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SLEEP BEHAVIOUR, ACTIVITY CIRCADIAN RHYTHM AND PSYCHOPHYSIOLOGICAL RESPONSES TO PHYSICAL ACTIVITY: THE CHRONOTYPE EFFECT.

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1. PROLOGUE

My first approach to the research on chronotype was in Alta (Norway) in February 2012 when I started the internship for the master degree in Sport Science. Prof. Franca Carandente introduced me to Prof. Andi Weydahl, physiologist at the Finnmark University College, and, during my Erasmus Placement, we studied together the effect of chronotype on physical activity. It was a great personal experience at the Finnmark University College and we also completed a pilot study where we observed that E-types showed a better predisposition when performing a physical task later in the day. Coming back to Milan, I started the Ph.D. career and worked with the research group composed by Prof. Franca Carandente, Prof. Andrea Caumo, Prof. Eliana Roveda and Prof. Angela Montaruli and we immediately started to study the differences in activity circadian rhythm and in sleep behaviour in different chronotypes with the aim to utilize this kind of information to the improvements of sport performance. We found large differences among chronotypes concerning the acrophases of the activity circadian rhythm and about the sleep parameters during both weekdays and weekend. This project took a long time but we wrote an important paper on this topic and, for our joy, it was published in Chronobiology International (leader journal of chronobiology). The collaboration with Prof. Weydahl continued with a study conducted in Alta on the effect of chronotype on self-paced exercises, and the paper was published in Perceptual and Motor Skills. Now, I am working to a new project with the aim to investigate the possible influences of circadian typology on the psychophysiological responses (sleep, HRV, cortisol, POMS and RPE) to a High Intensity Interval Training (HIIT) protocol performed in the morning and in the evening.

2. INTRODUCTION

1. Chronobiology: the science of rhythms

Chronobiology is the science that objectively quantifies and investigates mechanisms of biologic time structure, including rhythmic manifestations of life. Rhythms with different frequencies are found at all levels of biologic integration: ecosystem, population, group, individual, organ-system, organ, tissue, cell and subcellular structure. Their ubiquity and their critical importance to the survival of both the individuals and the species have prompted the development of a special methodology to study these temporal characteristics in the context of development, growth and aging, yet in a novel branch of biology separate from embryology, pediatrics and geriatrics. In physiologic terms, chronobiology provides generally applicable concepts and techniques for resolving predictable cycles in organisms and for isolating environmental effects from the underlying endogenous mechanisms. The basis properties of rhythms are important to education, ecology and medicine (Halberg, 1960; Halberg, 1969, Halberg & Reinberg, 1967).

1.1 The rhythm

The rhythm is a periodic component of (biologic) time series, demonstrated by inferential statistical means, preferably with objectively quantified characteristics (i.e., frequency f, acrophase Ø, amplitude A, mesor M, and/or waveform W). Rhythms thus include any set of biologic changes recurring systematically according to an (algorithmically) formulatable pattern or waveform that is validated in inferential statistical terms. Mathematically, more or less sinusoidal rhythms can be described by the use of approximating functions such as those of a form: $f(t) = M + A \cos(\omega t + Ø)$, where ω is the angular frequency and t = time. Confidence intervals also should be estimated for rhythm parameters. The frequent use of a cosine function, as the first step in a check of rhythmicity, does not imply more that this function approximates the data better than does a horizontal line. In other words, the microscopic fit of a cosine does not imply that the data are truly sinusoidal in shape, just as the use of a microscope in histology does not imply that the nucleus and cytoplasm are modelled by an objective and ocular (Halberg, 1966; Halberg et al., 1972). These are the characteristics of a rhythm (Figure 1):

- Acrophase, Ø: measure of timing. The lag from a defined reference timepoint of the crest time in function appropriately approximating a rhythm; the phase angle of the crest, in relation to the specified reference timepoint, of a single best fitting cosine (unless another approximating function is specified). The units of the acrophase could be angular measures, degrees, radians, time units (seconds, minutes, hours, days, months, years), or physiologic episodic units (number of heart beats, respirations, etc). Angular measures are directly applicable to any cycle length and hence are proposed for general use because of greater familiarity; degrees are preferred over radians (Deprins et al., 1977; Halberg et al., 1967).
- Amplitude, A: is a measure of one half the extent of rhythmic change in a cycle estimated by the (sinusoidal or other) function used to approximate the rhythm, e.g., difference between maximum and mesor of a best fitting cosine. The units for amplitude are original physiologic units, e.g. number of heart beats, mmHg in blood pressure, etc (Koukkari et al., 1973; Koukkari et al., 1974).
- MESOR (Midline Estimating Statistic of Rhythm), M: is the rhythm-determined average, e.g., in the case of a single cosine approximation. The value mid-way between the highest and lowest values of function used to approximate a rhythm. The units for M are original physiologic units. The M is equal to the arithmetic mean for equidistant data covering an integral number of cycles. (Bartter et al., 1976).

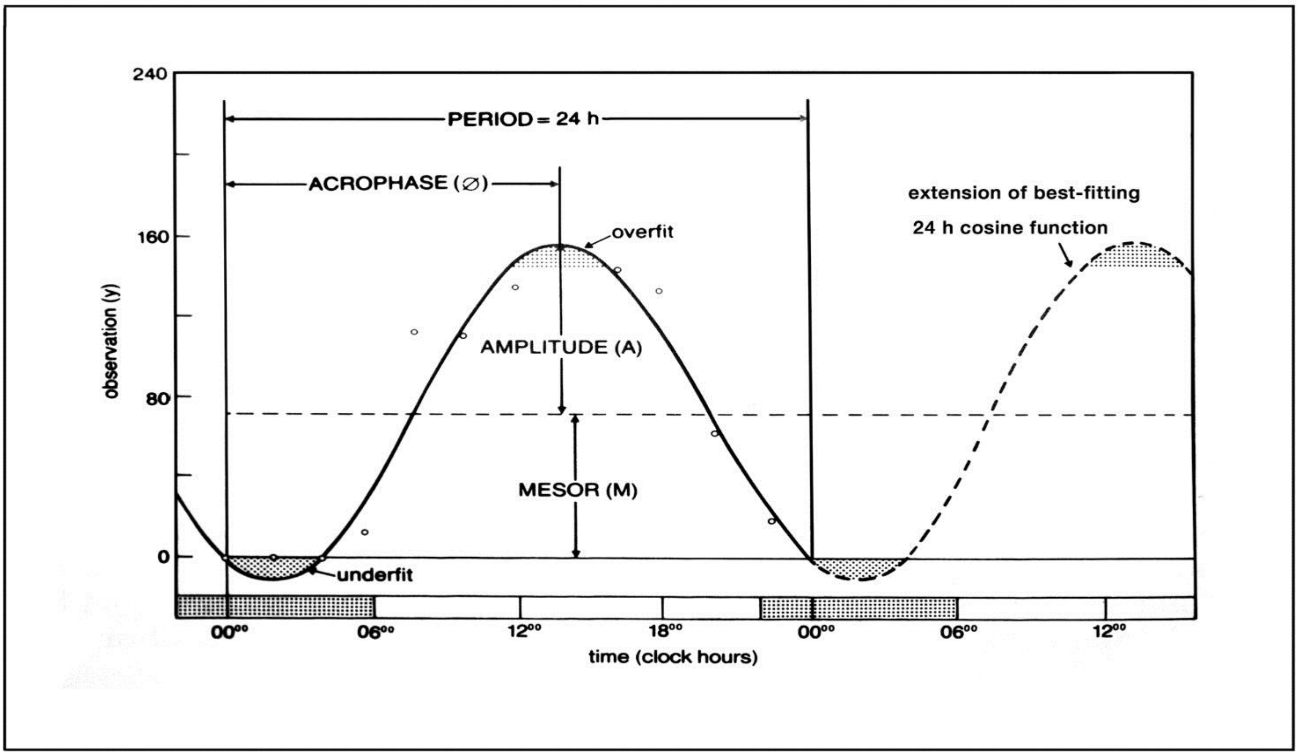


Figure 1. Exemplification of a biological rhythm and its parameters: Acrophase (Ø), Amplitude (A) and MESOR (M).

The biologic time structure is the sum of non-random and thus predictable time-dependent biologic changes, including, with growth, development and aging, a spectrum of rhythms with different frequencies (Aschoff, 1960). Time structure characterizes any biologic entity, including ecosystems and populations as well as individual or grouped organisms, organ systems, organs, tissues, cells and subcellular structures, exhibiting one or several of the frequencies listed here under:

- Ultradian: $\tau < 20$h; relating to biologic variations or rhythms with a frequency higher than circadian. Specifically, rhythms with frequencies greater than 1 cycle in 20 h. It is admitted arbitrary to set the low frequency limit of the ultradian range at 1 cycle in 20 hours (Halberg, 1964).

- Circadian: 20h ≤ τ ≤ 28h; relating to biologic variations or rhythms with a frequency of 1 cycle in 24 ± 4 h. This term describes rhythms with an about 24h cycle length, whether they are frequency-synchronized with environmental schedules (Pittendrigh, 1960).
- Dian: 23.8h ≤ τ ≤ 24.2h; relating to biologic variations or rhythms with a frequency of 1 cycle in 23.8 to 24.2 h, if not in precisely 24 h. It is a special case of circadian period with an inferential statistical 95% confidence interval for period length within 23.8 and 24.2h (Halberg, 1954).
- Infradian: τ > 28h; relating to certain biologic variations or rhythms with a frequency lower than circadian. Infradian rhythms include: circaseptan (τ = 7 ± 3 days), circadiseptan (τ = 14 ± 3 days), circavigintan (τ = 21 ± 3 days), circatrigintan (τ = 30 ± 3 days) and circannual (τ = 1 year ± months) (Halberg et al., 1965).

1.2 Endogenous and exogenous component of a rhythm

The time structure of all living organisms is the result of cooperation of neural, hormonal and cellular systems that interact with each other (Figure 2) (Halberg et al., 1977).

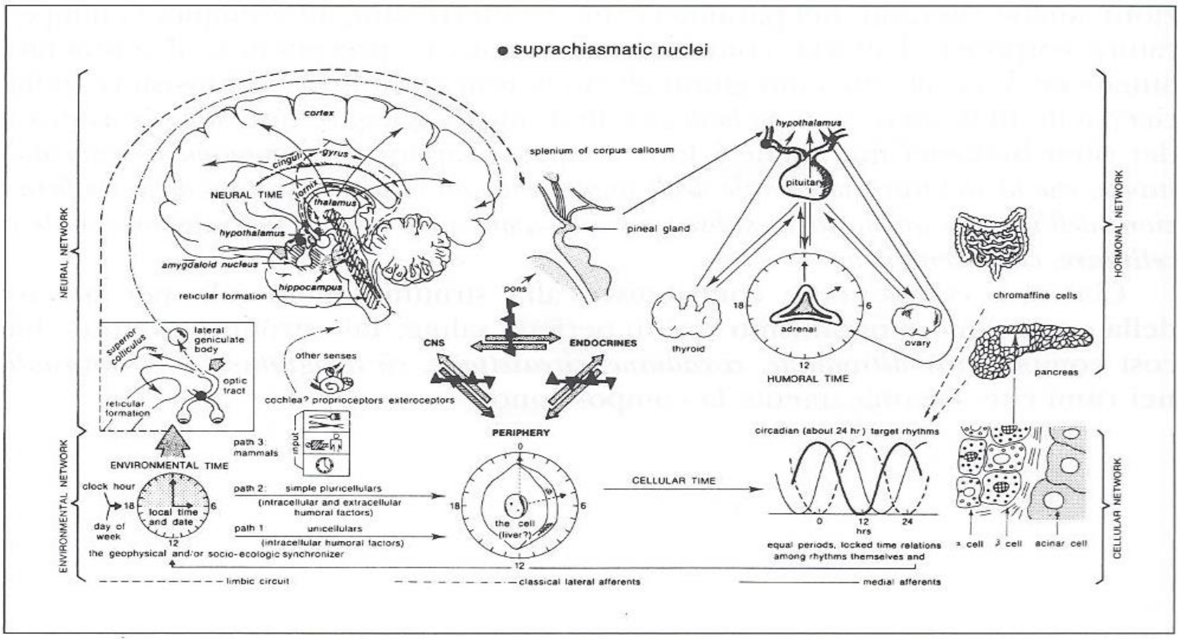


Figure 2. Schematic representation of the factors involved in the circadian synchronization.

The time structure shows periodic characteristics with different frequencies that could be influenced, regarding the length, by environmental factors: the synchronizers. The synchronizers could be defined as primary or secondary depending on their influence on a specific variable. For the human beings the primary circadian synchronizers are mostly of socio-environmental origin: the best known is the alternation of activity-rest. Figure 3 shows the circadian rhythm of core temperature in a group of subjects that first lived normally (full line) and then underwent a 24-hour “constant routine” (dash-line). First, it was supposed that temperature rhythm was the result of behavioural changes that are associated with the sleep-activity cycle but this kind of explanation is not exhaustive enough, the standard method for demonstrating this is the “constant routine”: in this protocol, a subject had to remain awake, sedentary and relaxed for at least 24 hours. When this protocol is undertaken, any rhythmic changes due to the individual’s lifestyle or environment are removed and it was observed that the rhythm of core temperature did not disappear (dash-line). By considering the two temperature profiles shown in Figure 3, we can conclude that: 1) the rhythm observed during the constant routine arises internally; it is the endogenous component of the temperature rhythm and its generation is attributed to a ‘body clock’ (Minors & Waterhouse, 1981. 2) The difference between the two rhythms is the exogenous component of core temperature rhythm that is dominated by the sleep-wake cycle (Reilly et al., 1997). These deductions are general, insofar as all rhythms show a mixture of endogenous and exogenous components when compared under normal living conditions and during a constant routine (Waterhouse et al., 2012).

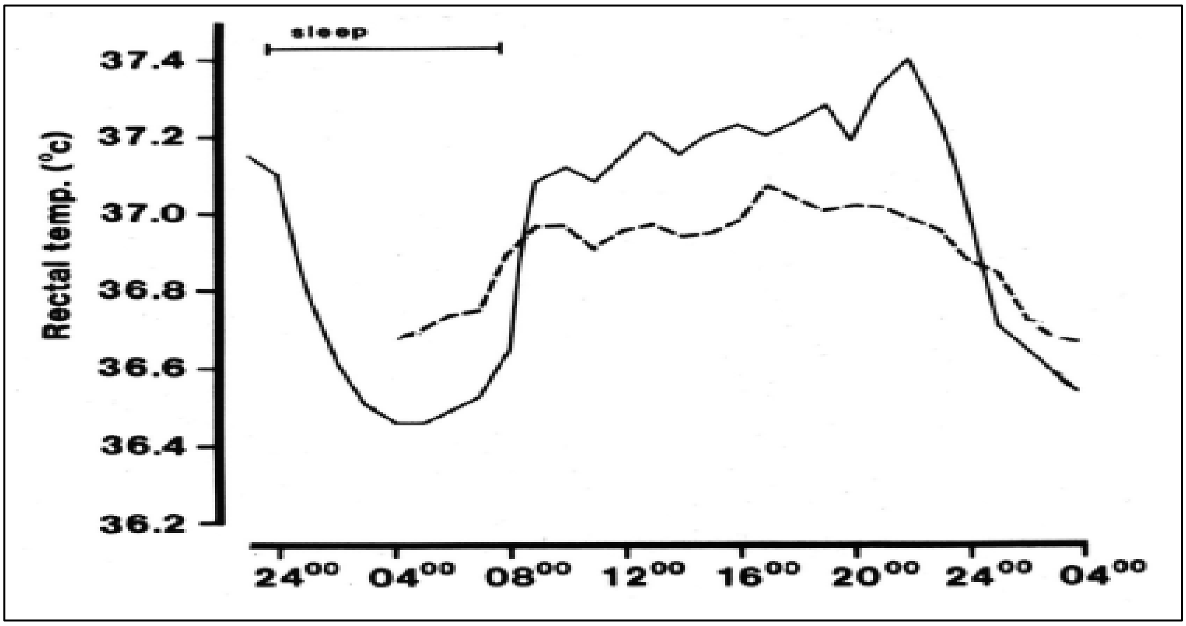


Figure 3. The daily rhythm of core temperature in a group of 8 young men. Full line: under normal conditions (sleep from 12 a.m. to 07 a.m., indicated by bar). Dashed line: undergoing a 24 hours constant routine starting at 4 a.m.

2. Chronotype

The chronotype, or circadian typology, is the expression of circadian rhythmicity in an individual and can differ among individuals. There are three different chronotypes: Morning-types (M-types), Evening-types (E-types) and Neither-types (N-types). The chronotype is determined using a number of self-assessment questionnaires that have been validated in several countries. In the last decades several studies have shown differences between M-types and E-types in the circadian rhythms of different physiological variables: M-types go to bed and wake up early and achieve their peak performance in the first part of the day (Taillard et al., 2004; Vitale et al., 2015) while E-types go to bed and wake up late and perform at their best toward the end of the day (Horne et al., 1980; Vitale et al., 2013, Rossi et al., 2015). The phase lags in circadian rhythmic functions between extreme groups range from 2 to 12 h, and this has been observed both in biological and behavioural parameters (Baher et al., 2000; Bailey & Heitkemper, 2001). In this chapter, that has been written following the scheme of Adan's review (2012), will be presented all the key concepts referring to the chronotype including: the methods for the measurement of the circadian tipology, the influences of individual and environmental factors on chronotype, the biological differences among M-, N- and E-types and the cognitive performances.

2.1 Assessing the chronotype

The Morningness-Eveningness Questionnaire (MEQ) (Horne & Östberg, 1976) is the most widely used morningness measure. Since its publication, the MEQ has been cited > 1000 times. The MEQ has 19 items, and the answer options include using a visual analog scale and choosing between four or five answer options. In their first study Horne and Östberg (1976) administered the MEQ to 150 adults aged 18–32 and the sample was gender balanced. 48 of these participants were randomly selected and 18 were found to be M-types and 10 were E-types. These participants recorded their oral temperature for 3 weeks and their sleep–wake behaviour. The oral temperature peaked at 19:30 h in M-types, 20:25 h in N-types and 20:40 h in E-types and M-types were found to go to sleep 99 min before E-types and woke 114 min earlier than E-types. The MEQ has received some criticism however. The scale contains 19 items and may be considered lengthy in some situations. These criticisms underpinned the development of the reduced Morningess–Eveningness Questionnaire (rMEQ) (Adan & Almirall, 1991). This scale contains five items and is considered a pure measure of M-types. The correlation between the rMEQ and the MEQ ranges from satisfactory to excellent (.69–.90) and also demonstrates good convergent validity (Adan & Almirall, 1991; Caci et al., 2009; Chelminski et al., 2000). Natale et al. (2006) reported the rMEQ discriminated M-types and E-types on the basis of objectively recorded motor activity.

Another way to evaluate the circadian typology is represented by the Composite Scale of Morningness (CSM), it was developed based on a psychometric assessment of the MEQ and Diurnal Type Scale (DTS): the CSM consists of 13 items; nine from the MEQ and four from the DTS (Smith et al., 1989). Smith et al. (1989) reported scale reliability of .87 and found significant differences between CT and bed/wake times, preferred class times and times when students felt at their mental and physical best. These results have been replicated elsewhere (Caci et al., 1999; Randler, 2009). One problem of the CSM is that all items assume all people work a diurnal schedule; shiftworkers may find it difficult to answer how they feel like when they wake in the morning. In addition, the varied response formats and the different number of answer choices may combine to increase the measurement error (Zickar et al., 2002).

The Preferences Scale (PS) was developed to address several concerns over the CSM (Smith et al., 2002) The PS addresses these criticisms by using a scale that does not refer to time of day. Instead, participants rate their preference relative to “most people” on a five-point scale. Across six countries, Smith et al. (2002) reported reliability coefficients between .80 and .90. The PS has demonstrated good convergent validity with the CSM (.69–.83) and .82 with the MEQ (Osland et al., 2011).

The most recent chronotype instrument measure is the Munich Chronotype Questionnaire (MCTQ). The MCTQ determines chronotype according to the mid-point of sleep (onset and offset) calculated on weekends and this is considered the best indicator of melatonin onset (Terman et al., 2001). Weekend sleep takes into account the fact that E-types accumulate a sleep debt that is recovered on weekends (Roenneberg et al., 2007).

2.2 Biological differences

The different circadian rhythmicity observed among chronotypes can be identified in a large number of biological markers. These markers include the sleep– wake cycle, body temperature and the hormones melatonin and cortisol.

Body Temperature, Cortisol and Melatonin

M-types, in normal day–night conditions (Baehr et al., 2000) and during a constant routine (Kerkhof & Van Dongen, 1996), have an earlier circadian temperature phase than E-types measured by both rectal (Duffy et al., 1999) and oral temperature (Gupta & Pati, 1994), this phase difference is around 2 h. Similar differences were observed, in healty adult men, for the acrophase of cortisol: M-types showed their peak in the morning 55 minutes earlier than E-types for both salivary (Bailey & Heitkemper, 1991) and plasma cortisol (Bailey & Heitkemper, 2001). Another physiological variable that is influenced by the chronotype is the melatonin: the onset, acrophase, and offset of the melatonin profiles, both in blood and in salivary measurements, occurred approximately 3 h delayed in E-types than in M-types, without differences in amplitude (Gibertini et al., 1999; Griefahn et al., 2002; Mongrain et al., 2004, 2005). A study showed an inverse relationship between MEQ scores and the time of the melatonin peak (Liu et al., 2000).

Clock genes

The clock gene was first identified in 1994 by Dr. Joseph Takahashi. Ko and Takahashi (2006) explained that circadian rhythms are generated by a core set of circadian clock genes and proteins that interact in a transcriptional/translational feedback loop to determine the circadian period. The clock genes also have roles in sleep regulation and homeostasis, therefore, because sleep and circadian systems interact to determine the chronotype, it should be expected that variation in clock genes could be associated with individual differences in circadian typology. Past studies has identified a catalog of polymorphisms in clock genes that show associations with the chronotype phenotype, including CLOCK (Katzenberg et al., 1998; Mishima et al., 2005), PER1 (Carpen et al., 2006), PER2 (Carpen et al., 2005) and PER3 (Archer et al., 2003, 2010; Johansson et al., 2003; Jones et al., 2007; Lázár et al., 2012; Pereira et al., 2005). Circadian typology is a complex phenotype and it is derived from a multiple underlying genetic factors.

2.3 Personal and environmental factors

The chronotype is influenced by individual factors, such as age and sex, as well as several environmental factors including the photoperiod at birth, the altitude/latitude of residence and the subjects' exposure to light.

Age

Morningness scores, after the end of adolescence, tend to increase with age (Kim et al, 2010; Merikango et al, 2012; Paine et al, 2006). There is a tendency to go to bed and wake up earlier as subjects grow older and to present the highest levels of activation at an earlier time (Park et al., 2002; Tonetti et al., 2008). Adolescence is a critical period when there is a pronounced tendency to eveningness (Achari & Pati, 2007; Borisenkov et al., 2010; Kim et al., 2002) and in women the peak of maximum eveningness appears earlier (Randler, 2011; Tonetti et al., 2008). This phenomenon may be interpreted as associated with pubertal development (Hagenauer et al., 2009).

