Original article

MOLECULAR METABOLISM

Demonstration of a day-night rhythm in human skeletal muscle oxidative capacity

Dirk van Moorsel [1,2,8], Jan Hansen [1,8], Bas Havekes [1,2], Frank A.J.L. Scheer [3,4], Johanna A. Jörgensen [1], Joris Hoeks [1], Vera B. Schrauwen-Hinderling [1,5], Helene Duez [6], Philippe Lefebvre [6], Nicolaas C. Schaper [2,7], Matthijs K.C. Hesselink [1], Bart Staels [6], Patrick Schrauwen [1,*]

ABSTRACT

Objective: A disturbed day-night rhythm is associated with metabolic perturbations that can lead to obesity and type 2 diabetes mellitus (T2DM). In skeletal muscle, a reduced oxidative capacity is also associated with the development of T2DM. However, whether oxidative capacity in skeletal muscle displays a day-night rhythm in humans has so far not been investigated.

Methods: Lean, healthy subjects were enrolled in a standardized living protocol with regular meals, physical activity and sleep to reflect our everyday lifestyle. Mitochondrial oxidative capacity was examined in skeletal muscle biopsies taken at five time points within a 24-hour period.

Results: Core-body temperature was lower during the early night, confirming a normal day-night rhythm. Skeletal muscle oxidative capacity demonstrated a robust day-night rhythm, with a significant time effect in ADP-stimulated respiration (state 3 MO, state 3 MOG and state 3 MOGS, $p < 0.05$). Respiration was lowest at 1 PM and highest at 11 PM (state 3 MOGS: 80.6 $\pm$ 4.0 vs. 95.8 $\pm$ 4.7 pmol/mg/s). Interestingly, the fluctuation in mitochondrial function was also observed in whole-body energy expenditure, with peak energy expenditure at 11 PM and lowest energy expenditure at 4 AM ($p < 0.001$). In addition, we demonstrate rhythmicity in mRNA expression of molecular clock genes in human skeletal muscle.

Conclusions: Our results suggest that the biological clock drives robust rhythms in human skeletal muscle oxidative metabolism. It is tempting to speculate that disruption of these rhythms contribute to the deterioration of metabolic health associated with circadian misalignment.

Keywords Biological rhythm; Mitochondria; Oxidative capacity; Skeletal muscle; Energy metabolism; Molecular clock

1. INTRODUCTION

Many metabolic processes are synchronized to day-night cycles by the circadian clock, thereby anticipating changes in metabolic activity associated with feeding or fasting and physical activity or rest [1]. In our modern "24/7" society, however, many individuals do not adhere to the lifestyle imposed upon us by nature. In this respect, epidemiological studies have shown that circadian misalignment — desynchronization between the intrinsic circadian and behavioral cycles, as is typical in shift-work — is associated with obesity, insulin resistance and type 2 diabetes mellitus (T2DM) [2—5]. Moreover, intervention studies have shown that challenging behavior by controlled circadian misalignment results in metabolic aberrations like decreased glucose tolerance and insulin sensitivity [6—8].

Circadian rhythms are governed by a central circadian clock, which is situated in the suprachiasmatic nucleus of the hypothalamus and is sensitive to light as the most important time cue (*Zeitgeber*) [9]. Interestingly, peripheral tissues have their own clocks. These peripheral clocks are synchronized by the central clock, but they can also be influenced by behavior, such as feeding or exercise [10,11]. The peripheral clock consists of transcriptional-translational feedback loops. The positive loop consists of the heterodimer of the CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle ARNT-like 1) proteins. The negative feedback loop is mediated via

[1]Department of Human Biology and Human Movement Sciences, NUTRIM School for Nutrition and Translational Research in Metabolism, Maastricht University Medical Center, PO Box 616, 6200 MD Maastricht, The Netherlands [2]Department of Internal Medicine, Division of Endocrinology, Maastricht University Medical Center, PO Box 5800, 6202 AZ Maastricht, The Netherlands [3]Medical Chronobiology Program, Division of Sleep and Circadian Disorders, Brigham and Women's Hospital, Boston, MA 02115, USA [4]Division of Sleep Medicine, Harvard Medical School, Boston, MA 02115, USA [5]Department of Radiology, Maastricht University Medical Center, PO Box 5800, 6202 AZ Maastricht, The Netherlands [6]Univ Lille, Inserm, Institut Pasteur de Lille, UMR1011-EGID, BP245, 59019 Lille, France [7]CAPHRI School for Public Health and Primary Care, Maastricht University Medical Center, Maastricht, The Netherlands

[8] Dirk van Moorsel and Jan Hansen contributed equally to this work.

