ( $P < 0.05$ ), † indicates results significantly different from 12:00 hours ( $P < 0.05$ ).

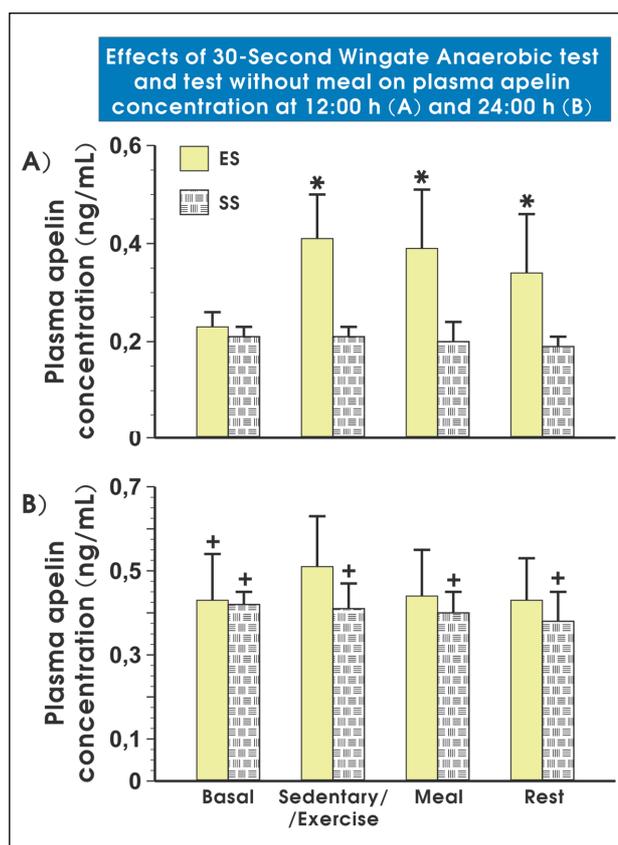


Fig. 4. Effects of the 30-second Wingate Anaerobic Test alone (without test meal) on plasma apelin concentrations at 12:00 hours (A) and 24:00 hours (B). Basal: represents values at baseline, Exercise/Sedentary: immediately post exercise (or at the same time in sedentary series), Without meal (at the same time as meal), Rest: 30 minutes later. ES, exercise series; SS, Sedentary series. Values shown are mean  $\pm$  standard deviations,  $n = 24$ . \* indicates results significantly different from the basal ( $P < 0.05$ ), † indicates results significantly different from 12:00 hours ( $P < 0.05$ ).

Table 2. Hunger ratings ('how hungry are you?') assessed with visual analog scale (VAS) at measurement 1 (Basal), 2 (Right after exercise or at the same time in sedentary series), 3 (Right after *ad libitum* meal or at the same time in the without-meal series) and 4 (Rest, 30 minutes later). Ratings for hunger are referenced by 0 mm = not at all hungry, 100 mm = extremely hungry.

	Basal	Exercise/ Sedentary	Meal/ Without meal	Rest
ES WM 12:00 h	62.0 $\pm$ 7.1	33.3 $\pm$ 5.9*	40.8 $\pm$ 4.7*	48.9 $\pm$ 3.9*
ES WM 24:00 h	58.9 $\pm$ 9.3	37.6 $\pm$ 7.4*	44.4 $\pm$ 6.4*	48.6 $\pm$ 4.4*
ES M 12:00 h	59.8 $\pm$ 8.9	31.5 $\pm$ 4.7*	19.7 $\pm$ 3.9*	16.7 $\pm$ 3.6*
ES M 24:00 h	62.5 $\pm$ 9.7	31.7 $\pm$ 3.9*	17.8 $\pm$ 3.2*	12.8 $\pm$ 1.9*
SS WM 12:00 h	68.8 $\pm$ 6.8	71.7 $\pm$ 8.8	71.5 $\pm$ 9.8	72.2 $\pm$ 8.7
SS WM 24:00 h	67.0 $\pm$ 9.4	70.9 $\pm$ 9.4	72.2 $\pm$ 9.5	71.5 $\pm$ 9.2
SS M 12:00 h	65.2 $\pm$ 8.1	72.1 $\pm$ 8.6	10.2 $\pm$ 4.1*	13.6 $\pm$ 2.1*
SS M 24:00 h	68.0 $\pm$ 9.9	67.2 $\pm$ 9.8	18.3 $\pm$ 3.6*†	19.8 $\pm$ 1.8*†

ES, Exercise Series; SS, Sedentary Series; M, Meal (with test meal); WM, Without Meal. Values shown are means  $\pm$  standard deviations. \* indicates results significantly different from the control (SS) group ( $P < 0.05$ ), † indicates results significantly different from 12:00 hours ( $P < 0.05$ ),  $n = 24$ .

group after the test meal were statistically lower at 24:00 hours than at 12:00 hours. Interestingly, these ratings were not influenced by exercise.