Sex

Many studies reported a larger number of E-types, mostly evaluated with the use of MEQ, among males, while morningness is more commonly observed among females (Adan & Natale, 2002; Borisenkov et al., 2012; Natale & Di Milia, 2011; Randler, 2011; Roenneberg et al., 2004; Tonetti et al., 2011; Vitale et al., 2015). On the other hand, some studies have not found any gender differences (Paine et al., 2006; Zimmermann, 2011). Nevertheless, the intrinsic circadian period is significantly shorter in women than in men (Duffy et al., 2011); this difference can be explained by the control of the circamensual rhythmicity associated with the menstrual cycle in women, which would act against the intensity of the rhythmic control of circadian periodicity (Adan & Natale, 2002). This argument is supported by the fact that sex differences on chronotype disappear following menopause in women (Roenneberg et al., 2004; Tonetti et al., 2008).

Photoperiod at Birth, Longitude and Altitude

The photoperiod at birth represents the duration of an organism's exposure to light at birth. The chronotype may be influenced by the photoperiod at birth: subjects born with a short photoperiod tends to be more M-types, while for those with a long photoperiod, it tends to be more E-types (Mongrain et al., 2006; Natale et al., 2002; Natale & Di Milia, 2011). However, other studies did not observe these differences (Achari & Pati, 2007; Harada et al., 2011) and one of the explanation of this discrepancy refers to the correct subdivision of photoperiod at birth. Usually in the past, all authors rely on the calendar seasons of the year, such as spring, summer, autumn and winter, to define the different photoperiods but this classification does not take into account the latitude of the place of birth and therefore is not accurate. Vollmer and colleagues (2012) were the first that grouped the subjects considering the latitude of their hometown (Vollmer et al., 2012) and, nevertheless, they observed the influence of photoperiod at birth on the circadian typology.

2.4 Cognitive performances

Studying the nature of time-of-day effect on cognitive performance in normal day-night conditions requires a large knowledge of these combined factors: endogenous rhythms, exogenous factors and the individual's motivation (Clarisse et al., 2010). The interest in cognitive efficiency at different time-of-day has a long history: first, Kleitman (1963) showed a strong correlation between body temperature and time-of-day: the decrease in reaction time response was significantly correlated to an increase in body temperature. This concept became the "arousal model": since body temperature increases during the day, also the performance efficiency should always increase during the day (Colquhoun, 1971). However, Horne and colleagues (1980) showed that E-types improved their motor component performance during the day, whereas M-types presented an opposite trend, with a phase advance up to 12 h. In support of this datum, other studies have shown a phase advance of the best performance time ranging between 2 and 6 h for M-types (Adan, 1991; Anderson et al., 1991; Natale & Lorenzetti, 1997; Petros et al., 1990). To explain this large gap in cognitive performance between M-types and E-types the role of alertness was considered and it was created a model known as "synchrony effect" (May and Hasher, 1998): cognitive performance are more efficient when testing in synchrony with individual's peak in alertness (Hasher et al., 2002; Rowe et al., 2009; Yang et al., 2007). Nevertheless, the synchrony effect did not always influence performance. In fact, synchrony effect was not documented in a constant way for all studies (Ciarkowska, 1997). Because of these results, we can assume that it is simplistic to link the cognitive efficiency of different chronotypes only to arousal or alertness, it is modulated by compensatory mechanisms such as expectancy due to the experience, motivational factors or it could be also associated to different cognitive styles.

2.5 Personality and mood

Sevaral studies tried to investigate the relationship between chronotype and peronsality/mood (disorders) and the results are not globally clear (Cavallera & Giudici, 2008). Generally, E-types reported higher scores in extraversion, obtained with the Eysenck Personality Inventory (EPI) (Eysenck & Eysenck, 1975) than M-types (Langford & Glendon 2002; Mitchell & Redman 1993; Tankova et al., 1994) and this result is mostly observed in women (Matthews, 1988). The EPI also give some informations about neuroticism of subjects but the results are contradictory: some studies have reported higher scores in E-types (Mecacci & Rocchetti, 1998; Tankova et al., 1994), others showed M-types had higher levels of neuroticism (Langford & Glendon, 2002; Tankova et al., 1994) while several authors did not report any kind of relationship (Mitchell & Redman 1993). Another method to describe the human personality is the Big Five Model (or Five-Factor Model) that is made up of the following factors: extraversion, agreeableness, conscientiousness, neuroticism and openness (Costa & McCrae, 1992). The studies conducted to evaluate the personality among chronotypes reported that E-types were more extroverted than M-types (Jackson & Gerard, 1996) and it was observed a positive relationship between agreeableness and morningness (Hogben et al., 2007; Randler, 2008; De Young et al., 2007); moreover, conscientiousness showed a positive correlation to morningness and it is considered the best predictor of morningness while E-types showed higher levels of neuroticism (Randler, 2008; Tonetti et al., 2009; De Young et al., 2007) and openness (Hogben et al., 2007). As a summary, research on personality and chronotype indicates that M-types tend to be more introverted, conscientious, agreeable, persistent and emotionally stable while E-types subjects seems to be extraverted, impulsive, novelty seeking and open-minded and they tend to be more prone to mood and eating disorders (Kim et al., 2010).

3. Sleep

Sleep is a biological process that is known to have underlying beneficial functions and appears to be evolutionary conserved across species (Siegel, 2009). An optimal sleep behaviour, without circadian disruption, is necessary to promote high levels of attention and cognitive performance, to prevent weight gain (Arble et al., 2009), obesity (Kobayashi et al., 2012), diabetes (Chao et al., 2011), and hypertension (Wang et al., 2012) and, last but not least, to reduce mortality (Cappuccio et al., 2010). Sleeping less than 7 hours during weekdays results in cumulative deficits in behavioural alertness and vigilant attention (Belenky et al., 2003). The link between the sleep-wake cycle and the rhythm of core temperature has been investigated and interpreted as a causal link (Kleitman, 1963) but this might be an oversimplification of the position since there are many other rhythms associated with the sleep-wake rhythm (Reilly et al., 1997; Van Someren, 2000): levels of plasma adrenaline, plasma melatonin, reciprocal activity of the sympathetic and parasympathetic branches of the autonomic nervous system. It is clear that many factors contribute to the physiological preparations that need to be accomplished to feel ready for sleep in the evening and core temperature is only one of these factors that reflect the activity of the body clock. One simple, and yet very effective, model of sleep rhythms is the two-process model of sleep homeostasis of Borbély (1982): sleep is the results of the interaction between a homeostatic process and the intrinsic circadian clock which combine to determine the timing of sleep onset and offset. The homeostatic process represents the drive for sleep that increases as a saturating exponential during wakefulness and decreases as a saturating exponential during sleep while the circadian clock represents the daily oscillatory modulation of these homeostatic processes and it is parallel to the rhythm of core temperature (Goel et al., 2013).

3.1 Actigraphy

The Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) and the Epworth Sleepiness Scale (ESS) (Johns, 1991) are two of the most common questionnaires that allow to evaluate sleep with subjective data. The PSQI assesses sleep quality over a 1-month time interval and it consists of 19 individual items generating scores about subjective sleep quality, latency, duration and sleep disturbances, while the ESS is intended to measure subjective daytime sleepiness. Even if the subjective data could be interesting to have a global picture of sleep behaviour, it has been demonstrated a discrepancy if compared to the actigraphy: Lockley and colleagues (1999) showed that values of sleep latency, number and duration of night awakenings or number of naps, evaluated both with actigraphs and questionnaires, were not similar in their sample. Actigraphy is used in research to study sleep/wake patterns for over 30 years and its advantage, compared to the polysomnography, is that it is possible to record continuously for 24 hours/day for days, weeks or even longer (Ancoli-Israel et al., 2003). In 1995, under the auspices of the American Academy of Sleep Medicine (AASM), Sadeh and colleagues (1995) realized a big review on the role of actigraphy and they concluded that it was not indicated for routine diagnosis but it might be useful to detect insomnia and circadian rhythm disorders (Standards of Practice Commitee, 1995). From that point acigraph technology improved and several studies concluded that actigraphy could usefully approximate sleep versus wake state during 24 hours (Broughton et al., 1996; Teicher, 1995). The first actigraphs were developed in the 1970’s (Colburn et al., 1976; Kupfer et al. 1974) and they were devices that could be placed on the wrist, ankle or trunk to record movements (Kripke et al., 1978; Webster et al., 1982); the data collected were then downloaded with specific software for the analysis of activity/inactivity. Today additional types of actigraphs are available and they have movement detector, accelerometers and the analysis are more specific and complete.

3.2 Chronotype effect on sleep timing and quality

Because of the interaction between sleep and circadian systems and the correlation between circadian rhythms and chronotype, it should be expected that inter-individual variability in circadian typology would be associated with differences in sleep–wake timing and in sleep behaviour. It was largely demonstrated that M-types go to bed and wake up earlier than E-types (Horne & Ostberg, 1976; Robiliard et al., 2002; Taillard et al., 2004) and that E-types use to sleep less during the working-days but more during the weekends (Roenneberg et al., 2007). While, concerning the relationship between circadian typology and sleep behaviour, several studies have evaluated the sleep quality in different chronotypes using either self-assessment questionnaires or actigraphy. Studies that used selfassessment questionnaires showed that E-types were more prone to sleep complaints, as measured by the PSQI (Buysse et al., 1989) and the ESS (Johns, 1991), than M-types (Barclay et al., 2010). Moreover, the prevalence of nightmares and insomnia symptoms, which was evaluated by self-reported questionnaires, was higher in E-types than in M-types (Merikanto et al., 2012). Lehnkering & Siegmund (2007) studied the influence of chronotype on sleep behavior in young adults using actigraphs (Actiwatch© actometers, CNT, Cambridge, UK): the results showed that there was a difference in sleep efficiency between M-types (87.9%, SD ± 1.3%) and E-types (84.3%, SD ± 0.87%). Martin et al. (2012) investigated the relationship between chronotype and sleep, but they did not find any significant differences in actigraphic sleep parameters, such as sleep duration, sleep efficiency and sleep latency, whereas sleep onset and sleep offset differed among the chronotypes. Few studies have investigated sleep parameters during weekdays and the weekend in relation to the circadian typology using both a self-assessment questionnaire and actigraphy. The results regarding sleep timing have shown that E-types generally go to bed and wake-up significantly later than M-types on both work and free days; therefore, eveningness is associated with a later bedtime and wake-up time and a shorter time in bed during the week (Giannotti et al., 2002; Kabrita et al., 2014; Lee et al., 2014; Roepke & Duffy, 2010). Thus, chronotype differences in sleep debt accumulated during workdays can affect sleep duration and timing. No data are reported regarding sleep quality. Social factors are crucial for sleep and they can lead to the desynchronization during normal working lives, E-types who go to bed late but rise early because of work times or family commitments will wake up at a time that is discordant with their circadian clock and also accumulate a sleep debt during the week. This "social jet lag" is common in our current society and has been shown to be associated with metabolic disorders and depression (Levandovski et al., 2011; Roenneberg et al., 2012; Wittmann et al., 2006).

3.3 Sleep and physical exercise: a reciprocal relationship.

Relatively few studies have investigated sleep quality and quantity of athletes even if it is considered a critical variable to have optimal performances (Halson, 2008; Venter, 2014). Past studies showed that athletes have better sleep behaviour that nonathletic subjects (Porter & Horne, 1981; Shapiro et al., 1986) and they should sleep for 9-10 hours/night, while 7-9 hours is recommended as enough for healthy adults (Ferrara, 2001); nevertheless, recent evidence suggests that athletes sleep far less in respect of these recommendations (Sargent et al. 2014). It is difficult to understand exactly how exercise impacts on sleep and vice versa: different volumes, intensities and types of physical activity could have some positive / negative effects on sleep (Ingersol, 2003; Matos et al., 2011) and sleep loss or restriction could influence both performances and physiological / cognitive performances (Mougin et al., 1991; Souissi et al., 2008). Chronic moderate-intensity exercise represent a non-pharmacological treatment for poor sleepers (Montgomery &

Dennis, 2004); in a study conducted by Kalak and colleagues (2012) it was shown that 30 minutes of moderate physical activity, performed every day for 3 weeks, were associated with increased sleep time and decreased sleep latency and these beneficial effects were observed both in young subjects and older population (Oudegeest-Sander et a., 2013). The beneficial effects of moderate-intensity exercise can be observed on body temperature, cardiac and autonomic function, endocrine system, metabolic function, immune-inflammation and on mood. Figure 4 represent the effects of acute and regular exercise on sleep (Chennaoui et al., 2015).

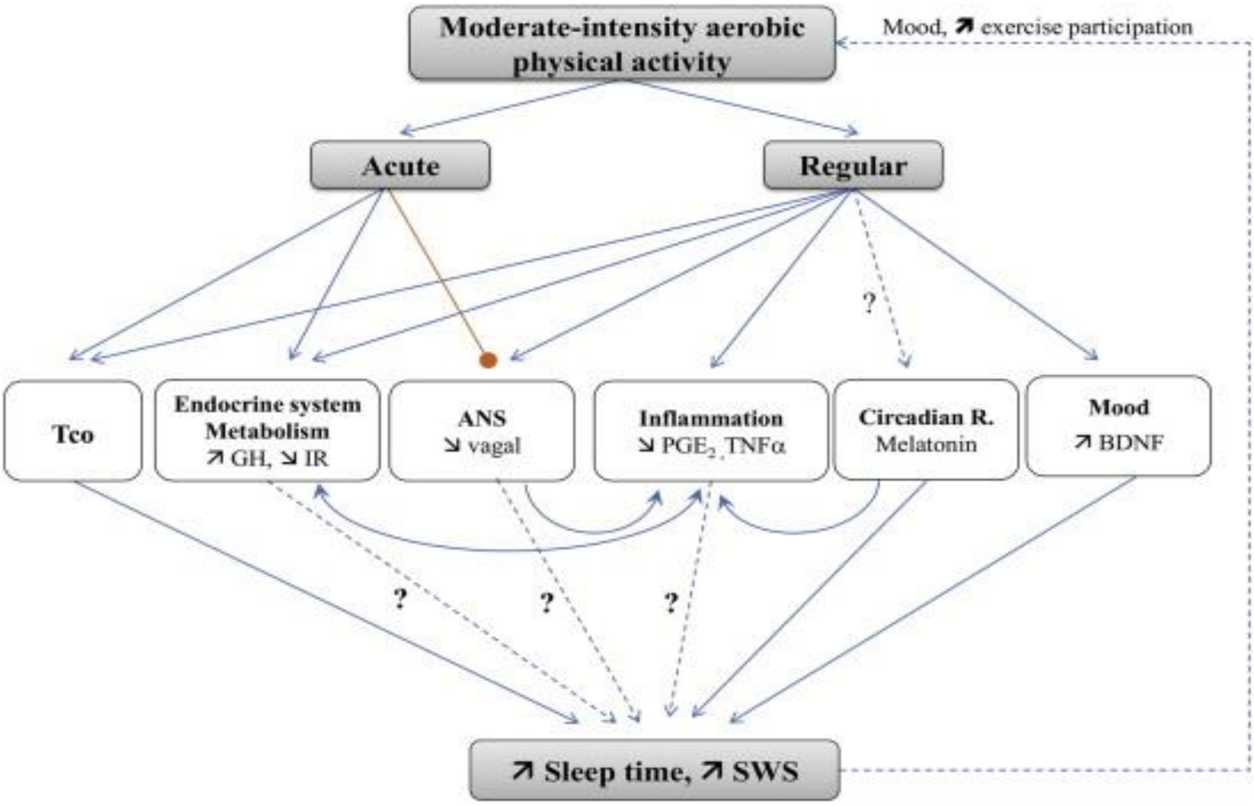


Figure 4. Possible effects of acute or regular moderate intensity aerobic physical activity on sleep. ANS = autonomic nervous system, BDNF = brain-derived neurotrophic factor, Circadian R. = circadian rhythm, GH = growth hormone, IR = insulin resistance, PGE_2 = prostaglandin E_2, SWS = slow wave sleep, Tco = body core temperature, TNF-α = tumor necrosis factor alpha.

On the other hand, sleep could influence physical exercise; good sleep behaviour is vital for high levels of mental and physical performance, general well-being and the recovery process (Skein et al., 2013). Some studies indicate that chronic or acute sleep loss is directly correlated to athletic injuries (VanHelder et al., 1993) while the direct effects on physical performance appear inconclusive: some authors explained the impact of sleep deprivation on exercise as an enhanced perception of the exertion during exercise known to decrease sub maximal performance (Marcora et al., 2009). Figure 5 shows the possible effects of acute / chronic sleep deprivation and loss on physical performance and exercise-induced disease (Chennaoui et al., 2015).

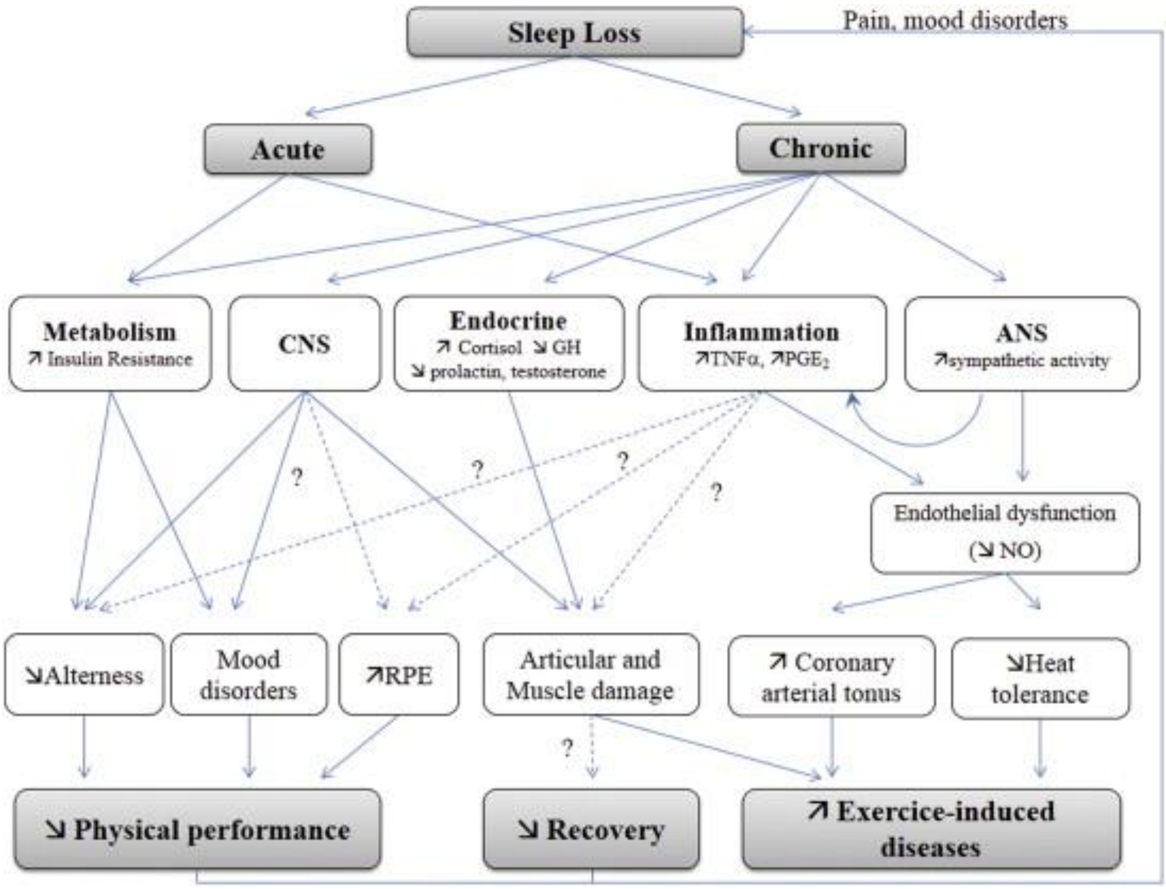


Figure 5. Possible effects of acute or chronic sleep deprivation/sleep loss on physical performance, muscle recovery and exercise-induced diseases. ANS = autonomic nervous system, BDNF = brain-derived neurotrophic factor, BP = blood pressure, Circadian R. = circadian rhythm, IR = insulin resistance, GH = growth hormone, HR = heart rate, $PGE_{2\,=}$ = prostaglandin E_2, RPE = rating of perceived exertion, TNF-α = tumor necrosis factor alpha.

4. Circadian rhythms in sport performance

Almost all physiological processes of the human body follow a circadian rhythm and the suprachiasmatic nucleus regulates sleep-wake cycle and other biological rhythms that are in line with solar time. At the same time, many physiological functions associated with physical performance showed a specific circadian rhythm, such as metabolic, neuromuscular and behavioural variables or perceptual performance. Late afternoon or early evening are the time-periods when best performances and even world records are most often likely to be set in competitions. A large number of studies demonstrated that exercise performances (or many aspects of the latter) display a peak in this moment of the day: peak force of leg and back muscles (Coldwells et al., 1994) and arm muscles (Gauthier et al., 1997), maximal anaerobic power output (Souissi et al., 2004), broad jump performances (Reilly & Down, 1986), running (Pullinger et al., 2013), swimming (Klince et al., 2007), cycling (Hachana et al., 2012), badminton (Edwards et al., 2005) and skilled tasks related to football (Reilly et al., 2007). These activities cover a range of skills from gross locomotor functions to fine and complex tasks. Table 1 shows some of the variables related to sport performance, with relative acrophases, that have been studied by different authors in relation to their circadian rhythm.

4.1 Possible mechanisms of circadian rhythm in sport

The reason why these variables show a circadian rhythm with an acrophase in late afternoon is complex and unclear but it can partially be explained by several mechanism (Teo et al. 2011; Youngstedt & O'Connor, 1999), including:

- External (exogenous) changes in the environment: light, temperature. External influences are usually incontrollable

- Internal (endogenous) changes due to the “body clock”. The body clocks exerts effects throughout the body via influences on temperature regulation, hormone secretion and the sleep/wake and feeding cycles (Reilly and Waterhouse, 2009).
- Lifestyle factor: nutrition, preferred time of training, changes related to sleep-wake cycle such as the ability to cope with sleep inertia.

Javierre and colleagues (1996) tried to demonstrate the influence of external factors on exercise: first, the authors investigated the 80-m sprints performance in competitive sprinters at several times of the day and showed that performance normally peaked at 19:00 and, subsequently, they observed that, when both the sleep-wake cycle and times of meals were either advanced or delayed by 2 hours, the time of peak performance changed in a compatible direction and by a similar amount.