*Corresponding author. Department of Human Biology and Human Movement Sciences, Maastricht University Medical Center, PO Box 616, 6200 MD Maastricht, The Netherlands. Tel.: +31 43 3881502. E-mail: p.schrauwen@maastrichtuniversity.nl (P. Schrauwen).

Abbreviations: BMAL1, brain and muscle ARNT-like 1; BMI, body mass index; CLOCK, circadian locomotor output cycles kaput; CRY, cryptochrome; FCCP, carbonyl cyanide-4-trifluoromethoxyphenylhydrazone; NADH, reduced nicotinamide adenine dinucleotide; PER, period; RER, respiratory exchange ratio; RT-QPCR, Real-Time Quantitative Polymerase Chain Reaction; T2DM, type 2 diabetes mellitus; TCA cycle, tricarboxylic acid cycle

Received June 6, 2016 • Revision received June 23, 2016 • Accepted June 26, 2016 • Available online 1 July 2016

http://dx.doi.org/10.1016/j.molmet.2016.06.012

heterodimers of the proteins PER (Period) and CRY (Cryptochrome), which repress CLOCK/BMAL1-controlled gene expression [9,12]. Interestingly, in mouse and cell models, several components of the molecular clock have been causally linked to mitochondrial metabolism [13], mitochondrial integrity and density [14], mitochondrial dynamics [15] and metabolic flexibility [16]. So far however, it is unknown whether mitochondrial metabolism also displays a day-night rhythm in human skeletal muscle. Such data would be relevant, since reduced skeletal muscle oxidative capacity is associated with T2DM [17,18]. It is tempting to speculate that disturbances in the day-night rhythm may affect muscle mitochondrial metabolism and thereby deteriorate metabolic health.

Here, we investigated whether skeletal muscle mitochondrial function displays day-night rhythmicity by taking multiple muscle biopsies from healthy, lean volunteers within a 24 h period, under tightly controlled experimental conditions. We used a research setting that reflects real life conditions, with regular meals, physical activity and a regular sleep/wake cycle. For this reason we use the term "day-night rhythm" instead of "circadian rhythm" [19]. We here show that gene expression in muscle displays rhythmicity, which is specifically evident for the core components of the molecular clock. Furthermore, we are the first to show the presence of a day-night rhythm in human skeletal muscle oxidative capacity.

2. MATERIAL AND METHODS

2.1. Participants

Twelve young lean male Caucasian individuals (age ± SD: 22.2 ± 2.3 years, BMI ± SD: 22.4 ± 2.0 kg/m^2) participated in this study. The participants did not engage in exercise more than 3 h per week, were non-smokers, had no active diseases and used no medication, verified by a medical questionnaire. Participants were selected for having a regular sleep duration (normally 7–9 h/night), not having done shift work or having traveled across more than one time zone for at least 3 months. A morningness-eveningness questionnaire (MEQ-SA) was used to exclude extreme morning larks or night owls (MEQ-SA score mean ± SD: 50 ± 7). All participants provided written informed consent. The study was approved by the Ethics Committee of the Maastricht University Medical Center, monitored by the Clinical Trial Center Maastricht and conducted in accordance with the principles of the declaration of Helsinki. All measurements were performed between November 2014 and July 2015. The study was registered at clinicaltrials.gov with identifier NCT02261168.