Studies performed in shift workers and patients with Night Eating Syndrome clearly suggest that nighttime meals may exert a deleterious metabolic consequences (50). Similarly, studies in

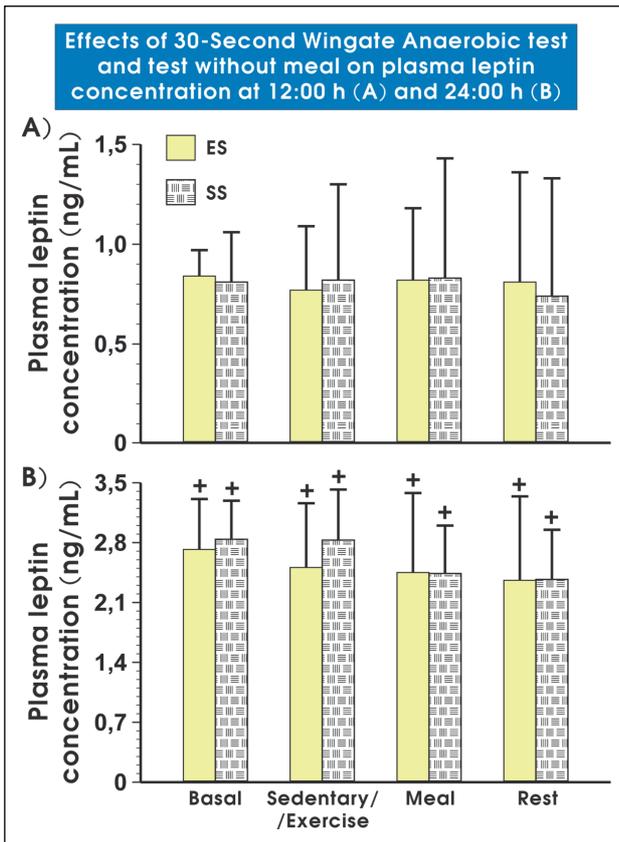


Fig. 5. Effects of the 30-second Wingate Anaerobic Test alone (without test meal) on plasma leptin concentrations at 12:00 hours (A) and 24:00 hours (B). Basal: represents values at baseline, Exercise/Sedentary: immediately post exercise (or at the same time in sedentary series), Without meal (at the same time as meal), Rest: 30 minutes later. ES, exercise series, SS, Sedentary series. Values shown are mean ± standard deviations, n = 24. † indicates results significantly different from 12:00 hours (P < 0.05).