Traditionally core temperature has been used as the primary indicator for circadian rhythm in biological processes and physical performance. The increasing of temperature through the day may lead to a subsequent increase of carbohydrate utilization over fat as a fuel source and also contributes to facilitate the actin-myosin crossbridge mechanics in the musculoskeletal unit (Starkie et al. 1999). To demonstrate the effects of core temperature on physical performance, Taylor and colleagues (2011) showed that extending warm-ups in the morning session could result in an attenuation of power and force loss in a countermovement jump test while Souissi and colleagues (2007) observed, during a Wingate test, that aerobic contribution was higher in the afternoon session in conjunction with increased body temperature and the power loss was greater in the morning.

Despite the large evidence on the implication of temperature in sport performances, recent studies have challenged this traditional view and tried to look at the relationship between diurnal fluctuations of testosterone and cortisol on neuromuscular adaptations to better explain the mechanisms that control circadian rhythms in sport. Testosterone is an hormone that contributes to maintain anabolism by promoting protein synthesis within the muscular system (Ferrando et al., 1998) and its effect on muscle strength is well documented (Bashin et al., 2001) while cortisol is a glucocorticoid that is commonly used as marker of stress and it has negative effects on neuromuscular system (Tafet et al., 2001). Sale (2008) provided evidence of the negative effects of increased cortisol on neuromuscular functioning, evaluated by stimulation of the primary motor cortex using transcranial magnetic stimulation, in the mornings. Another study by Bird and Tarpenning (2004) highlighted the importance of the levels of cortisol and the testosterone/cortisol ratio profile: they observed in the evening an increasing ratio, due to the lower concentration of cortisol pre-exercise, that contributed in reducing the catabolic environment that is beneficial for training adaptations.

In conclusion, we can assume that it is not easy to find fully satisfactory reasons that can explain the physiological mechanisms that govern circadian rhythms of variables affecting sport performance; an individual's chronotype is one of the factors that has to be investigated since it could strongly influence a physical performance.

Table 1. Summary of studies of circadian rhythms in sport performance and the relative acrophases of the investigated variables.

References	Variables investigated	Acrophase		
		Morning (07:00 - 13:00)	**Afternoon** (13:00 - 18:00)	**Evening** (18:00 - 00:00)
Trine & Morgan (1995)	HR and VO2		18	
Moussay et al. (2002)	Temperature and HR		18	
Reilly et al. (2007)	Temperature		18	
	Dribbling and Juggling (soccer)		16	
	Wall volley test (soccer)			20
	Flexibility		16	
	Self-perceived fatigue	8		
	Subjective alertness			20
Cable & Reilly (1987)	Submaximal VO2			20
Edwards et al. (2005)	Accuracy of serve (badminton)			20
Atkinson & Spears (1998)	Speed serve (tennis)			20
Rahnama et al. (2009)	Dribbling speed (soccer)			19
	Sergeant jump test			19
	20-meters sprint			19
Coldwells et al. (1994)	Back and legs strength		18	
Wyse et al. (1994)	Legs strength			19
Atkinson & Reilly (1996)	Isometric strength			20
	Reaction time			20
Gauthier et al. (1997)	Elbow flexors isometric strength		17	
Reilly & Down (1986)	Standing broad jump		18	
Taylor et al. (2010)	Countermovement jump		16	
Castaingts et al. (2004)	Drop jump		18	
Pullinger et al. (2013)	Distance cover during RSA test		18	
	Peak and average power /velocity		18	
Hachana et al. (2012)	Peak power in 15-s Wingate test		18	
Souissi et al. (2004)	Peak power in 30-s Wingate test		17	
	Mean power in 30-s Wingate test		18	
Klince et al. (2007)	Swimming speed		18	

5. Chronotype and sport

5.1 Methodological approach

Studies have taken different approaches in investigating how chronotype affect athletic performances: some studies have focused on physiological and psychological parameters, while others assessed directly the athletic performance. We searched in the database PubMed for articles using each of the following words: “chronotype”, “circadian typology”, “morningness” and “eveningness”, in combination with each of the words: “sport”, “performance” and “athletic”. The inclusion criteria were peer reviewed journal articles written and published in English, reporting data of objective and subjective measures of athletic performance and physiological responses to exercise. Studies were excluded if they reported: a) case studies, b) data about animals, children, shift workers eumenorrheic women and unhealthy subjects, c) studies in the effects of medications, such as caffeine and/or melatonin or other stimulants on performance and d) data on jet-lag or conducted in particular settings (forced light exposure and sleep deprivation or loss, expeditions, extreme competitions lasting for several days). The search yielded a total of 303 records, including duplicates and, after selecting relevant studies, a total of 14 articles were finally studied. Figure 6 shows the flow diagram and the results of the literature research.

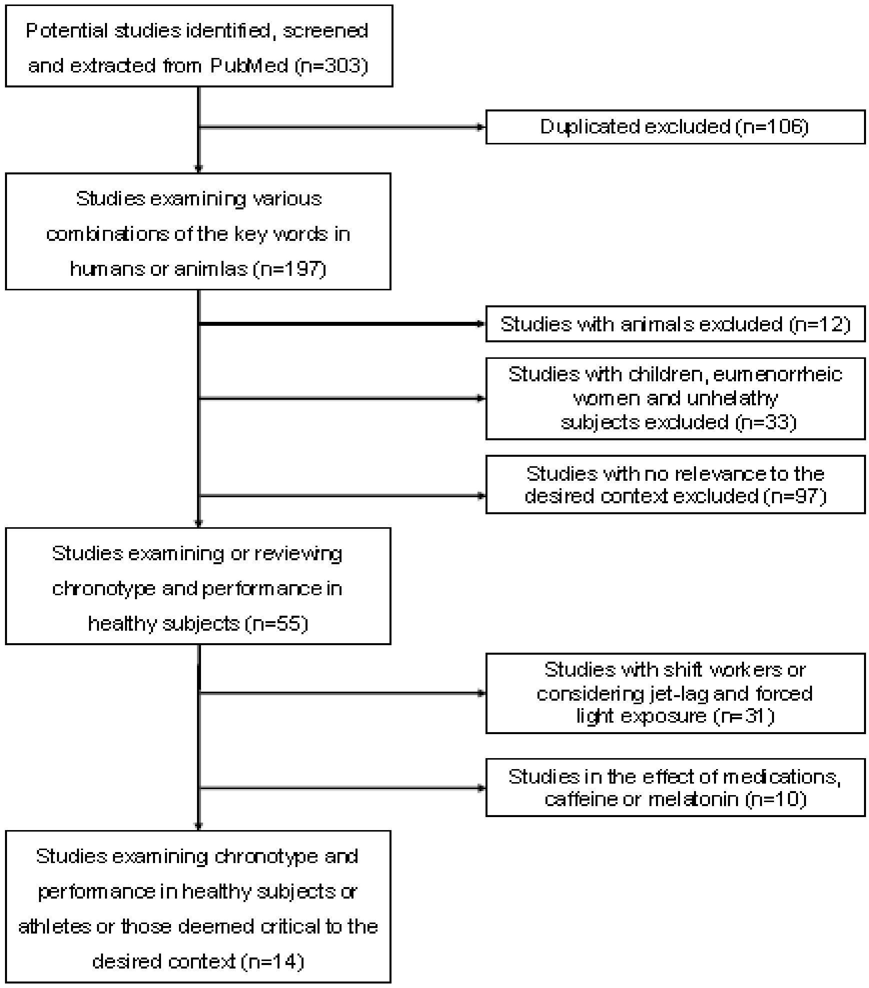


Figure 6. Flow diagram and results of the literature research to address the aim of this chapter to evaluate the importance of chronotype in sport performance.

5.2 The first studies in 80's and 90's by Hill and Burgoon

Studies that have considered the chronotype effects on physical performance are not totally clear and in agreement with each other. Hill and colleagues (1988) conducted the first study that evaluated the effect of college student's chronotype on physical activity. They recruited 32 subjects (mean age: 25 ± 4.5) that, after being classified as M-types or E-types, performed an incremental maximal cycle ergometer test both in the morning (06:00-08:30) and in the afternoon (15:30-18:00). During the maximal test, E-types had higher values in VO2 max in the evening compared to the morning session (+4%), while no other differences were observed. Nevertheless, an effect was found on oxygen consumption both in M and E-types, and on rating of self-perceived exertion (RPE) in M-types at a submaximal level. Similar results were observed in the study of Burgoon and colleagues (1992): the authors compared HR, respiratory parameters, RPE, and total exercise time recorded during a maximal graded treadmill test at two times of day (07:30 and 19:30) in 26 young men (mean age: 23 ± 4.4) grouped in M, N, and E-types. They found no statistical effect of chronotype on HR, respiratory parameters, and performance, but they did find it on RPE measured at maximal effort.

5.3 RPE and mood

In light of these findings, it seems that there could be an association between chronotype and the RPE or mood state. Two studies recently have confirmed this point: Kunorozva and colleagues (2014) reported that 20 trained males cyclists (mean age: 39.8 ± 7.7), categorized as M-types, had higher ratings of perceived exertion when cycling at 60%, 80%, and 90% of their HR max during the evening (18:00 and 22:00) compared to the other sessions (06:00, 10:00 and 14:00) even if the absolute power output, speed and cadence did not display any time-of-day effect. Figure 7 shows the means ± standard deviations of the subjects' RPE for the 3 stages of the 17-min Lambert & Lambert submaximal cycle test at different times of days. The M-type cyclists perceive the same relative intensity workload to be harder in the evening compared to the morning so, maybe, they may be more motivated and achieve greater intensities when sessions are scheduled early in the morning.

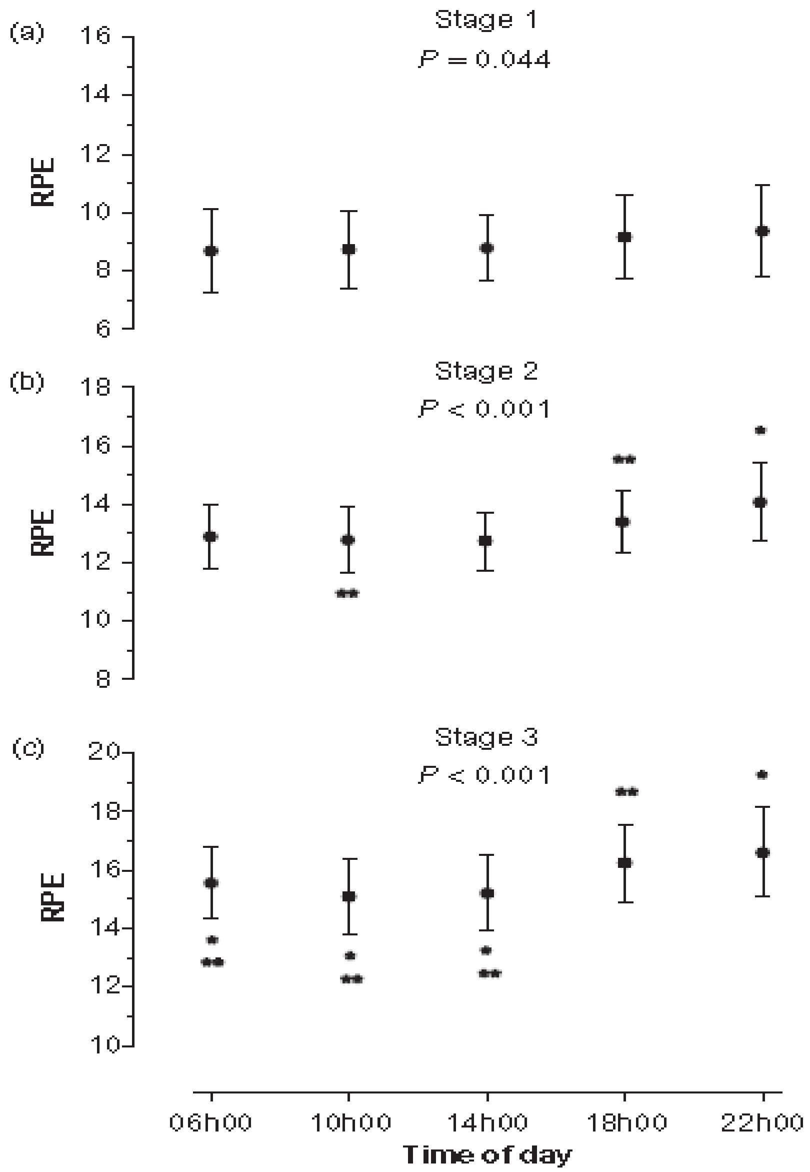


Figure 7. Time of day changes in RPE of M-types cyclists during stage 1(a), 2(b) and 3(c).

The second study that highlighted the effect of chronotype on RPE was conducted by Rae and colleagues (2015): the aim was to compare 200 meters swimming time-trial performance, rate of self-perceived exertion and mood state at 06:30 and 18:30 in 26 trained swimmers (mean age: 32.6 ± 5.7) taking into account the chronotype. The subjects completed the Profile of Mood States (POMS) questionnaire to assess their affective and mental state (McNair et al., 1971) before performing the 200 meters freestyle time-trials at different times of day. There were time-by-group interaction effects for both fatigue and vigour when chronotype was tested: specially M-types swimmers had lower fatigue scores prior to the 06:30 time-trial compared to the 18:30 time-trial (4.9 ± 3.2 vs 9.1 ± 5.9) while fatigue scores of N-types were similar in both sessions. M-types showed also lower total mood disturbance (TMD) compared to N-types, regardless of time-of-day (Figure 8).

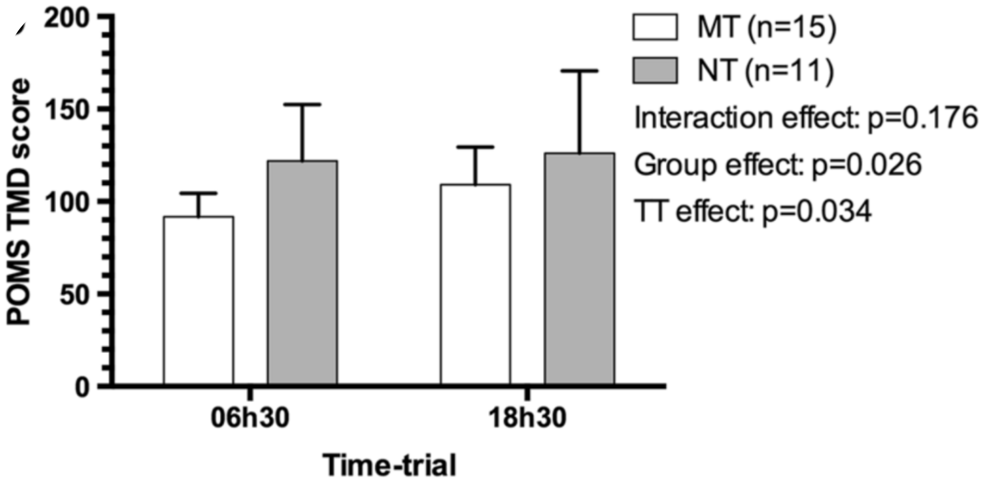


Figure 8. Total mood disturbance (TMD) scores for swimmers grouped by chronotype.

5.4 Results of physical performance

The effects of chronotype on physical performance are not yet entirely clear. Rae's study, in addition to analyse the results of POMS, showed that grouping the participants by chronotype produced a significant diurnal variation in performance with M-types swimming faster in the morning session and the N-types at 18:30. There was a weak but significant correlation between MEQ score and the time difference between morning-evening time-trials: swimmers with higher MEQ scores tended to swim faster in the 06:30 session. These results are in line with Brown's study (2008) where 16 collegiate rowers (mean age: 19.6 ± 1.5, 8 men and 8 women) had to perform a 2000 meters rowing test and a standing broad jump test both in the morning at 05:00-07:00 and in the afternoon 16:30-18:00. The analysis highlighted an interaction between chronotype and time indicating that M-types significantly slowed in rowing speed from morning to afternoon by 4.8 seconds and, in addition, they showed a larger decrement on performance across the day as compared with E-types and N-types; no significant changes in rowing speed were found for E-types and N-types and no statistically significant group difference occurred from morning to afternoon in broad jump distances.

Facer-Childs and Brandstaetter (2015) have conducted the most recent study that examined the results of a physical performance in different chronotypes. First, 121 competition level athletes were recruited and compiled a new chronometric questionnaire (RB-UB chronometric test) specifically designed to study sleep/wake-related parameters and performance variables in athletes. From this sample, 20 subjects (5 M-types, 10 N-types and 5 E-types) were selected to conduct BLEEP fitness tests at six different times of day (07:00, 10:00, 13:00, 16:00, 19:00 and 22:00). All 20 were field hockey players with an average age of 20.4 years competing at regional club level, with seven out of these 20 individuals additionally competing at international level. Analysis considering circadian phenotype revealed significant differences in peak performance, with the highest performance for M-types at 12.19 ± 1.43 hrs, N-types at 15.81 ± 0.51 hrs, and E-types at 19.66 ± 0.67 hrs (Figure 9a). Diurnal changes in performance were 7.62% ± 1.18% in M-types as compared to 10.03% ± 1.62% in N-types and a striking 26.2% ± 3.97% in E-types. The authors suggested that time of day is just an exogenous factor and is only partly related to the circadian physiology of an individual so they also evaluated the data as a function of time since awakening because this variable could be considered as an endogenous factor. It was observed that the average peak performance time for E-types was 11.18 ± 0.93 hrs after entrained wake-up, it was significantly delayed as compared to N-types and M-types peak performance times (Figure 9b). They concluded that it does not necessarily matter at what time of day personal best performance has to be achieved, what matters for an athlete is how many hours after entrained wake-up the competition or performance evaluation takes place.

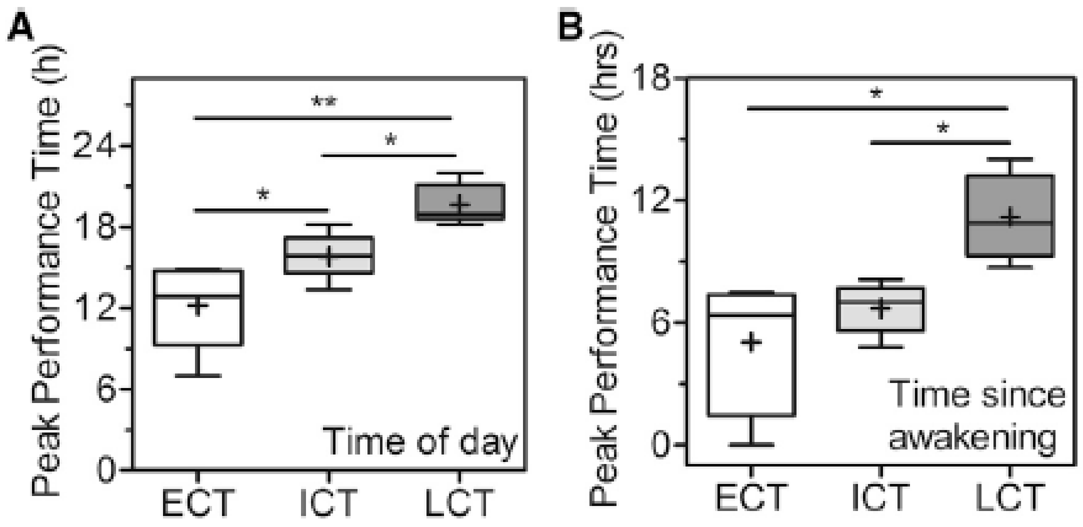


Figure 9. Peak performance times as functions of time of day and time since entrained awakening. **A**: peak performance times in real time, i.e., time of day in hours. **B**: peak performance times expressed as time since entrained awakening in hours. ECT: Early Circadian Phenotypes (M-types), ICT: Intermediate Circadian Phenotypes (N-types) and, LCT: Late Circadian Phenotypes (E-types).

5.5 Neuromuscular function, maximal torque and anaerobic power

Racinas and colleagues (2004) tried to establish the effect of time-of-day on maximal anaerobic leg power in a tropical environment in 23 physical education students (15 males and 8 females, mean age: 22.8 ± 3), all classified as N-types. Tests were scheduled at 08:00, 13:00 and, 17:00 on separate days and in temperature-controlled conditions (28°) and the subjects performed the vertical jump tests and a force-velocity test with the use of the cycle ergometer. The results showed a time-of-day effect on rectal temperature that was significantly higher at 13:00 and 17:00 compared to 08:00, nevertheless no variations across the day were observed among N-types in maximal anaerobic power under the influence of a tropical climate.

Tamm and colleagues (2009) designed some experiments to determine the influence of an individual's chronotype on the ability to generate torque during a maximum voluntary contraction and on cortical, spinal, and peripheral mechanisms that may be related to torque production. 9 M-types and 9 E-types (mean age: 26.3 ± 3) were recruited and participated in 4 data collection sessions (09:00, 13:00, 17:00, and 21:00) over 1 day. Magnetic stimulation of the cortex, electrical stimulation of the tibial nerve, electromyographic recordings of muscle activity, and isometric torque measurements were used to evaluate the excitability of the motor cortex, the spinal cord, and the torque-generating capacity of the triceps surae muscles. It was found that M-types had higher values of cortical excitability at 09:00, spinal excitability was highest at 21:00, and there were no significant differences in torque produced during maximum voluntary contractions over the day. In contrast, E-types showed parallel increases in cortical and spinal excitability over the day, and generated more torque at 21:00 (13%; $p = 0.0002$) and 17:00 (8%; $p = 0.04$) than at 09:00. E-types in this study demonstrated the largest diurnal increase in torque at 13% and this difference could have implications for maximizing human performance.

A recent study by Küüsmaa and colleagues (2015) examined the diurnal rhythms in maximal isometric force production both for the whole sample, composed by 72 men aged 32 ± 6, and by separating the high morning performance types (N=8) and the high evening performance types (n=19) based on their actual maximal isometric force levels. Measurements were performed in the morning (7:30) and in the evening (18:00) for maximal bilateral isometric leg press force (MVC_{LP}) and its relative myoelectric activity (EMG_{LP}), maximal unilateral isometric knee extension force (MVC_{KE}) and maximal voluntary activation level (VA%) together with myoelectric activity (EMG_{VA}). In the total group, MVC_{LP} and MVC_{KE} were higher in the evening compared to the morning while VA% did not show any circadian variation. The high morning performance types showed lower values in the evening compared to the morning for MVC_{LP} and MVC_{KE} while no differences were observed for VA%; the high evening performance types showed higher force values in the evening for MVC_{LP} and MVC_{KE} with a concomitant higher VA% in the evening. All the other neuromuscular values did not show significant circadian variations. The authors concluded that the questionnaires designed to determine the chronotype may not always be sensitive enough to determine “morningness” and “eveningness” in maximal neuromuscular performance.