2.2. Pre-study conditions

One week prior to the study, participants were instructed to maintain a standardized lifestyle. This lifestyle included (trying to) sleep every night from 11 PM until 7 AM, eating breakfast, lunch and dinner at regular times (at 9 AM, 2 PM and 7 PM) with no in-between snacks or drinks other than water. In this period subjects refrained from alcohol and caffeine. Participants were instructed not to engage in exercise three days prior to the study. Two days before the study, we provided standardized meals (see below) to ensure standardized caloric and macronutrient intake for all participants. Lifestyle was monitored by accelerometry (activPAL3 physical activity monitor, PAL Technologies, Glasgow, UK) together with food- and sleep-diaries, which were checked at the start of the first study-day.

2.3. Study design

Participants were admitted to the research unit at noon on study day 1 and stayed for 44 h in total, under standardized conditions mimicking a real-life situation. The first study-day was mainly used to standardize and monitor meals, physical activity and bedtime. Meals were provided at fixed times (9 AM, 2 PM and 7 PM). To prevent a sedentary lifestyle, participants went for a 15-minute low-intensity walk accompanied by a researcher, one hour after every meal. Directly hereafter, participants were instructed to stand for 15 minutes before they were allowed to sit again. In-between meals, physical activity and tests, the participants stayed in a respiration chamber; a small room with a bed, toilet, sink, desk, chair, TV and computer. During the first study-day we performed no measurements. At 11 PM, the lights of the respiration chamber were turned off and the participants were instructed to try to sleep. During this night, sleeping metabolic rate was measured by whole-room indirect calorimetry (Omnical, Maastricht Instruments, Maastricht, The Netherlands) [20].

The second study-day, participants were awakened at 6:30 AM. Hereafter, participants swallowed a telemetric pill for measurement of core-body temperature. Next, an intravenous cannula was placed in the forearm for subsequent blood-draws. The first blood-draw was at 8 AM, followed by an indirect calorimetry measurement using a ventilated hood while awake and at rest in supine posture to calculate resting energy expenditure and substrate oxidation. Directly hereafter, the first skeletal muscle biopsy was taken (described below). These measurements (blood draw, ventilated hood measurement and skeletal muscle biopsy) were repeated five times within 24 h: at 8 AM, 1 PM, 6 PM, 11 PM and 4 AM the next day. Additional blood samples were taken 2-hourly (10 AM, 12 PM, 2 PM, 4 PM, 8 PM, 10 PM, 0 AM, 2 AM, 6 AM and 8 AM). The timing of meals and physical activity was similar to study-day 1 and subjects stayed within the respiration chamber in-between measurements. After the 11 PM biopsy, participants went back to the respiration chamber to sleep with lights off. At 4 AM, the participant was awakened and the last measurements were performed, after which the subject was allowed to sleep until 7 AM. After the 8 AM blood draw the study protocol ended.

2.4. Study meals

Two days before the study and during the study participants were provided with standardized meals, according to Dutch and US dietary guidelines. Caloric intake for consumption at home was calculated by multiplying the estimated resting metabolic rate, obtained with the Harris-Benedict formula [21] with an activity factor of 1.5. Participants were provided with optional extra snacks to eat with their meals if they were still hungry, up to an activity factor of 1.7. For the first study-day in the laboratory, energy requirement was calculated by multiplying the estimated resting metabolic rate with an activity factor of 1.35, because of limited physical activity in the research facility. For the second study-day, energy requirement was calculated by multiplying the sleeping metabolic rate of the first study night (measured by whole-room indirect-calorimetry) by 1.5.

During the study days, participants received 3 meals daily. Breakfast accounted for ~21 energy%, lunch for ~30 energy% and dinner for ~49 energy%. Daily macronutrient composition was ~52 energy% as carbohydrates, ~31 energy% as fat (~9% saturated) and ~14 energy% as protein. No snacks or drinks other than water were provided in-between meals.

2.5. Skeletal muscle biopsies and respirometry

Five skeletal muscle biopsies were obtained from the m. vastus lateralis according to the Bergström method [22] under local anesthesia (1% lidocaine, without epinephrine). Each biopsy was taken from a separate incision at least 2 cm from the previous incision, moving from