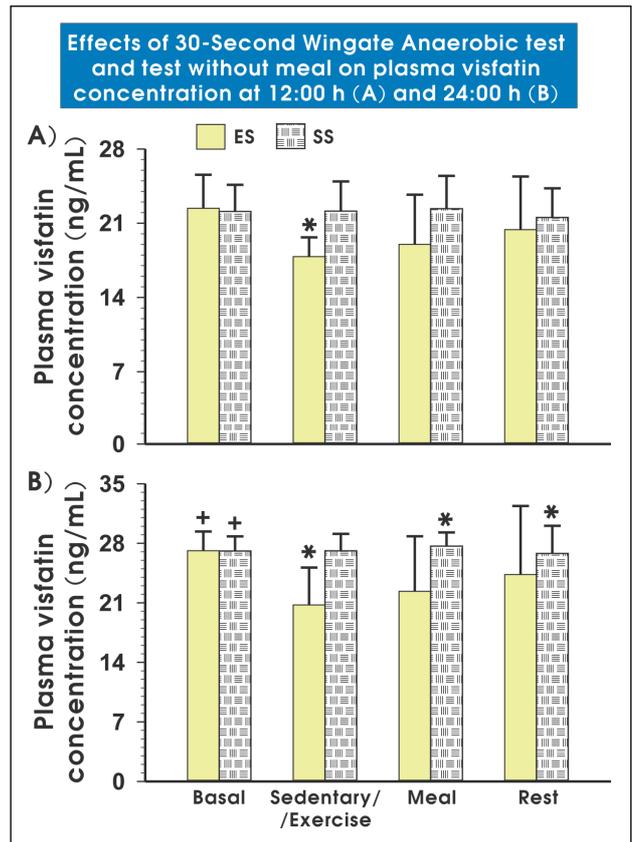


Fig. 6. Effects of the 30-second Wingate Anaerobic Test and test meal on plasma visfatin concentrations at 12:00 hours (A) and 24:00 hours (B). Basal: represents values at baseline, Exercise/Sedentary: immediately post exercise (or at the same time in sedentary series), Without meal (at the same time as meal), Rest: 30 minutes later. ES, exercise series; SS, Sedentary series. Values shown are mean ± standard deviations, n = 24. \* indicates results significantly different from the basal (P < 0.05), † indicates results significantly different from 12:00 hours (P < 0.05).

Table 3. Prospective consumption ratings ('how much do you think you could eat right now?') assessed with visual analog scale (VAS) at measurement 1 (Basal), 2 (Right after exercise or at the same time in sedentary series, SS), 3 (Right after *ad libitum* buffet or at the same time in the without-meal series, WM) and 4 (Rest, 30 minutes later). Ratings for prospective consumption are referenced by 0 mm = nothing at all, 100 mm = a very large amount.

	Basal	Exercise/ Sedentary	Meal/ Without meal	Rest
ES WM 12:00 h	60.7 ± 7.8	34.4 ± 3.8*	44.6 ± 5.2*	51.0 ± 5.9*
ES WM 24:00 h	60.2 ± 5.9	33.6 ± 4.2*	41.3 ± 4.9*	47.7 ± 5.3*
ES M 12:00 h	60.8 ± 6.9	32.9 ± 3.9*	16.3 ± 2.7*	23.7 ± 3.2*
ES M 24:00 h	61.5 ± 7.4	36.5 ± 4.3*	19.52 ± 2.1*	14.5 ± 2.6*
SS WM 12:00 h	59.9 ± 6.7	70.6 ± 7.4	77.1 ± 7.2	71.5 ± 7.6
SS WM 24:00 h	71.6 ± 8.1	74.5 ± 7.2	74.0 ± 7.8	73.8 ± 8.3
SS M 12:00 h	70.6 ± 7.9	71.7 ± 7.8	10.9 ± 2.4*	13.8 ± 2.7*
SS M 24:00 h	62.9 ± 7.0	67.0 ± 8.3	18.1 ± 1.8*†	15.8 ± 3.2*

ES, Exercise Series; SS, Sedentary Series; M, Meal (with test meal); WM, Without Meal. Values shown are means ± standard deviations. \* indicates results significantly different from the control (SS) group (P < 0.05), † indicates results significantly different from 12:00 hours (P < 0.05), n = 24.

healthy subjects demonstrate that postprandial satiety can fluctuate with time of day and that food consumption at nighttime is less satiating and leads to greater energy intake than morning

consumption (51). Kinsey *et al.* (52) observed the morning increments in insulin levels and insulin resistance in response to nighttime meals. A particularly interesting observation is that

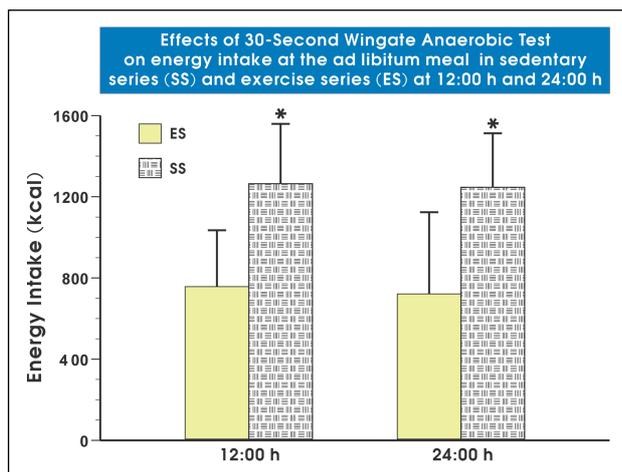


Fig. 7. Effects of the 30-second Wingate Anaerobic Test on energy intake at the *ad libitum* meal in sedentary series (SS) and exercise series (ES) at 12:00 hours and 24:00 hours. Values shown are means  $\pm$  standard deviations. \* indicates results significantly different from the control (SS) group ( $P < 0.05$ ),  $n = 24$ .

those effects were prevented by exercise training (52, 53). Wang *et al.* (51) demonstrated that morning food intake is less associated with the risk of obesity than an evening meal. Experiments on animals confirmed that meal timing is relevant to obesity. Mice on a high-fat diet fed at their physiological feeding time only (during the dark phase) weighed significantly less than the mice fed only during the time when feeding is normally reduced (during the light phase) although the caloric intake did not differ significantly between these two groups (54).