5.6 Distribution of chronotype among athletes.

Few studies tried to investigate and justify the distribution of chronotype among athletes. As many competitive events in South Africa for individual athletes are scheduled for the early mornings, Kunorozva and colleagues (2012) hypothesized that this might favour those athletes with a preference for morning activities. 125 white male cyclists, 120 runners and 49 Ironman triathletes compiled the MEQ and were compared with a control population of 96 active, non-competitive individuals. Moreover, since a link between diurnal preference and a variable number tandem-repeat (VNTR) polymorphism in the PERIOD3 (PER3) gene has been demonstrated, the PER3 VNTR genotype for each participant was determined. The athlete groups contained more M-type individuals than the control group and a strong relationship between chronotype and PER3 VNTR genotype was observed ($p<0.001$). Finally, the time of day at which the athletes preferred to train was related to their chronotype ($p<0.001$). These data suggest that white males of European descent participating in individual endurance sports in South Africa are more likely to be M-types and the PER3 VNTR may be one of the factors contributing to this observation.

Henst and colleagues (2015) argued that habitual early waking for training or endurance events in South African endurance athletes might have conditioned their chronotype. South African marathons typically start at about 06:30 while those in the Netherlands start later (11:00) therefore, they compared both South African marathon runners (n=95) with Dutch marathon runners (n=90) and active but non-competitive controls from South Africa (n=97) and Netherlands (n=98) to better understand the effects of marathon start time on chronotype. The main finding was that South African runners were significantly more morning-orientated than Dutch runners suggesting that participation in an endurance sport with an earlier start time may influence chronotype. Secondly, both the South African and Dutch runners were significantly more M-types than their respective control groups, indicating that individuals who train for and participate in recreational endurance sport races have an earlier chronotype than physically active but non-competitive males. Finally, the PER3 VNTR polymorphism distribution was similar between the four groups and was not associated with chronotype, suggesting that the difference in chronotype between the four groups in this study is not explained by the PER3 VNTR genotype.

3. ABSTRACT

In the past, several studies investigated the circadian rhythm of different physiological variables associated to sport, the time-of-day effects on physical performance and the reciprocal relationship between sleep and physical activity but there is a scarce literature on how the chronotype could influence all these aspects.

Therefore, the purposes of this Ph.D. thesis are: 1) to assess, with the use of actigraphy, the relationship between the three chronotypes and the circadian rhythm of activity levels and to determine whether sleep parameters respond differently with respect to the time (weekdays versus the weekend) in M-types, N-types and E-types; 2) to evaluate whether a linear regression formula using the MEQ score would predict the actigraphy-based acrophase in a young Italian population; 3) to investigate the effects of chronotype on psychophysiological responses (RPE, HR and walking time) to a submaximal self-paced walking task performed in two different times of day (08:30-09:00 vs 15:30-16:30).

The results showed that: 1) the acrophases of the activity levels were significantly different in M- (14:32h), N- (15:42h) and E-types (16:53h) ($p<0.001$) while MESOR and amplitude were similar among chronotypes; there was also a significant interaction between the chronotype and sleep parameters: Sleep Efficiency of the E-types was poorer than that of the M- and N-types during weekdays (77.9% ± 7.0 versus 84.1% ± 4.9 and 84.1% ± 5.2) ($p=0.005$) but was similar to that measured in the M- and N-types during the weekend. 2) There was a significant linear relationship between MEQ and the Acrophase thus, enabling us to use the equation of the regression line to obtain predictions. The predictive equation resulted as follows: 1238.7-5.487*MEQ. The precision of the estimates was excellent and the r^2 was 0.70, indicating that 70% of the variance in the acrophase was explained by MEQ. 3) It was found a significant interaction between chronotype and time of day. The

post hoc analysis showed a significant difference for RPE in the morning session, with E-types reporting higher RPE compared with the M-types (14.33 ± 2.45 vs 12.00 ± 1.66) ($p<0.01$).

This Ph.D. thesis highlights two key findings: 1) the chronotype influence the activity circadian rhythm and the sleep parameters suggesting that E-types accumulate a sleep deficit during weekdays, due to social and academic commitments and that they recover from this deficit during “free days” on the weekend; 2) the chronotype and the time of day when a physical task in undertaken can influence the RPE response.

4. EXPERIMENTAL STUDIES

1. INFLUENCE OF CHRONOTYPE ON RESPONSES TO A STANDARDIZED, SELF-PACED WALKING TASK IN THE MORNING VS AFTERNOON: A PILOT STUDY. (2013). Vitale JA, Calogiuri G, Weydahl A. *Perceptual and Motor Skills* 116: 1020-1028. (see Appendices)

Introduction

General knowledge of circadian variations of the physiological response to exercise may be inadequate, since it is potentially confounded with chronotype. Individuals' chronotype may affect the expression of their biological rhythms (Koukkari & Sothern, 2006), and therefore influence the individual's response to exercise stimuli and the benefit from physical performance. Some studies suggest that chronotype can influence not only performance but also the response to exercise at different times of the day in young adults (Brown et al., 2008). A study using arithmetic subtraction as a stressor (Roeser et al., 2012) found that chronotype had an effect upon heart rate (HR) response (Cohen's d = 0.72, comparing E-types vs M-types in the morning test) and subjective stress ratings (Cohen's d = 0.17), with E-types showing the largest response to the stress. In a study published in 2001, Sugawara and colleagues found a statistically significant interaction between chronotype and time of day for the beat-by-beat heart rate decrease for the first 30-sec. span after a 3-min. ergometer exercise test (intensity: 80% of the ventilatory threshold) in 12 healthy male college students. In evening-types the beat-by-beat heart rate decrease was larger in the morning than in the evening (M = 165.5 sec., SD = 45.2 vs M = 119.5 sec., SD = 25.7; $F=12.05$, $p < .05$), M-types did not show such a difference between morning and evening values (M = 94.4 sec., SD = 33.8 versus M = 91.2 sec., SD = 33.8). The E-types also showed statistically significant larger values compared to the morning types in the morning

(M = 165.5 sec., SD = 45.2 vs M = 94.4 sec., SD = 33.8; F = 5.06, p < .01, Cohen's d = 1.73) (Sugawara, et al., 2001). This study suggests that individuals with different chronotypes might have different psychophysiological responses to physical tasks, if performed at inconvenient circadian time. The literature concerning the influence of chronotype on the psychophysiological response to low-intensity physical activity and exercise stimuli is yet unclear, although understanding the relationship between individual chronotype, circadian fluctuations of performance, and psychophysiological responses to exercise stimuli is important for promoting physical activity for health and wellness. In 2012, this study was undertaken to investigate physiological and perceived responses to a standardized self-paced walking task in morning vs late afternoon in individuals with different chronotypes. The aim of this study was to investigate possible influences of individual chronotype on psycho-physiological responses to an exercise stimulus.

Methods

Twenty-two healthy college students [12 men, 10 women; M age = 23.2 yr., SD = 3.6; M body mass index (BMI) = 22.45 m2/kg, SD = 2.7] agreed to participate in the study and signed an informed consent form after receiving an explanation of the project's aim, purpose, methods, and possible risks. The Norwegian Social Science Data Service (Project #30516) approved the study. Each participant completed the Horne–Ostberg Morningness–Eveningness Questionnaire (MEQ) for assessment of chronotype by placing the scores on the Morningness–Eveningness scale (Horne & Ostberg, 1976). The participants were divided into three groups according to scores on the MEQ: Morning-type, including Definitely and Moderately M-types scoring 59 and above; Evening-type, including Definitely and Moderately E-types scoring 41 and below; and Neither-type, scoring 42 to 58. Individual chronotype scores and categories were communicated to the participants only after the completion of the experimentation. The walking task consisted of three repetitions up and down a hill at the participants' own voluntary speed. A self-selected walking speed was assumed to be a workload the participants felt comfortable with. This was preferred to investigate psychological responses to physical activity, such as affective states and perceived exertion (DaSilva et al., 2010). It has been observed that when participants are asked to walk at a self-selected speed, they tend to select a speed that is close to their anaerobic threshold, making the workload intensity fairly even across participants with similar characteristics (i.e., age, fitness, adiposity, etc.; DaSilva et al., 2009). During the walk, the students wore a heart rate (HR) monitor (Polar Team2, Polar Electro Oy, Kempele, Finnland®), set to record the beat-to-beat interval of every heartbeat. A rating of perceived exertion (RPE) was obtained for each session by showing a Borg Rating Scale (6–20; Borg, 1982) on a board when the participants were completing the task at the top of the last hill. The individual HR maximum (HRmax) was assessed from a running test, after the completion of the last walking session. The HR data collected during the walking sessions were downloaded to the Polar Team2 software. Variables describing the cardiac response to the physical task, the individuals' ratings of perceived exertion, and the behavioural output (time to complete the walking task, which is a direct reflection of voluntary speed), were studied through descriptive analysis.

Results

The scores on the MEQ showed that the sample comprised 14 N-types, 4 E-types, and 4 M-types. Figure 1 shows the distribution of the MEQ results. Looking at the data, it would seem that 3 of 4 M-types tended to report higher RPE when walking in the afternoon compared to the morning, although their HR during morning activity was higher and their walking speed faster as compared to the evening walk. The E-types gave a similar or higher RPE in the morning activity, although 3 out of 4 tended to walk faster and their HR was higher during the afternoon activity compared to the morning activity. For what concerns the 14 N-types, they tended to show HR and RPE patterns similar to either the M- or the E-types according to their placement along the chronotype scale (Table 1). A multivariate analysis of variance (MANOVA) was performed, with time for completion, HRave, and RPE as dependent variables (Table 2), to investigate possible influences of chronotype (MEQ score) in interaction with time of day on the psychophysiological response to the walking test. Although statistical significance was not achieved for the interaction, according with the guidelines proposed by Cohen (Cohen, 1988, pp. 284–287), the effect size was large. On the other hand, the estimated power was not sufficient (31%), suggesting that the not-achieved significance may be explained by a small sample size. Looking at the data, one may notice that the psychophysiological response results mostly in the individuals with lower MEQ score being disfavoured (lower walking speed with higher RPE) when testing in the morning, while in contrast, those with higher MEQ appear to be advantaged in the morning (with relatively lower RPE). This was confirmed by MANOVA, which did show a main effect for MEQ score ($p = .017$) with a fairly high effect size and adequate power (partial $\eta 2 = 0.75$; estimated power = 0.97). Including an experimental condition in the study design (i.e., walking test later in the evening) could lead to an inversion of the psychophysiological response in favour of the individuals with Evening preference.

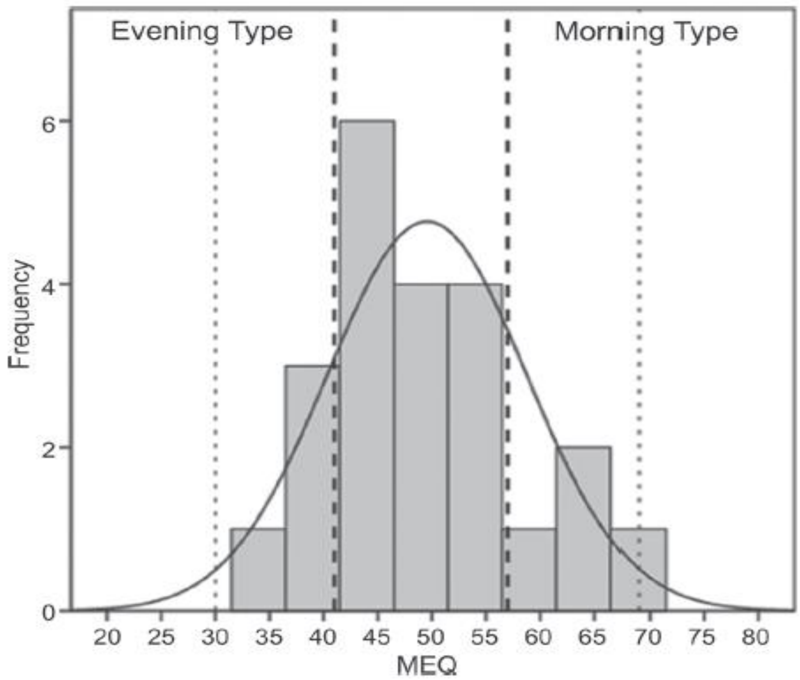


Figure 1. Frequency distribution of scores from the Horne and Ostberg Morningness–Eveningness Questionnaire (MEQ) in 22 college students (12 men and 10 women; M age = 23.2 yr., SD = 3.6) participating in the pilot study. Evening-type: MEQ < 41, Morning-type: MEQ > 59, Neither-type: 42 < MEQ < 58.

Fixed Factor	Wilks' λ	F	p	Partial η^2	Observed Power
Chronotype	0.705	2.35	.044	0.16	0.76
Time of day	0.985	0.18	.907	0.02	0.08
Interaction	0.920	0.51	.799	0.04	0.19

Table 2. Multivariate analysis of variance (MANOVA), with time for completion, HRave, and RPE as dependent variables. Note: HRave (Heart Rate Average) = heart rate average during the total walking sessions; expressed as % of individual maximal HR. RPE (Ratings of Perceived Exertion) = determined using the Borg Rating Scale (6–20; Borg., 1982) as the participants completed the walking task at the top of the third hill.

Table 1. Individual and average chronotype, time for completion, Heart-Rate response, and perceived exertion during a standardized, self-paced walking task in morning vs afternoon (n = 22).

Chronotype (Individual)	Subject	MEQ Score	Time for Completion (sec.)		HRave (% HR_{max})		HRpeak (% HR_{max})		RPE (Borg–20 Scale)	
			Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
	1	70	1,135	1,074	65.99	62.94	79.19	77.16	10	10
Morning-type	2	66	1,089	1,180	81.32	78.57	97.25	97.25	10	14
	3	62	1,062	1,125	88.95	88.95	99.47	98.95	13	14
	4	61	1,046	1,075	72.08	76.14	86.80	90.36	11	13
	5	55	1,006	963	75.39	69.27	88.80	82.12	10	13
	6	54	1,028	1,114	71.74	73.37	84.78	89.13	10	12
	7	54	980	930	72.00	76.00	87.00	88.50	13	15
	8	52	1,142	1,069	79.09	89.09	87.73	79.09	12	16
	9	51	1,029	1,039	69.19	70.27	85.95	89.73	15	16
Neither-type	10	51	1,112	1,114	77.55	79.59	88.78	91.84	16	14
	11	47	1,029	1,110	75.92	79.08	88.69	90.31	10	12
	12	47	1,026	1,103	72.77	72.28	88.61	89.60	13	11
	13	46	1,030	1,005	53.50	57.50	64.50	68.50	9	10
	14	46	949	973	82.11	82.63	93.68	95.79	14	11
	15	45	1,125	973	74.38	83.74	86.21	99.51	12	14
	16	45	924	945	83.90	84.88	94.15	98.05	14	12
	17	44	923	1,010	70.05	72.08	86.80	90.86	15	15
	18	42	1,095	1,079	78.84	80.00	97.37	98.42	12	12

(continued on next page)

Table 1 (Cont'd). Individual and average chronotype, time for completion, Heart-Rate response, and perceived exertion during a standardized, self-paced walking task in morning vs afternoon (n = 22).

Chronotype (Individual)	Subject	MEQ Score	Time for Completion (sec.)		HRave (% HR_{max})		HRpeak (% HR_{max})		RPE (Borg–20 Scale)	
			Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Evening-type	19	40	1,133	1,029	73.20	75.77	83.51	86.60	14	14
	20	40	974	957	72.99	77.59	88.51	95.98	15	15
	21	38	1,217	1,128	67.03	65.93	81.87	81.32	11	11
	22	34	988	963	68.21	74.36	82.05	88.72	15	13
Average Value										
Morning-types		64.75	1,083	1,113	77.08	76.65	90.67	90.93	11.00	12.75
Evening-types		48.50	1,028	1,029	74.08	76.41	87.36	89.39	12.50	13.00
MEQ > median		52.83	1,049	1,038	74.16	76.27	87.17	86.74	12.67	14.33
MEQ < median		45.25	1,013	1,023	73.93	76.03	87.50	91.38	12.38	12.13
Evening-types		38.00	1,078	1,044	70.35	73.41	83.98	88.15	13.75	13.25

Note: Time for completion = the duration of each walking session from the first uphill (when the HR started to increase) to the end of the third uphill. It was controlled by EKT system, used in the sport of orienteering. Heart Rate Peak (HRpeak) = highest heart rate value recorded during each session; this value is expressed as % of individual maximal HR. Heart Rate Average (HRave) = the average of the heart rate during the total walking sessions, expressed as % of individual maximal HR. Ratings of Perceived Exertion (RPE) determined using the Borg Rating Scale (6–20; Borg, 1982) as the participants completed the walking task at the top of the third hill. The board with the Borg RPE scale was shown to the participant while he/she was still walking.

Discussion

This pilot study had a number of limitations. The small sample size and the consequent small numbers of participants of each chronotype, especially the extreme M- and E-types, suggest the need of additional studies to test a much larger group of participants to include more extreme chronotypes. Another limitation was that the physical task was not performed at any truly extreme times. We selected the times of day when most people are usually starting and ending their daily work schedules in Norway, and most likely will do some type of exercise. This choice aimed to resemble real-life conditions of ordinary people, but on the other hand, it could have limited observation of possible differences across chronotypes. In future studies, it would be advisable to adopt a crossover design, and also include motivational determinants, such as enjoyment, affective responses, intention scales, or behavioural attitude (implicit cognition). One of the strong points in the study design was the employment of the MEQ scale, which is used to assign chronotype accurately. Furthermore, the choice of using a self-paced speed allowed the authors to include a behavioural component of physical activity that is also of relevance for health purposes (Tudor-Locke & Rowe, 2012). Using a self-paced speed in this pilot study allowed the authors to not only focus on the cardiac response to a physical task, but also to evaluate behavioural components such as the preferred walking speed. Interpreting the results of the pilot study, the physiological circadian variation of HR response to exercise stimuli, which has been shown independently (Calogiuri et al., 2011), and the individual chronotype have to be taken into account. In this light, the data suggest that chronotype may have an effect on psychophysiological responses to physical activity, mainly with E-types being more stressed when performing the task in the morning (see Table 1). To confirm these impressions, there is a need for a much larger and more representative sample that includes the full range of chronotypes, with representatives of extreme types, and different times of day for testing (e.g. late evening). On the base of the preliminary findings presented, this study can advance a number of hypotheses for future studies. (1) E-types will show a better predisposition, with a shorter time for completion and/or lower RPE, when performing a physical task later in the day (with the task occurring in the evening or late evening). On the contrary, M-types will meet more of a burden when undertaking a physical task late in the day, while showing a better predisposition in performing a physical task early in the day (morning). (2) Participants will report a preference of engaging in an exercise program in accordance with their circadian typology. (3) Performing a physical task at the individual’s favourable circadian time will be associated with a better profile of factors connected to the motivation to exercise, such as enjoyment, affective response, and behavioural attitude towards exercise, and higher intention to engage in exercise in future. The compatibility between time of day for exercising and chronotype may predict the adherence to an exercise program in the long term.

2. CHRONOTYPE INFLUENCES ACTIVITY CIRCADIAN RHYTHM AND SLEEP: DIFFERENCE IN SLEEP QUALITY BETWEEN WEEKDAYS AND WEEKEND. (2014). Vitale JA, Roveda E, Montaruli A, Galasso L, Weydahl A, Caumo A, Carandente F. *Chronobiology International* 32: 405-415. (see Appendices)

Introduction

Chronotype is the expression of circadian rhythmicity in an individual and can differ among individuals. There are three different chronotypes: Morning-types (M-types), Evening-types (E-types) and Neither-types (N-types). Several studies have shown differences between M-types and E-types in the circadian rhythms of different physiological variables. For instance, M-types have, under normal conditions, an earlier oral temperature peak approximately 2h before the E-types (Baehr et al., 2000), and the acrophase of cortisol in serum is 55min earlier in M-types than in E-types(Bailey & Heitkemper, 2001). M-types generally wake up and go to bed early (Taillard et al., 2004) and have their best performances in the first part of the day, whereas Etypes go to bed and wake up late and have their peak performances in the evening (Horne et al., 1980; Vitale et al., 2013). In addition, the acrophase of melatonin profiles in blood and salivary samples, which is the best predictor of sleep onset, occurs approximately 3h later in E-types than in M-types (Mongrain et al., 2004). Chronotype is usually evaluated by self-assessment questionnaires. The most used and cited questionnaire is the Morningness–Eveningness Questionnaire (MEQ), which was validated by Horne and Ostberg in 1976 (Horne & Ostberg, 1976). Morningness is related with difficulty in maintaining sleep, and eveningness is related to difficulty in initiating sleep (Taillard et al., 2001). Chronotype is influenced by individual and environmental factors: morningness increases with age (Kim et al., 2010); thus, older people tend to go to bed and wake up earlier. Additionally, eveningness is observed in a larger proportion of males than females (Adan & Natale, 2002). Furthermore, chronotype may be influenced by the photoperiod at birth, i.e. the duration of an organism's exposure to light at birth. In fact, individuals born during a short photoperiod at birth are more likely to be M-types than are those born during a long photoperiod at birth, who, quite to the contrary, show a predisposition toward eveningness (Mongrain et al., 2006; Natale & Adan, 1999). Individuals show variation in their preference for the daily timing of activity; there is also an association between chronotype and sleep duration/sleep complaints. Several studies have evaluated the sleep quality in different chronotypes using either self-assessment questionnaires or actigraphy. Studies that used selfassessment questionnaires showed that E-types were more prone to sleep complaints, as measured by the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) and the Epworth Sleepiness Scale (ESS) (Johns, 1991), than M-types (Barclay et al., 2010). Moreover, the prevalence of nightmares and insomnia symptoms, which was evaluated by self-reported questionnaires, was higher in E-types than in M-types (Merikanto et al., 2012). Actigraphy has been used to study sleep/wake patterns for many years (Calogiuri et al., 2011; Lehnkering et al., 2006; Montaruli et al., 2009; Paquet et al., 2007; Roveda et al., 2011). Compared with the traditional polysomnography (PSG), actigraphy is more advantageous because the recording lasts for 24h a day for days, weeks or longer periods. Actigraphy is also able to assess the circadian rhythm of the activity-rest cycle expressed by the subjects over 24h. Although actigraphy is rarely used for clinical research (Sadeh et al., 1995), it is commonly used for the diagnosis of insomnia, circadian rhythm disorders and excessive sleepiness (Standards of Practice Commitee, 1995). Under the auspices of the American Academy of Sleep Medicine (AASM), Ancoli-Israel and colleagues concluded that actigraphy can provide information, particularly on the diagnosis of sleep disorders, circadian rhythm disorders and sleep variability in patients with insomnia, that are not obtainable in any other practical way (Ancoli-Israel et al., 2003). Lehnkering & Siegmund (2007) studied the influence of chronotype on sleep behavior in young adults using actigraphs (Actiwatch actometers, CNT, Cambridge, UK): the results showed that there was a difference in sleep efficiency between M-types (87.9%, SD=1.3%) and E-types (84.3%, SD=0.87%). Martin et al. (2012) investigated the relationship between chronotype and sleep, but they did not find any significant differences in actigraphic sleep parameters, such as sleep duration, sleep efficiency and sleep latency, whereas sleep onset and sleep offset differed among the chronotypes. Few studies have investigated sleep parameters during weekdays and the weekend in relation to the circadian typology using both a self-assessment questionnaire and actigraphy. The results regarding sleep timing have shown that E-types generally go to bed and wake-up significantly later than M-types on both work and free days; therefore, eveningness is associated with a later bedtime and wake-up time and a shorter time in bed during the week (Giannotti et al., 2002; Kabrita et al., 2014; Lee et al., 2014; Roepke & Duffy, 2010). No data were reported regarding sleep quality. In the light of what has been reported in the literature, we focused our attention on the relationship between chronotype and the circadian rhythm of activity levels and the differences in sleep quality in relation to circadian typology. The purpose of this study was to provide a thorough circadian characterization of chronotypes and sleep parameters in university students as follows: (1) to determine the prevalence of the three chronotypes (M-, N- and E-types) in a student population in southern Europe; (2) to evaluate the influence of sex and photoperiod at birth on chronotype; (3) to use actigraphy to assess the relationship between the three chronotypes and circadian rhythm in activity levels; and (4) to use actigraphy to monitor sleep quality to determine whether sleep parameters respond differently between weekdays and weekend in the three chronotypes.

Materials and Methods

Study subjects. The study subjects were college students of the School of Sports Science of the University of Milan who were enrolled in the academic year 2012–2013 (N=502; 347 males and 155 females; mean age (±SD) was 21.3 (±2.37yrs). After receiving an explanation of the project's purpose and methods, the participants signed an informed consent and completed the Horne–Ostberg MEQ for the assessment of chronotype (Horne & Ostberg, 1976). The subjects were categorized as M-types (scores between 59 and 86), N-types (scores between 42 and 58), and E-types (scores between 16 and 41). A subsample of 50 subjects was recruited to undergo a 7-day monitoring period (from Monday to Sunday) using the actigraph (Actiwacth actometers, CNT, Cambridge, UK) to evaluate the circadian rhythm of their activity levels and their sleep parameters. The subgroup included 16M-types (7 males and 9 females), 15N-types (5 males and 10 females) and 19E-types (10 males and 9 females). We recruited the M-types with the highest scores on the MEQ, the E-types with the lowest scores on the MEQ and the N-types with closest scores to the median of all MEQ scores. All of the subjects were in good physiological and psychological health, and they were not under any pharmacological therapy. In February 2013, each participant wore the actigraph on their non-dominant hand for 7 days and was given a diary to record information regarding their bed time, wake-up time, hours spent napping, hours without wearing the actigraph and number of nocturnal awakenings. The subjects had similar university time schedules, with lectures starting at approximately 09:00h, and none of the sub-group subjects had a part-time job. The students were told to abstain from sport activities during the monitoring. None of the subjects knew their own chronotype before the monitoring period, nor did the staff who analyzed these data. The study protocol and procedures complied with the guidelines required by the journal (Portaluppi et al., 2010).

Evaluation of the photoperiod at birth. The photoperiod at birth represents the duration of an organism's exposure to light at birth. To investigate the influence of the photoperiod at birth on the chronotype, the students were asked to provide their date of birth, including the day, month and year. The subdivision of the different categories of photoperiod at birth do not rely on the calendar seasons of the year, such as spring, summer, autumn and winter, because this classification does not take into account the latitude of the place of birth and therefore is not accurate. Similar to the protocol of Vollmer and colleagues in their study, we grouped our subjects, considering the latitude of Milan (45°N), into the following categories of photoperiod at birth: increasing photoperiod (February, March and April), long photoperiod (May, June and July), decreasing photoperiod (August, September and October) and short photoperiod (November, December and January) (Vollmer et al., 2012).

Actigraphy. The actigraph (Actiwacth actometers, CNT, Cambridge, UK) was used to evaluate the circadian rhythm of the activity levels and the sleep parameters. The Actiwatch Software was used to obtain the activity data, which were expressed in activity counts and recorded for every one-minute throughout the monitoring period (7 days). For each subject, the data were then processed to evaluate the circadian rhythm of activity levels. The Actiwatch Sleep Analysis Software (Cambridge Neurotecnology, Cambridge, UK) was used to evaluate the sleep patterns. We considered seven sleep parameters for further analysis: (1) Sleep start (Ss): the start of sleep was derived automatically using the Sleepwatch algorithm (expressed in hours and minutes). (2) Sleep end (Se): the end of sleep was derived automatically using the Sleepwatch algorithm (expressed in hours and minutes). (3) Assumed Sleep (AS): the difference in hours and minutes between the Sleep end and Sleep start times. This parameter was calculated automatically using the Actiwatch Sleep software. (4) Sleep Latency (SL): the period of time required for sleep onset after retiring to bed. SL is the period between Bed Time and Sleep Start. It was automatically calculated by an algorithm based on the lack of movement following Bed Time. (5) Movement and Fragmentation Index (MFI): the MFI is the addition of the Movement Index (percentage of time spent moving) and the Fragmentation Index (percentage of immobile phases of one minute). MFI is used as an index of restlessness. (6) Immobility Time (IT): the total time, expressed in hours, spent without recording any movement within the period from Sleep start to Sleep end. (7) Sleep Efficiency (SE): the percentage of time in bed spent actually sleeping. For each subject, the data recorded during the 7 nights of monitoring were divided in two periods: five nights from Sunday to Thursday (Weekdays – WD) and two nights from Friday to Saturday (Weekend – WE).

Statistical analyses

Effect of sex on the MEQ score. The MEQ scores were reported as the mean±SD. The normality of the distributions of the MEQ scores obtained in the female and male students were checked using graphical methods and Shapiro–Wilk's test. The MEQ scores in females were normally distributed, whereas the MEQ scores in males were not. Because the deviation from normality of the MEQ scores in males was mild (p¼0.031), the unpaired Student's t-test was used to compare the mean MEQ score obtained in male and female students (of note, the Mann–Whitney nonparametric test led to the same conclusions).

Chronotype versus sex and photoperiod at birth. To evaluate the influence of individual and environmental factors on chronotype, a chi-square test for association was conducted between sex and chronotype and between the photoperiod at birth and chronotype.

Analysis of circadian rhythmicity. To determine the circadian rhythmicity in the three chronotypes, the activity data provided by the actigraph were analysed using the single cosinor method (Halberg et al., 1977; Nelson et al., 1979). Based on the least squares method, the single cosinor method identifies and evaluates the cosine mathematical function that best fits the data as a function of time. The function $f(t)=M+A\cos(\omega t+Ø)$ defines three parameters that are characteristics of each statistically significant rhythm: M is the Midline Estimating Statistic of Rhythm (MESOR); A is the amplitude and Ø is the acrophase. The MESOR is a rhythm-adjusted mean that approximates the arithmetical mean of the data for a 24-h period, and the amplitude is the measure of one-half the extent of the rhythmic variation in a cycle. The acrophase indicates, with 95% confidence limits (CL), the time interval within which the highest values of the variable are expected. The three parameters are usually indicated with the relevant 95% confidence intervals. The rhythmometric parameters of activity levels (MESOR, amplitude and acrophase) were then processed with the average of the population mean cosinor. This method, applied to the rhythmometric parameters of each subject's circadian variables, evaluates the rhythmometric characteristics of the activity levels of the population (Nelson et al., 1979). The statistical analyses were carried out using the Time Series AnalysisSeriel Cosinor 6.0 (Expert Soft Technology, Richelieu, France). The rhythmometric parameters were expressed as the mean±SD. The normality of the distribution of each parameter was checked using graphical methods and Shapiro–Wilk's test. Whereas the amplitude and acrophase were normally distributed in the three chronotypes, the MESOR deviated significantly from normal in both the M-types and E-types due to the presence of two outliers (one M-type subject with a rather low MESOR and one E-type subject with a very high MESOR). To compare the amplitude and the acrophase among the three chronotypes, we used a one-way ANOVA followed by the Tukey–Kramer post-hoc test. To compare the MESOR among the three chronotypes, we used a Kruskal–Wallis non-parametric test followed by pairwise comparisons performed using Dunn's procedure with a Bonferroni correction for multiple comparisons.

Analysis of sleep parameters. The actigraphy-based sleep parameters were expressed as the mean±SD. Each sleep parameter was calculated twice (WD and WE) for the three chronotypes (M-types, N-types and E-types). The normality of the distribution of each sleep parameter was checked using graphical methods and Shapiro–Wilk's test. Each parameter was checked for normality six times (three chronotypes multiplied by two time frames). The most severe deviations from normality were displayed by the sleep latency values and, though to a lesser extent, by the assumed sleep values. The other parameters showed normal distributions apart from some sporadic and modest deviations from normality. The analysis of each sleep parameter was conducted using the mixed ANOVA procedure. The mixed ANOVA procedure is somewhat robust to deviations from normality. Nonetheless, we preferred to be conservative and thus also tested sleep latency and assumed sleep using non-parametric methods. Because the results provided by the non-parametric methods confirmed those obtained by the mixed ANOVA, we focus only on the latter procedure in the following discussion. Briefly, the mixed ANOVA considered each sleep parameter to be dependent on two factors: a between-subjects factor (i.e. chronotype) and a within-subjects factor (i.e. the time of the week). The primary purpose of the mixed ANOVA was to establish whether there was an interaction between the two factors, that is, whether the modality of change of the sleep parameter over time (from WD to the WE) was dependent on the chronotype. Therefore, a statistically significant interaction indicated that the impact of chronotype on the sleep parameter depended on the level of time. This is usually visualized as three nonparallel lines (one line per chronotype) connecting the levels of the sleep parameter measured at the two time points (WD and WE) (Figure 5). To single out the individual effects of chronotype and time on the sleep parameter, we considered whether the interaction was significant. When a significant interaction was identified, we determined the simple main effects of chronotype and time. The simple main effect of chronotype was determined by testing for differences in the sleep parameter among the chronotypes at each level of time (two separate one-way ANOVAs were performed, one on the data recorded during WD and the other on the data recorded during the WE). The simple main effect of time was determined by testing for differences in the sleep parameter between WD and the WE for each chronotype (three separate repeated-measure ANOVAs were performed, one for each chronotype). When the interaction was not significant, the main effects of time and chronotype were determined. The main effect of time was tested by evaluating the differences in the sleep parameter measured during WD and the WE collapsed across the chronotypes (i.e. regardless of the chronotype). Thus, the chronotype was ignored, and the two levels measured during the WD and the WE were compared (in a similar manner to a repeated-measure ANOVA or, as in this case in which only two time levels were present, to a paired t-test). The level of significance of this comparison was found in the within-subjects table provided by the mixed ANOVA. The main effect of chronotype was tested by evaluating the differences in the sleep parameter among the three chronotypes collapsed across time (i.e. regardless of the time point). Thus, time was ignored, and the three chronotypes were compared (in a similar manner to a one way ANOVA). The level of significance of this comparison was found in the between-subjects table provided by the mixed ANOVA. The statistical analyses were conducted using SPSS version 21 software (IBM Corporation, Armonk, NY). A p value less than or equal to 0.05 was considered statistically significant.

Results

MEQ scores and the chronotype distribution. The overall average MEQ score was 48.2±8.7. Based on the MEQ scores, the subjects were categorized as follows: 335N-types (66.7%) consisting of 228 males and 107 females, 118E-types (23.5%) consisting of 92 males and 26 females, and 49M-types (9.8%) consisting of 27 males and 22 females. Figure 1 shows the distribution of the chronotype scores.

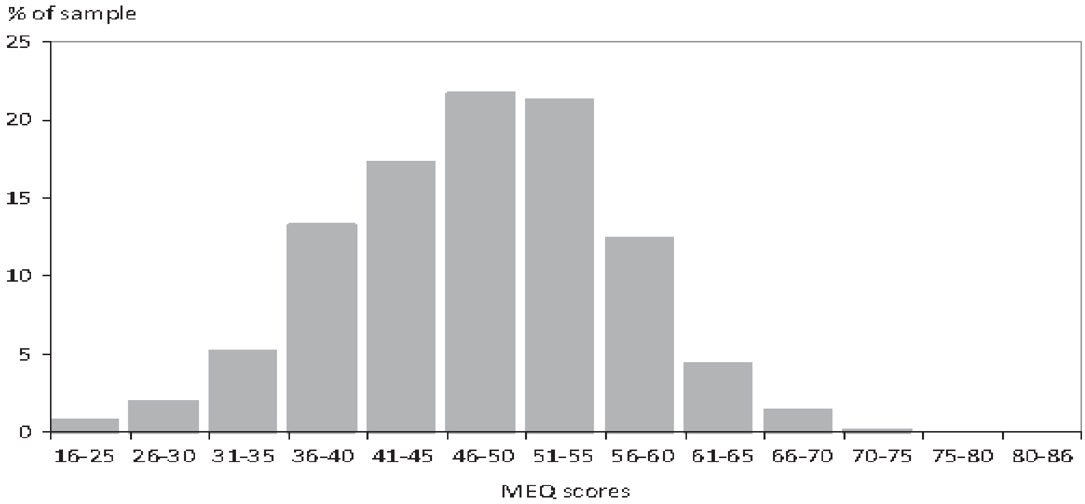


Figure 1. Frequency distribution of subjects by MEQ scores.

<u>Association between chronotype and sex</u>. The study subjects included 347 males (69.1%) and 155 females (30.9%). The average MEQ score was slightly but significantly higher in females than in males (49.5±8.6 versus 47.0±8.7, respectively, p=0.003). Figure 2 shows the distribution of the chronotypes in males and females. There was a significant association between chronotype and sex (p<0.011). Males were more likely to be E-types, whereas females showed a predisposition towards morningness.

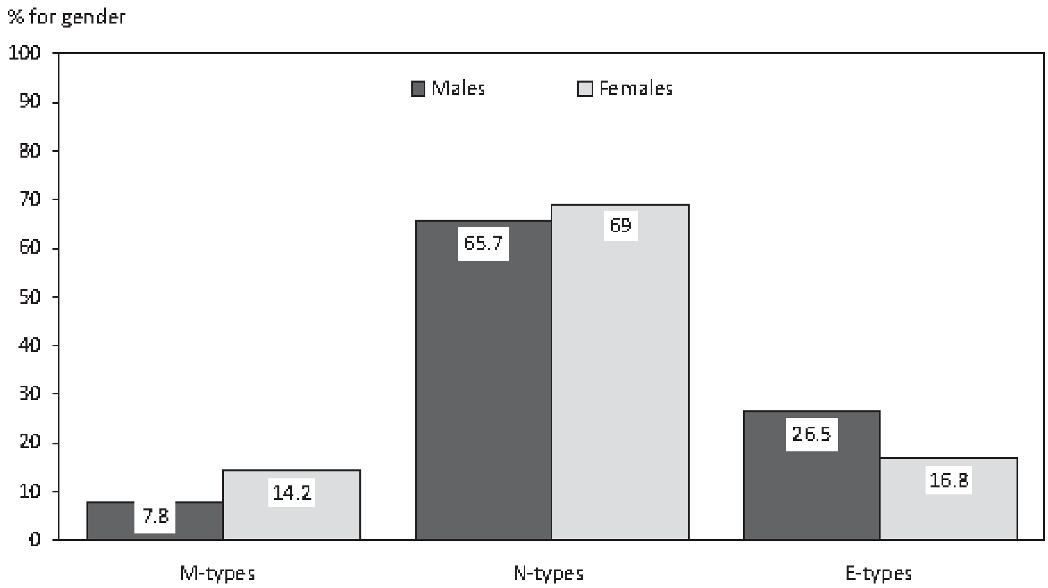


Figure 2. Distribution of chronotypes in males (N¼347) and females (N¼155) as a percentage of each gender.

Association between chronotype and photoperiod at birth. The subjects were uniformly distributed among the four categories of photoperiod at birth: 127 students were born during the short photoperiod, 129 were born during the long photoperiod, 124 were born during the decreasing photoperiod, and 122 were born during the increasing photoperiod. Figure 3 shows the distribution of chronotypes for each category of photoperiod at birth. There was an association between chronotype and the photoperiod at birth ($p<0.05$). The subjects born during the long photoperiod at birth were more likely to be E-types, whereas the subjects born during the short photoperiod at birth showed a predisposition toward morningness.

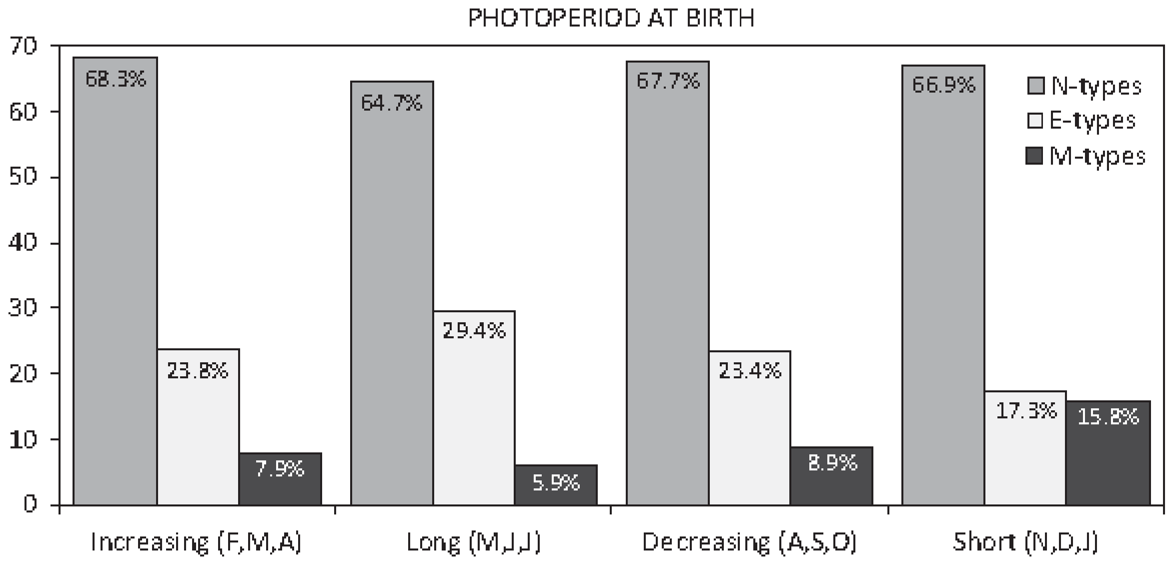


Figure 3. Distribution of chronotypes (percentage for each type) by photoperiod at birth for 502 subjects. The time scale indicates 3-month spans and the indication of daylight length.

Circadian rhythm of activity levels. The single cosinor method revealed the presence of a statistically significant circadian rhythm ($p<0.001$) in each of the 50 subjects, who were recruited to evaluate the rhythm of their activity levels. The population mean cosinor applied to the three chronotypes revealed the presence of a significant circadian rhythm in all three chronotypes ($p<0.001$). Table 1 and Figure 4 report the rhythmometric parameters measured in the three chronotypes. Although the MESOR and amplitude were not different among the three chronotypes, the acrophase results were significantly different (<50.001). The ANOVA post-hoc test revealed the presence of a significant difference (<50.001) between the M- and E-types. Specifically, the M-types had an early acrophase of their circadian rhythm of activity levels (14:32h), whereas the E-types showed an acrophase more than 2h later (16:53h). The N-types showed an intermediate acrophase between the M-types and E-types (15:42h).

Chronotype	*N*	PR (%)	*p*-value	Mesor (a.c.) (mean and 95% CI)	Amplitude (a.c.) (mean and 95% CI)	Acrophase (hr:min) (mean and 95% CI)
M-type	16	37	<0.001	265.6 [224.3–306.9]	222.8 [176.4–269.2]	14.32 [13:39–15:24]
N-type	15	50	<0.001	239.7 [204.2–275.2]	236.0 [200.9–271.1]	15.42 [15:10–16:14]
E-type	19	41	<0.001	277.8 [222.3–333.3]	216.8 [180.7–253.0]	16.53 [15:55–17:52]

Table 1. Rhythmometric analysis (population mean cosinor) for M-types, N-types and E-types. PR: percentage of rhythm. MESOR (activity counts): Midline Estimating Statistic of Rhythm. Amplitude (activity counts): half the difference between the highest and the lowest points of the cosine function best fitting the data. Acrophase (h:min) indicates the time in which the highest values occurs.

Sleep parameters. The results of the analysis of the sleep parameters are reported in Figure 5 and Table 2. The panels of Figure 5 (one panel for each parameter) display the sample average responses for each chronotype (M/N/E) and each time frame (WD/WE). Table 2 shows the values (mean±SD) of the actigraphy-based sleep parameters and the results of the mixed ANOVA procedure with the associated p values. There was a significant interaction between chronotype and the four sleep parameters Se, AS, IT and SE. Therefore, the changes that these parameters displayed from the WD to the WE differed among the three chronotypes. This can be visually appreciated by examining Figure 5. In fact, for the four above-mentioned parameters, the line segments deviated significantly from being parallel.

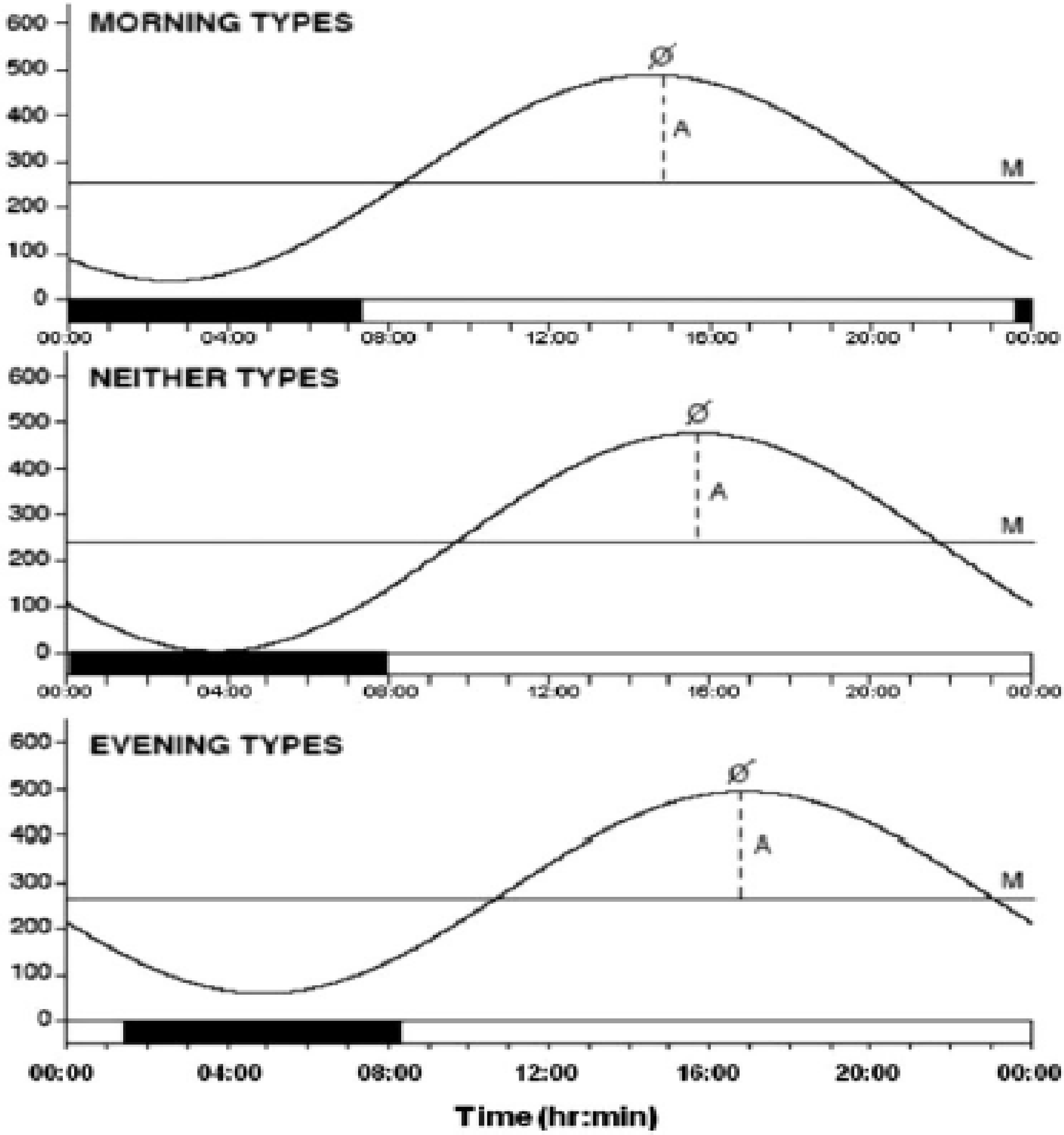


Figure 4. Activity counts (a.c.) circadian rhythms: data collected by the actigraph in M-, N- and E- types. Ø: Acrophase; A: Amplitude; M: MESOR.