The mechanisms linking meal timing and weight control are not well understood but the role of hormonal factors have been implicated. Alterations in timing of food intake modify the diurnal rhythms of many hormones involved in metabolism such as insulin, glucagon, glucocorticosteroids and adipokines (22).

In the present study we observed that at rest leptin, visfatin and apelin concentrations fluctuated with the time of day, at 24:00 hours being higher than at 12:00 hours. The leptin diurnal rhythm, present in humans and rodents, is thought to be the consequence of both the internal clock-regulated and the feeding-regulated mechanisms (55). It has been shown that plasma leptin exerts a strong diurnal rhythm with a zenith at 24:00 hours, and nadir between 09:00 and 12:00 hours (55). Similarly, in the present study plasma leptin at 24:00 hours after twelve hours of fasting was much higher than at 12:00 hours. Neither exercise nor test meals significantly changed plasma leptin levels, although they had a tendency to increase after meals at 12:00 hours. Physiologically, the highest concentrations of plasma leptin levels are observed during the sleep phase, when hunger is blocked and adipose tissues use lipolysis to sustain metabolic turnover. Disrupted leptin diurnal rhythms, linked with reduced satiety and obesity, are frequently described in shift workers (9, 13).

Large body of experimental evidence indicates that another adipokine, visfatin (identical to the cytokine pre-B-cell colony-enhancing factor, PBEF), and the enzyme nicotinamide phosphoribosyltransferase (Namp1) can play an important role in the intracellular and extracellular metabolic effects associated with obesity (56). Visfatin was initially described as having insulin-mimetic properties but that claim has been retracted (56, 57). Elevated plasma visfatin concentrations in metabolic and inflammatory disorders are well documented because visfatin is often overexpressed in tumor tissues and its targeting is considered a promising anti-tumor strategy (58-62). The elevation

of plasma visfatin levels seen in subjects with type 2 diabetes and obesity may be due to visfatin's proinflammatory action (63).

In the present study, the short-term intensive exercise caused a significant decrease in plasma visfatin. In previous studies contradictory effects of exercise on plasma visfatin levels were observed. Some authors reported reduction (64-66) in plasma visfatin, while others found no change (67, 68) or even elevation of plasma visfatin (69). Such contradictory results can be explained by the use of different bout of exercise in various studies. However, Vatani *et al.* (66) compared different exercise protocols and observed reduced serum visfatin in all studies suggesting that this decrease in plasma visfatin levels seems to be unrelated of the type of exercise. The observed differences may also result from the fact that visfatin can be produced not only by adipose tissue but also by skeletal muscle, liver and immune cells (56,70). It was demonstrated that skeletal muscle exhibited a greater amount of visfatin compared with visceral adipose tissue and exercise further enhanced the expression of skeletal muscle visfatin (71). Differences in experimental data can also result from the time of testing. It is well known that visfatin concentrations follow a diurnal rhythm, peaking in the afternoon (23). In our study, we observed that the level of visfatin was higher at night than at noon, but the difference was relatively small compared to other examined adipokines at the same conditions. This is consistent with the fact that our test time occurred much later than the visfatin acrophase. The observation that plasma visfatin levels seem to be affected by sleep duration allowed us to propose its involvement in deleterious metabolic effects of sleep deprivation (23, 62, 72). In our study, we observed a marked increase in the level of visfatin after the test meal. To our knowledge, this is the first study to examine the effect of high-intensity exercise on postprandial release of visfatin. We have also demonstrated that intense short exercise prevented the postprandial rise of visfatin.

Apelin is involved in the regulation of cardiovascular and fluid homeostasis, food intake, cell proliferation, and angiogenesis (73). In addition, apelin is considered as an adipokine playing an important role in the regulation of glucose energy metabolism. Plasma apelin concentrations have been shown to increase in individuals with obesity and type 2 diabetes (74). Apelin has been shown to increase insulin sensitivity *via* the activation of its receptor and was claimed as a promising target for type 2 diabetes treatment (73).