The results of the parameter Ss indicated that E-type students began sleeping later than M-type and N-type students did. In all of the chronotypes, Ss was extended on the WE compared with WD (by an average of 51min). The results of the parameter Se showed that E-type students slept later than M-type students during WD and later than both M-type and N-type students during the WE. Delays in sleep–wake timing were common for the three chronotypes (Se was extended on the WE compared with WD by an average of 1h and 22min). This tendency was more appreciable in the E-type students, who showed the greatest Se delay of the three chronotypes (an average of 2h and 14min). The AS parameter results indicated that during WD, E-type students slept approximately 1h less than their M-type or N-type colleagues. During the WE, E-type students increased significantly their sleep duration, which became greater, though not significantly so, than that of the two other chronotypes. The two parameters SL and MFI were not different among the three chronotypes and did not change on the WE compared with WD. The parameter IT showed the same pattern of results as the AS. This similarity can also be appreciated by comparing the two panels devoted to these parameters in Figure 5. Interestingly, the parameter SE (i.e. an index of sleep quality) showed the same pattern of results as did the AS (i.e. an index of sleep quantity). In fact, the SE of the E-types was poorer than the M-types and N-types during WD but was similar to that measured in the other two groups during the WE.

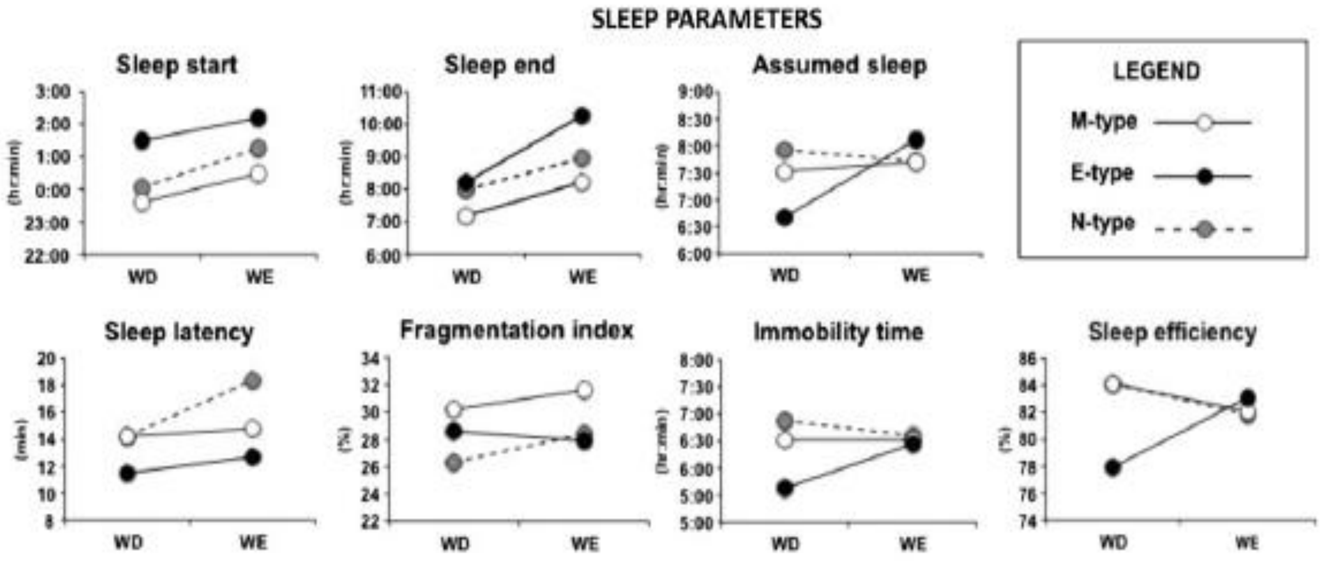


Figure 5. Mean values of the actigraphy-based sleep parameters recorded in the three chronotypes (M-type, E-type and N-type) in the two time spans, i.e., weekdays (WD) and the weekend (WE).

	M-types		N-types		E-types					
	WD	WE	WD	WE	WD	WE	Interaction (group x time)	Effect of chronotype	Contrasts	Effect of time
Ss (h:min)	23:38 ± 0:30	0:30 ± 0:58	0:03 ± 0:48	1:15 ± 0:58	1:28 ± 1:05	2:13 ± 1:02	ns	$p<0.001$	E≠M, E≠N	$p<0.001$
Se (h:min)	7:10 ± 0:41	8:13 ± 0:43	7:59 ± 1:04	8:58 ± 1:03	8:13 ± 1:22	10:17 ± 1:17	$p=0.01$	WD: ($p=0.04$) WE: ($p<0.001$)	E≠M E≠M, E≠N	M: ($p<0.001$) E: ($p<0.001$) N: ($p=0.002$)
AS (h:min)	7:32 ± 0:47	7:42 ± 0:48	7:55 ± 1:05	7:43 ± 1:07	6:40 ± 1:16	8:07 ± 0:57	$p=0.03$	WD: ($p=0.002$) WE: ns	E≠M, E≠N	M: ns E: ($p=0.001$) N: ns
SL (min)	14.2 ± 13.2	14.8 ± 12.6	14.2 ± 9.3	18.4 ± 18.4	11.5 ± 10.4	12.7 ± 14.5	ns	ns		ns
MFI (%)	30.2 ± 10.4	31.6 ± 11.2	26.3 ± 9.2	28.4 ± 11.6	28.6 ± 9.7	27.9 ± 11.3	ns	ns		ns
IT (h:min)	6:31 ± 0:38	6:32 ± 0:52	6:52 ± 0:34	6:36 ± 0:40	5:38 ± 0:55	6:27 ± 1:08	$p=0.01$	WD: ($p<0.001$) WE: ns	E≠M, E≠N	M:ns E: ($p=0.013$) N: ns
SE (%)	84.1 ± 4.9	82.1 ± 6.3	84.1 ± 5.2	81.8 ± 8.7	77.9 ± 7.0	83.1 ± 6.8	$p=0.001$	WD: ($p=0.005$) WE: ns	E≠M, E≠N	M: ns E: ($p=0.002$) N:N: ns

Table 2. Results of the actigraphy-based sleep parameters that were recorded in the three chronotypes (M-type, E-type and N-type) in the two time spans, i.e. weekdays (WD) and the weekend (WE). Sleep start (Ss), Sleep end (Se), Assumed Sleep (AS), Sleep Latency (SL), Movement and Fragmentation Index (MFI), Immobile Time (IT), Sleep Efficiency (SE). The table also reports the results of the mixed ANOVA procedure (see the ‘‘Statistical analyses’’ section for details) with the associated p-values: (1) the interaction between the chronotype and the time of the week; (2) the effect of the chronotype per se (with the associated contrasts among the three chronotypes); (3) the effect of the time of the week per se (with the associated contrasts between the two week periods).

Discussion

This study is highlighted by two key findings: first, we observed that the circadian rhythm of activity levels is influenced by chronotype. Second, chronotype had a significant effect on sleep parameters: E-types had a lower sleep quality and quantity than did M- and N-types during weekdays, whereas during the weekend, E-types reached the same levels as the other chronotypes. According to the literature (Merikanto et al., 2012; Paine et al., 2006; Tonetti et al., 2008), because morningness scores tend to increase with age, in our sample of 502 young students, there was a larger number of E-types (23.5%) than M-types (9.8%). Several authors who used the MEQ found that morningness was more commonly observed among females and that males were more likely to be of E-types (Borisenkov et al., 2012; Randler, 2011; Roenneberg et al., 2004; Tonetti et al., 2011). Our data confirmed that males showed a predisposition towards eveningness and females to morningness: among E-types, there was a larger proportion of males (27.5% males versus 16.8% females), and there was almost double the number of female M-types than male M-types (14.2% females versus 7.8% males). Different studies have investigated the influence of the photoperiod at birth on circadian typology. However, the evidence is not yet convincing because different methodological approaches have been used, such as different categorizations of the seasons or different measures to define chronotypes. Boresinkov et al. (2012) did not find any effects of the photoperiod at birth on chronotype in adolescents, as defined by the Munich Chronotype Questionnaire; however, other studies have shown that more E-types were born during the seasons associated with a longer photoperiod (spring and summer) and that people born during a short photoperiod (autumn and winter) were linked with morningness (Caci et al., 2005; Natale & Di Milla, 2011; Natale et al., 2002). In the present study, we used the MEQ questionnaire (Horne & Ostberg, 1976), and we grouped the different photoperiods at birth in accordance with the more accurate categorization of

Vollmer's study (Vollmer et al., 2012); our results showed that the subjects born during a long photoperiod were more likely to be of E-types, and students born during a short photoperiod showed a predisposition toward morningness.

Several studies have investigated the differences among chronotypes regarding the circadian rhythmicity of a large number of biological markers, such as the sleep–wake cycle, melatonin, cortisol or body temperature. The results showed that M-types had earlier peaks of these variables than did E-types (Duffy et al., 1999; Gibertini et al., 1999; Horne & Ostberg, 1976; Kudielka et al., 2006). In this study, using actigraphy we demonstrated that the circadian rhythms of activity levels have different characteristics in the three chronotypes. In particular, we observed a significant difference in the acrophase: the M-types showed an early acrophase (14:32h) compared with both the N-types (15:42h) and the E-types who had a delayed acrophase that was more than 2h later than the M-types (16:53h). No significant differences were demonstrated in the MESOR or the amplitude for all three groups. Therefore, all of the subjects, apart from their chronotype, had similar levels of activity throughout monitoring period. It is likely that these results are due to the characteristics of the participants: university athletes. Daily training would be effective to promote the circadian amplitude, and this masking effect would diminish the difference between chronotypes. The data of the circadian rhythm of activity levels demonstrated that there was a clear difference between ‘‘larks’’ and ‘‘owls’’; these results are in line with other studies that showed biological differences among chronotypes: Lee et al. (2014), using actigraphy, argued that the mean activity acrophase of E-types was nearly 2h later in the morning than M-types. Moreover, Gupta & Pati (1994) demonstrated that M-types have an earlier circadian oral temperature acrophase than E-types, and this difference was approximately 2h (Baehr et al., 2000). We can conclude that M-types are more active in the early afternoon and that E-types have their peak activity in the late afternoon. The use of the actigraph to monitor the activity–rest cycle could represent an objective method to determine the chronotype, and it can be integrated into a subjective method, such as a selfassessment questionnaire. Finally, we evaluated the sleep parameters, both during the weekend and weekdays, for the different groups of chronotypes using an actigraph. The chronotype influences the sleep timing and duration during WD and the WE: M-types went to bed approximately 2h earlier than did E-types both during WD (23:38h versus 01:28h) and the WE (00:30h versus 02:13h). All three groups showed delayed Ss during Friday and Saturday nights compared with weekdays. The E-types woke up significantly later than did the M-types during WD (08:13h versus 07:10h) and the WE (10:17h versus 08:13h), and the E-types had longer AS during the weekend compared to weekdays (06:40h versus 08:07h). During the weekend, AS increased 87min for the E-types and 11min for the M-types. These results are in line with other studies showing a strong association between chronotype and sleep–wake timing and sleep duration (Besoluk et al., 2011; Park et al., 1998; Robillard et al., 2002; Taillard et al., 2004). Roepke & Duffy (2010) observed that the E-types had a shorter sleep duration during working days but slept longer during the weekend than the other chronotypes; they had accumulated a sleep debt during the weekdays that they attempted to compensate for on free days. Regarding sleep quality, we observed that M-types and N-types had higher SE of 84.1%±4.9 and 84.1%±5.2, respectively, than did E-types (77.9%±7.0) during WD. Nevertheless, the latter significantly increased their SE during the weekend to 83.1%±6.8. The M-types and N-types did not significantly modify their SE from WD to the WE. We found similar results regarding the IT parameter: the E-types spent less immobile minutes than did the M- and N-types during WD (05:38h±55 versus 06:31h±38 and 06:52h±34). We did not find any significant differences among the three groups during the WE. Therefore, only the E-types significantly increased their IT during Friday and Saturday nights (06:27h±68). MFI and SL were similar for all chronotypes during both WD and the WE. The results in the scientific literature regarding this topic are still unclear because few studies considered the difference between the WD and the WE in sleep parameters using an objective method, such as the actigraph. Merikanto used questionnaires and reported insomnia symptoms more frequently in E-types than in M-types, with no differences between WD and the WE (Merikanto et al., 2012). Other authors did not find any significant differences in actigraphic sleep parameters (SE, SL and sleep duration) between chronotypes during the whole week (Martin et al., 2012).

Conclusion

Based on our findings, we can conclude that sleep quality is influenced by chronotype. The M-types tended to sleep better and to spend more Immobile Minutes (IT) in bed than did the E-types during the Week Days (WD), whereas the E-types had the same sleep quality as the M- and N-types during the Week End (WE) by increasing their Sleep Efficiency (SE) and IT. This finding suggests that E-types accumulate a sleep deficit during WD due to their social and academic commitments that force them to wake up earlier with respect to their preferred sleeping times. The data support that the E-types recover from this deficit during the WE because they sleep better and longer. Although the simple use of questionnaires to determine chronotype has long been established, we suggest that actigraphy can be an additional important procedure in the study of both chronotypes and various parameters that can be used to describe sleep quality. Actigraphy can easily and non-invasively obtain a large amount of data on around-the-clock activity levels for the quantification of circadian activity parameters and for the objective determination of a multitude of sleep parameters.

3. THE EFFECT OF CHRONOTYPE ON PSYCHOPHYSIOLOGICAL RESPONSES DURING AEROBIC SELF-PACED EXERCISES. (2015). Rossi A, Formenti D, Calogiuri G, Vitale JA, Weydahl A. *Perceptual and Motor Skills* 121 (3): 840-55. (see Appendices)

Introduction

Under normal conditions, people's physiological functions change periodically over a daily cycle of about 24 hr. (approximately 22–26 hr.), with patterns that have been defined as circadian rhythms (Koukkari & Sothern, 2007) . Circadian rhythms have been shown in basic physiological functions such as body temperature, heart rate (HR) at rest, oxygen consumption, metabolic rate, sweat rate, and cardiac output during exercise (Cohen, 1980; Reilly & Brooks, 1990; Cappaert, 1999; Drust, Waterhouse et al., 2005; Reilly et al ., 2006; Calogiuri et al., 2011). Differences in circadian rhythms were found between sexes, with a shorter intrinsic rhythm and larger fraction of sleep in women than in men (Wever, 1984). In both sexes, the alteration of sleep–wake rhythms caused by aging, ultra-endurance performance, or behaviours (such as training at different times of day) induces modifications in the circadian rhythms (Schmidt et al., 2012). Furthermore, the circadian rhythms can influence behaviours such as food intake (Garaulet & Gómez-Abellán, 2013) , sleep patterns (Kloog et al., 2011) , and participation in physical activity (Hill et al., 1988; Burgoon et al., 1992) . In addition, sport performances can take advantage of circadian rhythms; e.g., short-term, high-intensity exercises should be conducted in the afternoon because the peak of strength has been shown to be higher in the afternoon than in the morning (Cappaert, 1999). Individual differences in the way in which the circadian rhythms manifest themselves are evidenced in people's preferences for morning or evening activities (i.e., chronotype or circadian preference ; Adan, 1994 ; Amorim, Byrne, & Hills, 2009). In 1976, Horne and Östberg developed a questionnaire to determine morningness–eveningness preference (the Morningness–Eveningness Questionnaire; MEQ), which assigns a person to a different chronotype group: morning (M), evening (E), or neither (N) type. M-types prefer to wake up early in the morning and reach the peak body temperature at rest earlier in the day than N- and E-types (Baehr, Revelle, & Eastman, 2000). The delayed phase of the temperature would explain the fact that E-types, in contrast to M-types, feel less energetic early in the morning, and habitually stay up late at night (Baehr, et al. , 2000) . N-types do not show strong preferences for either earlier or later activities, and they can adapt as easily to advanced or delayed sleep–wake schedules, although they tend to wake up and fall asleep later than M-types but before E-types (Muro, Gomà-i-Freixanet, Adan, & Cladellas, 2011; Vitale, Roveda, Montaruli, Galasso, Weydahl, Caumo, et al., 2015). The circadian rhythm for body temperature is associated with melatonin production and rest–activity rhythms, as well as with the physiological responses to exercise (E scames, Ozturk, Baño-Otálora, Pozo, Madrid, Reiter, et al ., 2012). Therefore, given that most of the components of a sports performance, such as flexibility, muscle strength, and short-term, high-power output, peak together with body temperature (Winget, DeRoshia, & Holley, 1985; Atkinson & Reilly, 1996; Waterhouse, et al ., 2005), the chronotype-related variations in body temperature might influence the physiological and perceptual responses to a physical task performed at a given time of day. In other words, because the E-types have a "delayed" circadian phase, when they exercise in the early morning their bodies are simply not physiologically prepared to perform at their best: lower body temperature and baseline HR would result in E-types expressing lower cardiac and metabolic outputs, and greater effort would be required to achieve given levels of performance. On the other hand, the perceived exertion associated with a physical task performed at a given time of day might also be influenced by processes that are more cognitive or emotional in nature, such as the levels of alertness and energetic feelings. It has in fact been hypothesized that chronotype-related differences in the expression of the body temperature rhythm (as an indicator of the circadian pacemaker) could explain the lower alertness recorded in E-types in the morning (Baehr, et al. , 2000) . Either way, this phenomenon might have a number of implications, such as a negative effect on the reliability of measurements and tests, reduced training gains, and consequences for the individual's emotional experience associated with an exercise session. As the diurnal variation of various physiological variables differs between chronotypes, it is reasonable to assume that chronotype may have an effect on daily sports performance (Rae, Stephenson, & Roden, 2015). Therefore, knowledge about possible effects of chronotype on psychophysiological responses to a physical task performed at different times of the day is important. To date, only a few studies have investigated the possible influence of chronotype on physical performance and other responses to exercise stimuli (Winget, et al., 1985; Hill, et al ., 1988; Burgoon, et al ., 1992; Brown, Neft, & LaJambe, 2008). In light of these study findings, although it is still unclear whether chronotype can affect physical performance, the studies consistently found a significant association between chronotype and the rating of perceived exertion (RPE), measured using a 20-point Borg scale (B org, 1982). Kunorozva, Roden, and Rae (2014) showed that, when a 17min. submaximal cycling test at fixed workloads was performed (60, 80, and 90% of HR max), 20 M-type male cyclists (M age = 39.8 yr., SD = 7.7) reported a higher RPE in the evening compared with the morning, despite the fact that absolute power output, speed, and cadence did not vary at different times of the day. In another study, Rae, et al. (2015) observed, in a group of 26 amateur male and female swimmers (M age = 32.6 yr., SD = 5.7), an interaction effect on the RPE between time of day (06:30 vs 18:30) and chronotype: swimmers identifying themselves as M-types perceived less exertion when performing a 200-m swimming trial in the morning compared with the evening, whereas N- and E-types showed a similar RPE for the morning and the evening performance. Burgoon, et al. (1992) compared HR, respiratory parameters, RPE, and total exercise time recorded during a maximal graded treadmill test at two times of the day (07:30 and 19:30) in 26 young men (M age = 23.3 yr., SD = 4.4) grouped in M-, N-, and E-types. They found no significant effect of chronotype on HR, respiratory parameters, and performance, but they did find it on RPE measured at maximal effort. n summary, these studies indicate that the chronotype might mainly affect the perceptual component of a physical task (i.e., the perceived effort) rather than the physiological and performance outcomes. The chronotype-related differences in body temperature might not be sufficiently large to sensibly affect the performance of a physical task, especially when such a task is set on a standardized basis. On the other hand, the chronotype appears to have a more substantial effect on the psychological/perceptual component of a physical task, resulting in greater perceived effort. However, Burgoon, et al. (1992) cautioned that the particular form and intensity of the activity used in an experimental design might determine whether an effect is found. Specifically, use of maximal exercise tests may override smaller effects associated with chronotype on physiological response, whereas submaximal exercise may emphasize possible differences in performance in relation to chronotype (Burgoon, et al ., 1992); e.g., in a study on 32 university students (M age = 25.0 yr., SD = 4.5) investigating the chronotype effects on cardiorespiratory, metabolic, and perceptual exertion response (i.e., RPE) at two different times of the day (06:00–08:30 and 15:30–18:00), Hill, et al. (1988) found no statistical difference in the maximal graded test, whereas an effect was found on oxygen consumption for M- and E-types, and on the RPE in M-types at a submaximal level. The possible effects of the individual's chronotype might be emphasized even more when performing spontaneous low-intensity physical activity, such as self-paced walking (Cappaert, 1999). The characteristic of self-paced walking is that individuals can express their preferred pace without the examiner imposing a high-intensity effort. Self-paced walking tasks have been used previously in studies investigating psychophysiological responses in association with different conditions (Parfi tt, Rose, & Burgess, 2006; DaSilva, Guidetti, Buzzachera, Elsangedy, Colombo, Krinski, et al., 2009; DaSilva, Guidetti, Buzzachera, Elsangedy, Krinski, De Campos, et al., 2011). Such physical tasks might, for example, provide evidence of differences in HR or walking speed because the individual could regulate performance on the basis of the perceived effort rather than the external imposition. In a pilot study, possible chronotype differences of HR, walking speed, and RPE were investigated during a self-paced walking task performed in the morning and the afternoon (Vitale, Calogiuri, & Weydahl, 2013). In this pilot study, the participants' chronotype had some effect on their psychophysiological responses to the walking task. Specifically, when walking in the morning the E-types reported a higher RPE compared with the M-types. On the other hand, there was no significant effect of chronotype on HR or walking speed. The study was, however, limited by a small sample size. The present study aims to continue and extend the previous work investigating the effects of chronotype on psychophysiological responses to a submaximal self-paced walking task. Based on the literature and the findings of this pilot study, the following hypothesis is proposed.