In the present study, we observed a marked significant increase in plasma apelin levels after Wingate test at 12:00 hours, but surprisingly, it was not altered at 24:00 hours. Our observation is consistent with the results of recent studies showing an increase in plasma apelin levels in response to maximal exercise in humans (75). Thus apelin release may be required to meet cardiovascular demands of exercise (75). Studies in type 2 diabetes patients demonstrated that chronic exercise training can lead to an increase in plasma apelin levels. After 12 weeks of aerobic training a significant increase in plasma apelin levels was observed in obese patients with type 2 diabetes (76), however, this rise in plasma apelin increments remained without effect on the body weight changes observed after training in those patients. In another study, the aerobic (but not resistance) training caused a significant rise in plasma apelin levels (77). Similar observations were made in an animal study, in which both, the chronic and the acute exercise elevated apelin serum levels in healthy rats (78, 79). In contrast, another human study has revealed that after eight weeks of aerobic training in obese women, a decrease in plasma levels of apelin was observed only if the body mass index and body fat mass decreased simultaneously (80). Besse-Patin *et al.* (81) reported lack of major changes in plasma apelin concentrations after exercise training in obese non-diabetic patients, however, they have demonstrated that exercise training indeed increased expression of this adipokine in the skeletal muscle but not adipose tissue. This apelin expression

was induced by exercise signaling pathways *in vivo* and this peptide secreted *in vitro* using human primary myotubes. Thus, the data accumulated so far suggest that apelin acts as an exercise-regulated myokine (81). Similarly as in the case of visfatin, contradictory results can be explained by the different bouts of exercise employed in various studies as well as possible different sources of apelin (adipose tissue vs. skeletal muscle) depending on the duration and intensity of exercise.

The effects of feeding on the diurnal plasma apelin levels in relation to exercise have not been extensively studied. In the present study, we observed that test meals led to a small, yet sustained increase in plasma apelin levels during daytime, but not during nighttime. When short intense exercise preceded the meal, there was no postprandial increase in plasma apelin levels. Another important finding of the present study is the observation that apelin concentrations have exhibited a diurnal rhythm showing values higher during the night than at noon. To our best knowledge this is the first such observation in humans. To date, the only similar observation has been made in mice, with acrophase in the afternoon (82). It is interesting that in the present study stimulatory effects of exercise and feeding on plasma apelin concentrations were present only at 12:00 hours and not at 24:00 hours.

The observed differences in the concentrations of adipokines may be, at least in part, due to changes in performance associated with the time of day. It has been well established that there are diurnal changes of muscle power with morning nadirs and afternoon maximum values. Several studies demonstrated that during the Wingate test, the values of  $P_{peak}$  and  $P_{mean}$  fluctuate with time of day, with an acrophase at about 18:00 hours (83, 84). However, in the present study we did not observe any significant time-of-day effect on peak power or mean power although their values were slightly lower at 24:00 hours, yet that is not inconsistent with the mentioned observations (80, 81). In those studies,  $P_{peak}$  and  $P_{mean}$  were measured at 6:00, 10:00 and 14:00 and later at 18:00, 22:00 and 2:00 hours, and the predicted values for the 12:00 and 24:00 hours were very close to those observed in our experiment (83, 84).

Scheduled feeding and exercise are powerful synchronizers of peripheral clocks (31). Experiments on rodents have shown that timed food intake could be an appropriate method to minimize adverse effects of shift work (85, 86). It has also been demonstrated that physical activity could reverse disruption of peripheral circadian clocks caused by exposure to constant light (31). Previous studies recommended that regular physical activity can protect against several chronic diseases such as obesity and type 2 diabetes, leading to enhanced insulin sensitivity and metabolic flexibility (87). The advantageous effects of physical activity may be partly mediated through the release of myokines (33, 88). Myokines may counteract the deleterious effects of some adipokines participating in the cross-talk between skeletal muscle and adipose tissue (35, 36). Apelin may also be released during skeletal muscle contraction, which suggests that apelin may act as a myokine (81).

The results of the present study show that time of day is an important aspect to consider in the relationships among exercise, metabolism and appetite. Further studies are needed to explain the specific mechanisms underlying the effects of acute exercise on postprandial physiology at different times of day.

The present study is limited to healthy non-obese young men and therefore, these findings may not apply to women or obese subjects. Furthermore, the present investigation examined acute responses to a single bout of exercise not providing the information about the hormonal alterations associated with chronic exercise. Therefore, future studies should address these issues in different populations subjected to various bouts of exercise. It seems possible that a better understanding of the circadian regulation of the human metabolism will allow to propose of a timed dietary

intake and exercise as physiological recommendations to neutralize some harmful effects of circadian desynchronization which affects a large part of modern westernized society.

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