Hypothesis

1. The individual's chronotype will have an effect on the perceptual (RPE), physiological (HR), and performance component (walking time) associated with a submaximal self-paced walking task performed at different times of the day; specifically, (a) the E-types will report a higher RPE and lower HR and record longer walking times in the morning compared with the M-types; (b) no significant difference will be found in the afternoon; and (c) the N-types will show more stable responses across different times of day, with no significant difference when compared with the M- or E-types.

Methods

Participants and Preliminary Measurements. The participants were 46 students at Finnmark University College, 27 men and 19 women (M age = 24.8 yr., SD = 7.2), who voluntarily participated in this study. As background information, the authors collected information about the participants' sex, age, and Body Mass Index (BMI), and measured their maximal cardiac output (HR max). The BMI was assessed using self-reported height and weight. To assess the HR max, all participants performed an all-out-uphill-run test (1,042 m with an altitude gap of 45 m overall, calculated using the map from the Alta town web site. The test was preceded by 10 min. of jogging from the university to the place where the test started, two episodes of about 15 sec. run with increasing speed, and a little leg stretching, performed voluntarily. This served as a warm-up. The test was concluded when the participant achieved maximum exertion, which was achieved by everyone by the time they reached the top of the hill or before. During the test, the HR was recorded at 0.2 Hz using an HR monitor (Polar Team 2, Polar Electro Oy, Kempele, Finland). The test was performed at completion of the experiment, on a separate day to avoid any long-lasting fatigue interfering with the measurement. Furthermore, as in the pilot study, physical tasks performed in the early afternoon were less subject to chronotype-related influences (Vitale, et al., 2013), the test was performed at 14:00. The participants' descriptive information is provided in Table 1. All participants were declared healthy and in good physical condition. They received an explanation of the project's aim, methods, and possible risk, and signed a written informed consent to participate in this study, which was approved by the Ethical Committee of the Norwegian Social Science Data Service (Project no. 30516).

Chronotype	*n*	MEQ Score		Sex				Age (yr.)		BMI (kg/m²)		HR_{max} (beats/ min.)	
				Women		Men							
		M	*SD*	*n*	%	*n*	%	*M*	*SD*	*M*	*SD*	*M*	*SD*
E type	10	35.42	4.12	3	30	7	70	25.73	9.65	23.74	3.13	187.2	9.65
N type	27	48.72	4.00	7	26	20	74	23.34	3.61	22.50	2.64	193.1	10.59
M type	9	65.44	3.71	8	89	1	11	26.6	7.66	22.98	1.63	185.9	7.49

Table 1. Participants' descriptive data. *Note:* E-type: evening-type participant; M-type: morning-type participant; N-type: neither-type of participant. Morningness-Eveningness Questionnaire (MEQ) scale: E-type 16–41; N-type 42–58; E-type 59–86. No statistical difference found among the chronotype groups in age, BMI, and HR max ($p > .05$).

Research Design and Procedure. The study was designed as a within-subjects experiment, in which all participants underwent two submaximal self-paced walking tasks at two different times of day. In a preliminary meeting with the researchers, the participants filled in the MEQ (Horne & Östberg, 1976). The MEQ score was not communicated to the participants until completion of the experiment to avoid any influence on the performance of the walking test. Then, on separate days, all the participants carried out two self-paced walking sessions: one in the afternoon (15:30–16:30) and one in the morning (08:30–09:30). These specific times of day were chosen because the light intensity was fairly equal. It was anticipated that the time of day chosen for the afternoon session was not late enough to emphasize any strong differences between chronotype groups (Vitale, et al. , 2013); this session served as a control, however, to ensure that any differences in study variables were not the result of individual or more generic circadian variations. The walking task consisted of walking three times up and two times down a steep hill; the first down-hill round was used to allow the participants to become acquainted with the track and as a warm-up. The participants were asked to walk at a pace with which they felt comfortable, with just the clear specification that running was not allowed. The hill chosen for the test was situated near the university building in Alta, Norway. It was 306 m long with a slope of 14.2% and a rise of 42.5 m (altitude difference between top and bottom of the hill, calculated using the map from the web site mentioned earlier). The walking sessions occurred in February and March of two consecutive years (2012–2013). During the period of the experiment, the surface of the hill was covered with packed snow. All participants carried out the afternoon walking session first (Day 1); then, on the following day, they carried out the morning walking session (Day 2). The intensity and overall duration of the walking task were such that long-lasting fatigue was not expected, which was confirmed by feedback from the participants. During both walking sessions, HR, time for completion, and RPE were measured (more details are provided below). At completion of the experiment (Day 3), the all-out uphill-run test was performed. The participants were asked to avoid alcohol, caffeine, and medicine that might affect the cardiac response to physical activity, and strenuous physical activity for 24 hr. before both the walking sessions and the all-out uphill-run test. The study protocol and procedures complied with the guidelines for biological rhythm research dictated by Portaluppi, Smolensky, and Touitou (2010), which formulated clear hypotheses and application of appropriate chronobiological methods, including adequate sampling frequency and proper time series.

Measures and Instruments

Chronotype. The participants' chronotype was determined using the MEQ (Horne & Östberg, 1976), which is a questionnaire containing 19 items inquiring about participants' preferences for engaging in different activities (e.g. "At approximately what time of day do you usually feel your best?" and "You have to do 2 hr. of hard physical work. You are entirely free to plan your day. Considering only your internal 'clock,' which one of the following times would you choose?"). Each item is provided with a set of closed answer alternatives, used to calculate a total score ranging from 16 to 86. On the base of the total MEQ scores, the authors grouped the participants as M, N, and E types that accorded with the morningness– eveningness scale: 16–41 = E types; 42–58 = N types; 59–86 = M types. The instrument showed good internal consistency (Cronbach's α = .87).

Walking time. Time of completion of the walking sessions was recorded, starting from the bottom of the first valid uphill until the end of the third uphill. A marker system (EKT, Orienteering system with timing, Emit AS, Oslo, Norway) was used, with two markers positioned at the start and end of the hill.

Heart rate. The HR was recorded at 0.2 Hz using an HR monitor (Polar Team 2). The HR data recorded during the walking sessions were downloaded and macroscopically examined using Polar Team 2 software (Polar Electro Oy). Subsequently, the HR data were exported and processed using Microsoft Office Excel 2010, where mean HR values for each walking session were calculated and expressed in relation to their HR max values (percentage HR max).

Perceived exertion. The RPE (Borg, 1982) was measured at completion of both walking sessions using a 20-point Borg scale. This instrument consists of a 6:20 visual rating scale, with commentaries cueing different levels of effort (e.g., 7: Very, very light, 13: Somewhat hard, and 20: Very, very hard) that measure a person's perceived effort, and it showed valid assessments of the perceived exertion during both aerobic and resistance training (Borg, 1982). A Borg 6:20 scale was shown on a large panel to the participants while they were still walking, approximately 10 m before the finish line. They were asked to declare what the level of their perceived exertion was, and this was noted by the researchers on a pre-prepared sheet. All participants were familiar with the instrument because of previous investigations, so a memory-anchoring procedure was used (Haile, Gallagher, & Robertson, 2015).

Analysis

The data were tested for normal distribution by examining distribution graphs and performing a Shapiro–Wilk test. An independent-sample Student's t test was used to establish possible differences between chronotypes for the background information (age, BMI, and HR max). Test-retest reliability was assessed for walking time, HR mean, and RPE using a one-way intra-class correlation coefficient (ICC) based on single measurements (ICC 1,1), in order to compare the results obtained at different times of the day. To investigate the possible effects of the participants' chronotype on the indicators of psychophysiological response to the walking test at different times of the day, a mixed between–within-subjects multivariate analysis of variance (MANOVA) was performed, after ensuring that the assumption of linearity, homogeneity of variance, multicollinearity, and singularity, and the homogeneity of variance–covariance matrices were met. Walking time, mean HR, and RPE were the dependent variables; chronotype (E-, N-, and M-type) was set as the between-subjects factor, and time of day (morning or afternoon session) was set as the within-subjects factor. If Wilks's λ achieved statistical significance, the univariate test (ANOVA) was examined for the individual dependent variables, applying a Bonferroni's adjustment of alpha (α/ n dependent variables = .02) to investigate possible major effects on the individual variables. In addition, to detect possible differences between chronotypes in the morning or afternoon sessions separately, a post hoc analysis was performed using an independent-samples Student's t test for all dependent variables (HR mean , walking time, and RPE) comparing all possible combinations of chronotype groups (M-, N-, and E-types). Bonferroni's correction of alpha (α/ n comparisons = .02) was applied to reduce the risk of a type 1 error. If significance was achieved for any of these comparisons, the effect size was calculated and reported as Cohen's d. the level of significance was set at $p < .05$ (95% confidence interval). Statistical analysis was performed using SPSS Version 20.0 for Windows (IBM SPSS Statistics, Inc., Chicago, IL; West, Welch, & Galecki, 2006).

Results

On the basis of the MEQ scores, 10 E-types (21.74%: 7 men and 3 women), 27 N-types (58.70%: 20 men and 7 women), and 9 M-types (19.56%: 1 man and 8 women) were identified. HR mean, walking time, and RPE mean values are provided in Table 2. The intensity of the physical task was fairly moderate during both sessions (M HR = 71.51%, SD = 8.19% and M HR = 72.76%, SD = 9.06%; M RPE = 12.98, SD = 2.10 and M RPE = 13.15, SD = 1.93 in the morning and afternoon, respectively). Mean HR and walking time showed good test-retest reliability (ICC = 0.82 and 0.72, respectively), whereas reliability for RPE (ICC = 0.42) was low. The MANOVA showed a significant interaction of chronotype and time of day with the dependent variables (Wilks's $\lambda = 0.73$; $F_{6,82} = 2.35$; $p = .04$; partial $\eta^2 = 0.15$; power = 0.78). However, when considering the results for the dependent variables separately, the univariate test (ANOVA) did not achieve significance for any of the dependent variables. The post hoc analysis found a significant difference between M- and E-types only in the morning session for RPE, with E-types reporting higher RPE compared with the M-types (E-types: M = 14.33, SD = 2.45; M-types: M = 12.00, SD = 1.66; $p = .01$; Cohen's d = 1.10). A significant difference was not found for walking time and mean HR between any of the compared groups, either in the morning or in the afternoon. The results are provided in Table 2 and Figure 1.

	M (*n*=9)		N (*n*=27)		E (*n*=10)		Total Participants (*N*=46)	
	Type							
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Morning session								
Walking time (sec.)	1,129.78	116.39	1,100.27	125.85	1,188.22	145.59	1,124.30	129.94
HR_{mean} (% HR_{max})	75.32	8.52	71.04	8.23	69.07	7.22	71.51	8.19
*RPE (Borg's 6:20 scale)	12.00	1.66	12.85	1.91	14.33	2.50	12.98	2.10
Afternoon session								
Walking time (sec.)	1,124.11	50.05	1,069.83	97.86	1,130.00	213.67	1,093.49	121.70
HR_{mean} (% HR_{max})	72.47	11.00	73.32	9.08	71.41	7.49	72.76	9.06
RPE (Borg's 6:20 scale)	13.33	2.45	12.92	1.69	13.63	2.13	13.15	1.93
Morning–afternoon difference								
Walking time (sec.)	5.67	66.34	30.44	27.99	58.22	68.08	31.37	99.29
HR_{mean} (% HR_{max})	2.85	2.48	–2.28	0.85	–2.34	0.27	–1.29	6.06
RPE (Borg's 6:20 scale)	–1.33	0.79	–0.07	0.22	0.70	0.37	–0.11	2.20

Table 2. Walking time, HR mean, and RPE measured during self-paced walking tasks in the morning and afternoon for individuals with different chronotypes (n = 46). *Note:* E-type: evening-type participant; HR: heart rate; M-type: morning-type participant; N-type: neither type of participant; RPE: rating of perceived exertion. * $p < .05$: difference between M- and E-types.

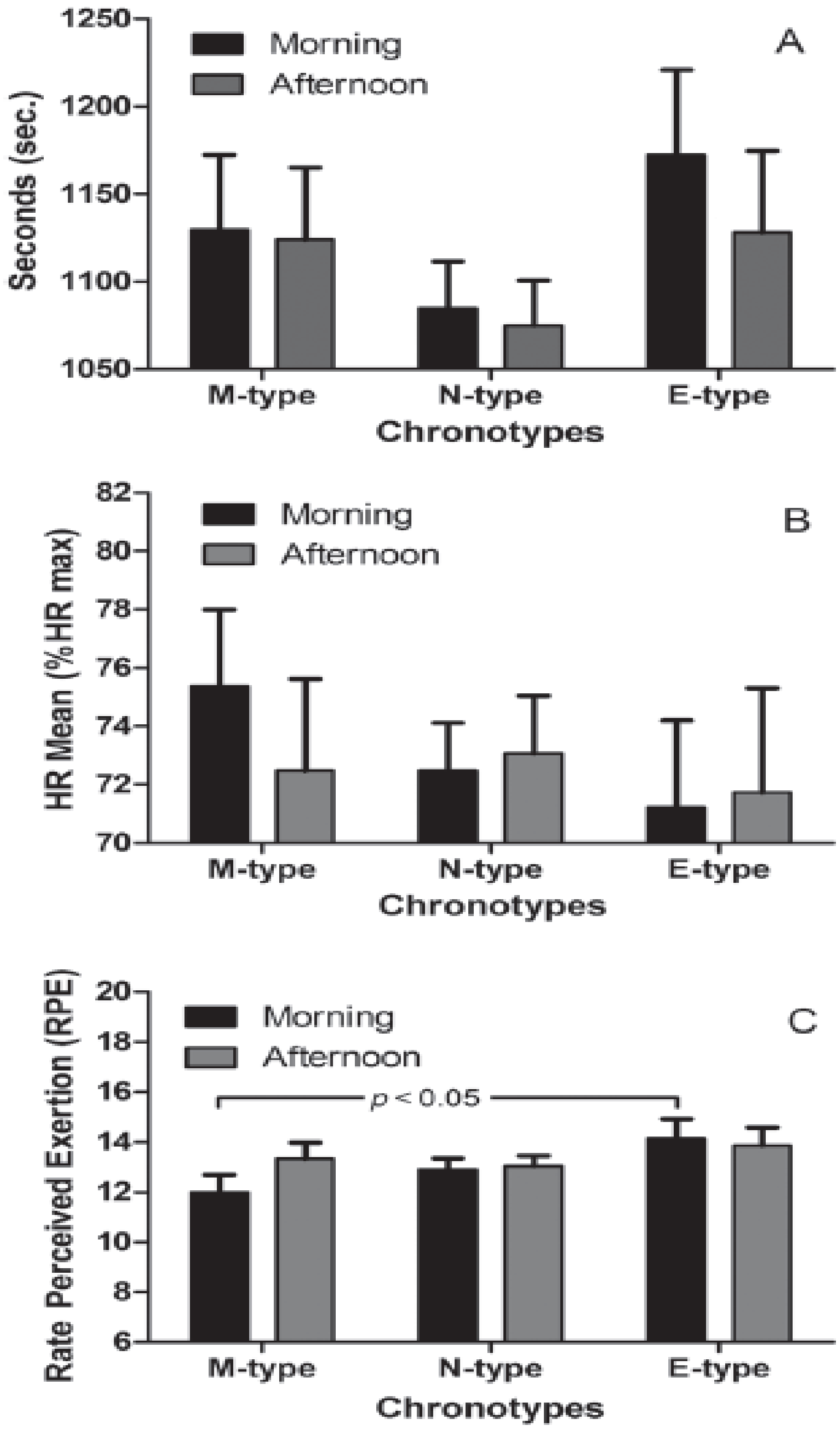


Figure 1. Values are presented as estimated marginal means and standard deviations resulting from a two-way ANOVA (between–within). (A) Time in seconds to perform the walking session, (B) mean heart rate (HR mean), and (C) rating of perceived exertion (RPE) were used as dependent variables.

Discussion

The purpose of this study was to investigate whether a person's chronotype influences psychophysiological parameters recorded during a submaximal self-paced walking task done at different times of the day. In particular, given that individuals with evening circadian typology typically have a delayed phase of body temperature and alertness (Baehr, et al., 2000), it was expected that, compared with the M-types, the E-types would report greater RPE and lower HR and take more time to complete the walking task when performing it in the morning. On the other hand, given that the body temperature circadian rhythm peaks in the late afternoon/ early evening (Winget, et al., 1985; Atkinson & Reilly,1996; Waterhouse, et al., 2005), no significant difference across chronotypes was observed at this time. Eventually, given that N-types do not show strong preferences for either earlier or later activities, although they show patterns that stand somewhere midway between the M- and the E-types (Muro, et al., 2011; Vitale, et al., 2015), no significant effects of time of day on walking were expected in this group. he MANOVA showed an overall effect of chronotype on the different psychophysiological responses (walking time, HR, and RPE together) in interaction with the time of day for the walking task. However, no significant effect was found when considering the individual variables separately in univariate tests. When looking at the morning or afternoon sessions separately, the post hoc analysis showed a significant difference between E- and M-types only for RPE in the morning, whereas no difference was found for walking time and HR or for any of the comparisons in the afternoon (Table 2 and Figure 1). Although significant differences were found in the multivariate test and the post hoc analysis, the fact that significance was not achieved for any of the individual parameters in the univariate tests could depend on the nature of the non-standardized (self-paced) physical task used in this study's design: the large inter-individual differences (shown by the large standard deviations) are likely to have hampered the ability of the statistical test to detect significant difference in the individual parameters, whereas the differences were more evident when taking all the parameters together. The post hoc analysis was then able to emphasize the differences between the extreme chronotypes (i.e., M- vs E-types) tested either in the morning or in the afternoon. The findings only partly support the hypothesis, specifically the prediction that E-types will report higher RPE in the morning compared with N-types. Otherwise, the findings are more in line with those of the pilot study (Vitale, et al ., 2013), as well as previous studies using maximal and submaximal physical tasks in their designs. In fact, previous studies found that there were no differences in physiological parameters or exercise performance in relation to chronotype, whereas an interaction effect was found for RPE between chronotype and time of day (Burgoon et a ., 1992; Kunorozva et al., 2014; Rae et al., 2015). In the current study, it is especially remarkable that despite the participants not being encouraged to maintain a given exercise intensity, the E-types still chose to maintain a fairly equal pace and HR at both times of day, rather than regulating the intensity of the exercise based on the perceived exertion. Racinais, Hue, Hertogh, Damiani, and Blonc (2004) found no differences in anaerobic performance (vertical jump test and maximal cycling test) in individuals with different chronotypes, despite the differences found in rectal temperature under resting conditions. These authors concluded that the lack of chronotype-related differences in anaerobic performance were probably due to the fact that the participants still achieved higher body temperatures during the physical tasks offering optimal conditions for exercise. In agreement with such an assumption, in the current study the higher RPE reported by the E-types when exercising in the morning would not be the result of the cognitive processes themselves, such as the delayed phase of alertness. Instead, as E-types in general have a lower body temperature in the morning compared with M-types, they would actually need to engage in more effort to achieve an optimal body temperature for exercising. The findings have numerous implications for sport and exercise research and practice. First, the findings indicated that HR- or performance based tests (e.g. cardiorespiratory fitness tests) are not significantly affected by an individual's chronotype; in contrast, the reliability of RPE measurements can be negatively affected when a person undergoes a physical task at an unfavourable circadian time. Also, the greater perceived effort experienced by E types in the morning could, in some cases, have implications for future exercise behaviour. Specifically, given that the perceived effort might influence a person's affective responses to exercise and future exercise behaviour (Ekkekakis, Backhouse, Gray, & Lind, 2008 ; Kwan & Bryan, 2010), the individual's chronotype should be taken into consideration when prescribing exercise or planning interventions to promote exercise in an inactive individual. As expected, the chronotype-related differences were more pronounced in the morning than in the afternoon when comparing M- with E-types. The N-types, in fact, showed quite stable values for all measured parameters (i.e., walking time, HR, and RPE), indicating that these individuals do not really have a particular predisposition to perform physical tasks at a given time of day. Furthermore, in agreement with the previous pilot study (Vitale, et al. , 2013), it appears that engaging in a physical task in the afternoon is less subject to the effects of chronotype on psychophysiological parameters. The body temperature circadian rhythm, along with components of sports performance such as flexibility, muscle strength, and short-term high-power output, peaks in the late afternoon/early evening (Winget et al.,1985; Atkinson & Reilly, 1996; Waterhouse, et al., 2005). The phase differences of such components associated with the individual's chronotype are likely to be more pronounced, and therefore more subject to chronotype influences, at times of day that are closer to nocturnal sleep, i.e., early morning and late evening.

Strengths and Limitations of the Study

The design, based on a submaximal self-paced walking task, and taking place in an outdoor setting, is a novelty that adds new knowledge and a different understanding to the field of chronobiology and chronotype. The intervention reflected a form of spontaneous physical activity that people could do in their everyday life, for either leisure or transportation purposes. The fact that the RPE responses ranged from 12 to 14 on the Borg scale indicated that the exercise was of moderate intensity, covering the overload training zone for cardiorespiratory fitness (Purvis & Cukiton, 1981) and adding relevance to the findings in an exercise-prescription context. The lack of a walking session later in the day (e.g. at 20:00 or later) is a weakness in the design. As a result of the mechanisms discussed above, the afternoon session was too early in the afternoon to assess any possible inverse effects of chronotype. However, the timing for the sessions was chosen so that fairly equal amounts of daylight were assured to avoid the influence of the light–dark cycle on the performance. Starting all participants with the afternoon session may have introduced a bias, and constitutes a weakness of the study design. A cross-over design that started randomly picked participants either with the morning or afternoon session may have been preferred. The mechanisms underlying the greater RPE reported by E-types in the morning still have a theoretical base and are inferred based on previous studies. Is the higher perception of effort reported by E-types the result of an actual “physiological requirement” of having to fill the larger gap between baseline body temperature and body temperature optimal for exercise? Or is the RPE associated with the circadian phase of processes that are more cognitive or emotional in nature? Such mechanisms are still not fully understood. For better understanding of these mechanisms, future studies should consider including in their design measurements of baseline temperature versus body temperature taken during and after exercise. Qualitative approaches (i.e., in-depth interviews about participants' perceptions of exercising at unfavourable circadian times) can also bring understanding to the cognitive and emotional processes underlying these mechanisms.

Practical Implications

Based on the findings, together with state-of-the-art studies in the literature, the following recommendations for research and exercise prescription are outlined: (1) The reliability of RPE measurements can be negatively affected when administered to individuals with more extreme circadian preferences (i.e., M- and E-types) who exercise at an “unfavorable” circadian time; (2) When exercising at a time of day that does not correspond to one's circadian preference, the chronotype does not negatively affect the ability to achieve exercise intensities that can lead to fitness and health benefits; (3) Individuals assessed as N-types are not subject to chronotype-related effects of exercising at “unfavorable” circadian times, at least with respect to circadian output, performance, and perceived effort; and (4) Exercise at those times of day that do not correspond to one's circadian preference can lead to increased perceived effort, which might in turn influence a person's affective responses to exercise and future exercise behavior. The individual's chronotype should therefore be taken into consideration in contexts prescribing and promoting exercise; e.g., scientists or exercise supervisors could choose timings that are less subject to chronotype-related influences (e.g. afternoon) or assess a person's morningness or eveningness preference.

4. DEVELOPMENT OF A FORMULA TO PREDICT THE ACTIGRAPHY-BASED ACROPHASE FROM THE MORNINGNESS-EVENINGNESS QUESTIONNAIRE (MEQ) SCORE IN A YOUNG ITALIAN POPULATION. Roveda E, Vitale JA, Montaruli A, Carandente F, Caumo A.

Introduction

In the two last decades hand-wrist actigraphy has been increasingly used in several studies to identify circadian rhythms (Ancoli-Israel et al., 2003), as well as to evaluate sleep patterns (Lehnkering & Siegmund, 2007; Montaruli et al., 2009; Roveda et al., 2011). In particular, actigraphy has proven capable to provide a detailed and objective portrait of the individual's circadian rhythms. Actigraphy-based data, analyzed by the cosinor method, yield a portrait of circadian rhythmicity entailing parameters such as the individual's cosinor amplitude, the MESOR and the Acrophase. Albeit the cost of actigraphy is constantly decreasing, its widespread adoption remains a challenge for many reasons. Actigraphy-based monitoring requires proper instruction and care, as well as the subject's compliance and collaboration. In addition, data recording has to be carried out for at least 7 consecutive days and data analysis has to be accomplished by specialized software.

One alternative approach to assess the circadian structure of a subject is based on self-assessment questionnaires. The most used questionnaire is the Morningness-Eveeningness Questionnaire (MEQ) by Horne and Ostberg (Horne & Ostberg, 1976). The MEQ score is used to classify subjects according to circadian typology (Baehr et al., 2000; Bailey & Heitkemper, 2001; Mongrain et al., 2004). Individuals with an early circadian phase are morning-types (M-types), those with a delayed circadian phase are evening-types (E-types) and those with an intermediate circadian phase are neither-types (N-types) (Kerkhof & Van Dongen, 1996; Tankova at al., 1994). MEQ has been used in many researches

(Jankowsky & Ciarkowska, 2008; Lee et al., 2014; Taillard et al., 2004; Vitale et al., 2013). It stands to reason that MEQ might be related with the circadian parametric portrait provided by actigraphy. Indeed, in a recent report by our group, it was found that the circadian typology brought about by the MEQ was associated with significantly different mean values of the Acrophase, while the other two circadian parameters, i.e. Amplitude and MESOR were not different among M, E, and N types (Vitale et al., 2015). In keeping with our findings, Lee et al. had previously found a significant difference among chronotypes concerning the mean Acrophase of sleep-wake rhythm and a strong negative association between the MEQ score and the activity Acrophase (Lee et al., 2014). Such evidence points to the notion that in the absence of a direct actigraphy-based assessment of circadian rhythmicity, a predictive equation might provide a cost-effective means of estimating the activity Acrophase from the easily measured MEQ score.

Aim of this study was to evaluate whether a linear regression formula using the the MEQ score would predict the actigraphy-based Acrophase in a young italian population.

Methods

Study subjects. The subjects participating in this study were college students of the School of Sports Science of the University of Milan who were enrolled in the academic year 2013-2014 (N=804; 572 males and 232 females; mean age 22.12 ±2.13 yrs). All of the subjects were in good physiological and psychological health and they were not under any pharmacological therapy.

Morningness-Eveningness Questionaire (MEQ). After receiving an explanation of the project's purpose and methods, the participants signed an informed consent and completed the Horne-Ostberg Morningness-Eveningness Questionaire (MEQ) for the assessment of chronotype (Horne & Ostberg, 1976). The subjects were categorized as M-types (scores between 59 and 86), N-types (scores between 42 and 58), and E-types (scores between 16 and 41). The study group included 17 M-types (8 males and 9 females), 18 N-types (9 males and 9 females) and 19 E-types (10 males and 9 females).

Actigraphy. The subjects underwent a 7-days monitoring period (from Monday to Sunday) using the actigraph (Actiwacth® actometers, CNT, Cambridge, UK) to evaluate the circadian rhythm of their activity levels. In April 2014 each participant wore the actigraph on their non-dominant hand for 7-days and was given a diary to record information regarding their bed time, get up time, hrs spent napping, hrs without wearing the actigraph and number of nocturnal awakenings. The subjects had similar university timetables with lectures starting approximately at 09.00 and none of the sub-group subjects had a part-time job. None of the subjects knew their own chronotype before the monitoring period, nor did the staff who analyzed these. The study protocol and procedures complied with the guidelines required by the journal (Portaluppi et al., 2010).

The Actiwatch Software was used to obtain the activity data, which were expressed in activity counts and recorded for every one-minute throughout the monitoring period (7 days). To determine the circadian rhythmicity, the activity data provided by the actigraph were analyzed using the single cosinor method (Halberg et al., 1977; Nelson et al., 1979). Based on the least-squares method, the single cosinor method identifies and evaluates the cosine mathematical function that best fits the data as a function of time. The function $f(t) = M + A \cos(\omega t + \phi)$ defines three parameters that are characteristic of each statistically significant rhythm: M is the MESOR; A is the amplitude; and ϕ is the Acrophase. The MESOR (Midline Estimating Statistic of Rhythm) is a rhythm-adjusted mean that approximates the arithmetical mean of the data for a 24-hour period, and the amplitude is the measure of one half the extent of the rhythmic variation in a cycle. The Acrophase indicates, with 95% confidence limits (CLs), the time interval within which the highest values of the variable are expected. The three parameters are usually indicated with the relevant 95% confidence intervals. The rhythmometric parameters of activity levels (MESOR, Amplitude and Acrophase) were then processed with the average of the population mean cosinor. This method, applied to the rhythmometric parameters of each subject's circadian variables, evaluates the rhythmometric characteristics of the activity levels of the population (Nelson et al., 1979). The statistical analyses were carried out using the *Time Series Analysis-Seriel Cosinor 6.0* (Expert Soft Technology, Richelieu, France).

<u>Linear model for predicting the acrophase from MEQ</u>***.*** A linear model was used to describe the relationship between the MEQ score and the Acrophase. The MEQ score played the role of the predictor (independent variable), while the Acrophase played the role of the predicted variable. The linear model was given by the equation:

$$Acrophase = a + bMEQ + e$$

where *a* and *b* are parameters the intercept and the slope of the linear model, respectively, and *e* is the experimental noise (assumed to have a normal distribution with zero mean and constant variance). Parameters *a* and *b* where estimated from the experimental data by linear least squares (R software). A distinct advantage of the linear model is that the coefficients are easily interpretable. Parameter *b*, representing the slope of the regression line, is such that a unit increase in MEQ would, on average, increase the Acrophase of b units. Another advantage of this model is that its mathematical nature allows one to compute standard errors and prediction intervals provided that certain assumptions about the distribution of the model residuals are made.

In order to quantify the quality of the model, various approaches were used. First of all, the parameter estimates of a and b were accompanied by their standard error (SE). The statistical significance of each parameter was evaluated by a Student's t-test with n-2 degrees of freedom. The t-score derived as the ratio between the parameter estimates and its standard error was used to test each parameter against the null hypothesis (H0: population parameter =0). In particular, by testing the slope of the regression line against 0, we were able to assess the statistical significance of MEQ as predictor of the Acrophase (Kuhn and Johnson). The standard error was also be used to derive the percent coefficient of variation (CV%), that is an easily interpretable index of precision given by: CV%= 100*SE(parameter)/abs(parameter).

To characterize the fit, i.e., the model predictive capability, we calculated the coefficient of determination, the mean square error and the root mean square error. The coefficient of determination, r^2, which coincides with the square of Pearson's correlation index, measures the fraction of the overall variance around the average Acrophase that is explained by the regression line. The more r^2 approaches 1, the better the data fit. Indeed, an r^2 value of 1 would indicate that the variability in the predicted variable (i.e., the Acrophase) is completely accounted for by the predictor (i.e., the MEQ score). The means square error (MSE) is an estimate of the variance surrounding the regression line. This metric is a function of the model residuals, which are the observed minus the model-predicted values. The mean squared error (MSE) is calculated by dividing the sum of squared residuals by n-2. The root mean square error (RMSE) was calculated by taking the square root of the MSE, so that it is in the same units as the original data. RMSE estimates the standard deviation surrounding the regression line and is usually interpreted as either how far (on average) the residuals are from zero or as the average distance between the observed values and the model predictions.

An important step in evaluating the quality of the model is to visualize the results. A plot of the residuals against the predicted values helps one to understand how well the model fits the data and can help uncover systematic patters in the model predictions. The residual plot was generated to determine the quality of the fit and allowed determination whether systematic deviations or outliers were present. Assumptions of normality and homoscedasticity of the residuals were examined using the Kolmogorov–Smirnov and Levene tests respectively.

Results

Figure 1 shows the regression line and the prediction limits. Both parameters resulted significantly different from 0. The fact that the slope was significantly different from 0, confirmed the presence of a significant linear relationship between MEQ and the Acrophase thus, enabling us to use the equation of the regression line to obtain predictions.

The predictive equation resulted as follows:

$$1238.7-5.487*MEQ.$$

The CV% of the two parameters are the following:

*Intercept: 100*25.83/1238.70=2.1%*

*Slope: 100*0.496/5.49=9.0 %.*

The precision of the estimates was excellent. The r^2 was 0.70, indicating that 70% of the variance in the Acrophase was explained by MEQ. The MSE was 50.97 and the RMSE was 7.1. The residuals did not show any systematic pattern and the distribution was congruent with the hypothesis of homoschedastic variation around the population line (Figure 2). The Durbin-Watson test for autocorrelation of the residuals was not significant (p=0.175).

Discussion and conclusion

The Acrophase is a key parameter of the chronobiological parametric portrait that can be gleaned from of an actigraphy–based evaluation. Our aim was to work out a simple and practical prediction model of the Acrophase based on the questionnaire-based MEQ. Provide an equation capable to predict the Acrophase based on MEQ in a young Italian population. The linear regression was significant and able to provide a good description of

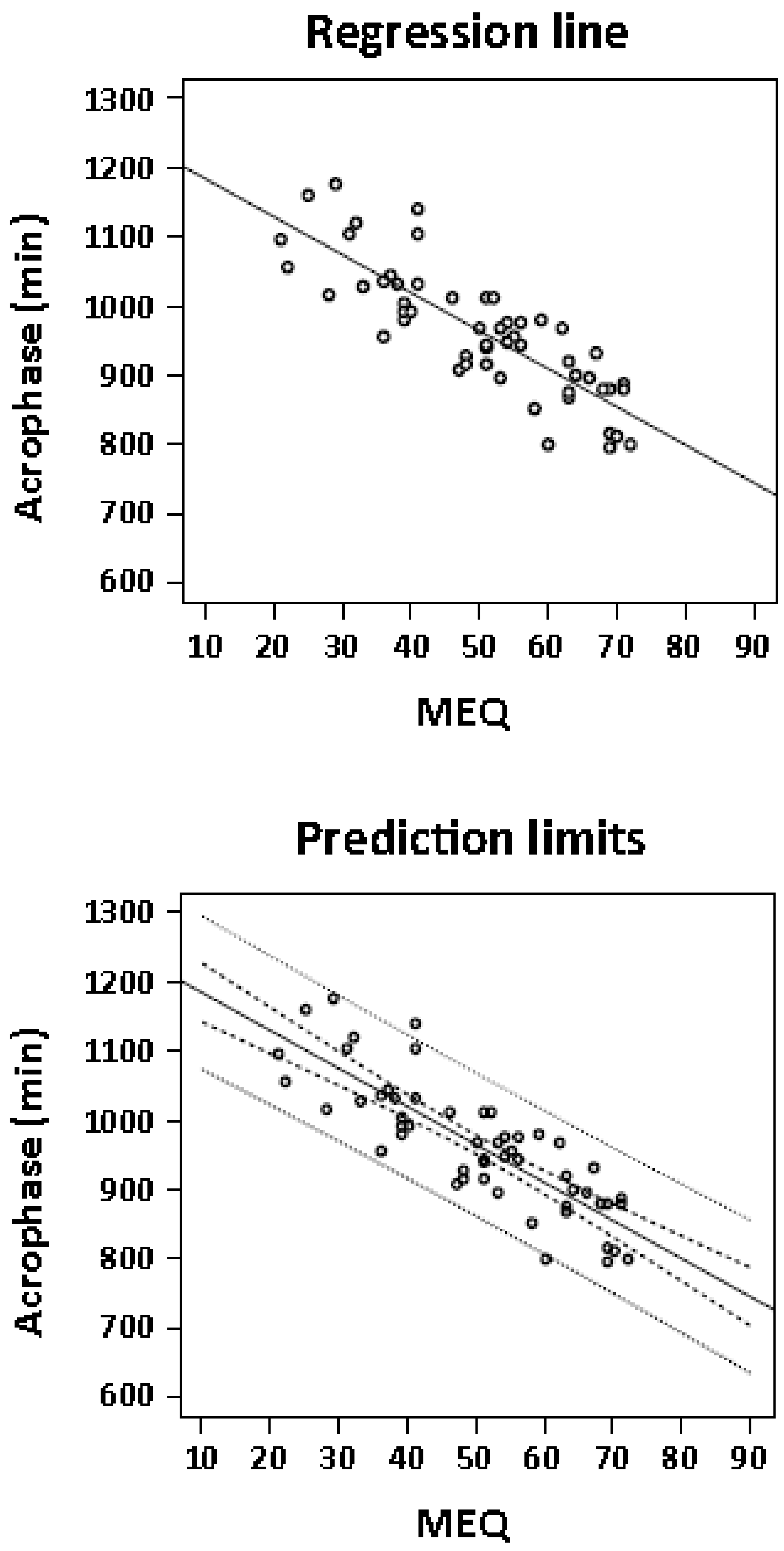
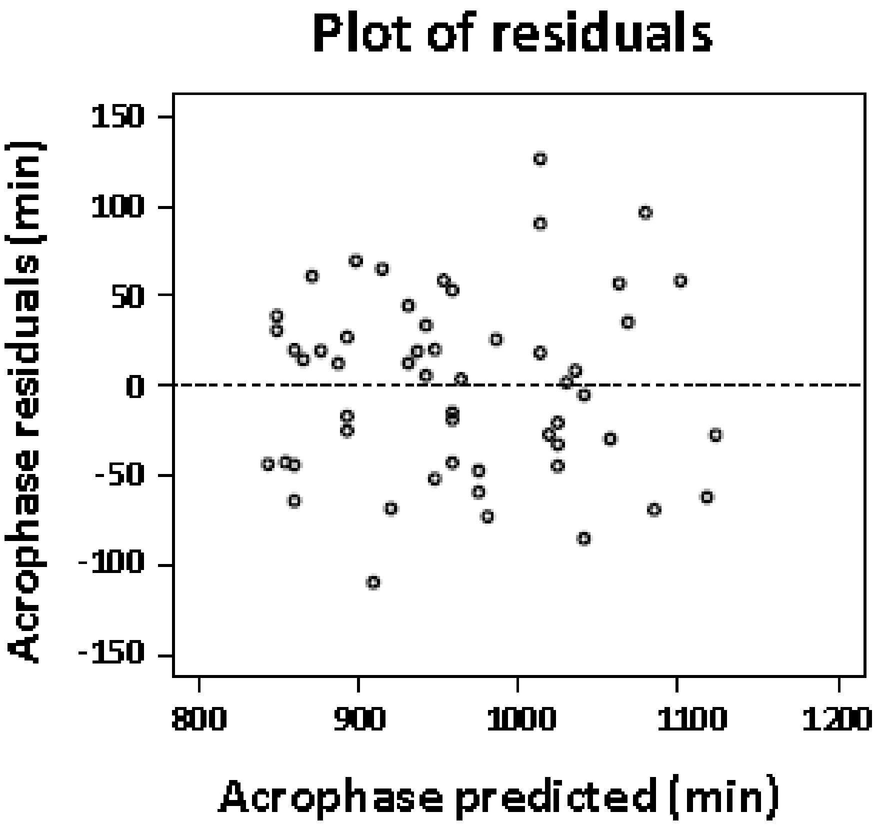


Figure 1. Regression line and prediction limits.



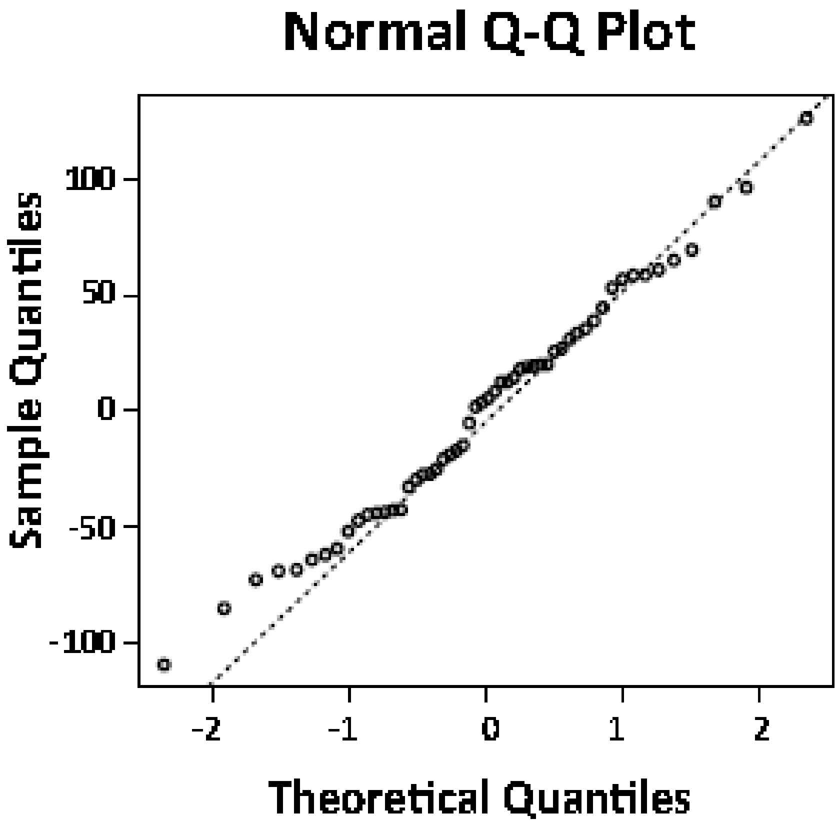


Figure 2. Plot of residuals and normal probability plot of residuals.

the relationship between MEQ and the acrophase. To the best of our knowledge, our study is the first to derive an equation for acrophase estimation based on MEQ.

The actigraphy-based evaluation of the acrophase is cost- and labor-intensive and due to the limited availability of actigraphs, its use has been restricted to relatively few investigators. Our aim was to determine whether it is possible to use questionnaire-derived estimates of MEQ to calculate the Acrophase without administering actigraphy to each subject and to define the precision associated with the prediction. Stepwise multiple linear regression indicated that the relationship was not improved including sex differences and photoperiod at birth.

Circadian rhythms and the key parameter Acrophase are influenced by genetic and environmental components. Thus, our predictive equation derived for a young Italian population is not necessarily suitable for being applied to another population from a different part of the world.

The main limitation of our study is that we used the same dataset for model training and validation. This is due to the relatively low number of the subjects participating in the actigraphy-based determination of circadian rhythms. Future studies with a greater number of subjects will allow to refine our results. The accuracy and precision of a predictive model is known to be dependent on the population. The validity of the model cannot be safely extrapolated to different populations. In particular, since circadian rhythmicity is known to depend on age, our predictive model cannot be safely adopted to predict the Acrophase outside the age range of our study.

What is the impact of using MEQ rather than actigraphy-based Acrophase? In order to appreciate the degree of approximation inherent to the use of the linear equation based on MEQ to predict the acrophase, we show the scatter diagram between the actigraph-based acrophase and its surrogate based on MEQ. The diagram has the same scale on the x and y axis so that the unity line (45° slope) can be drawn. The correlation is quite good and no systematic deviations are visible. This is just a corollary result of the linear regression analysis conducted between MEQ and actigraphy-based Acrophase.

An instructive example of the bias and variance inherent to the use of the predictive equation as a surrogate of the reference actigraphy-based assessment of the Acrophase is given below. The subjects participating in the study were classified according to their chronotype. Subjects were categorized as M-types (scores between 59 and 86), N-types (scores between 42 and 58), and E-types (scores between 16 and 41). As a result, we had 16 M-types (7 males and 9 females), 15 N-types (5 males and 10 females) and 19 E-types (10 males and 9 females). In each subject, we assessed the chronotype twice: one based on the actigraph data and one based on the equation. The mean values of the two assessments of the Acrophase are reported side to side. One can see that the mean values of the two assessments of the Acrophase are virtually the same in the two chronotypes. However, it is quite interesting to notice that the standard deviations of the MEQ-based surrogate estimates of the Acrophase are almost one-half of the reference, actigraphy-based Acrophase values. The reason is simple and instructive. Two subjects having the same MEQ are mapped on the same Acrophase value. However, these two subjects may have rather different actigraphy based Acrophases. For instance, the model predicts that two M-type subjects having the same value of MEQ=63 have the same MEQ based estimate of the Acrophase: 893.02. However, the actigraphy-based estimates of the Acrophase of these two subjects were 868 and 920. Another example. The model predicts that two E-type subjects having the same MEQ=51, have also the same Acrophase= 958.86. However, the actual acrtigraphy-based values of the Acrophase in these two subjects are 980 and 1004. Thus, one must keep in mind that using the equation produces little bias, but artificially delivers a reduced variance. As a result, the natural variability present in the population is somewhat compressed when the MEQ-base equation id used. This is an anvoiable corollary of the model prediction scheme. The only way to reduce this effect would be to use a better model capable to increase the R^2 and thus reduce the “unexplained” variance. We attempted to use a richer model incorporating also the sex of the subjects and their chronotype at birth. However, the resulting multiple regression model failed to improve over the simple one predictor model based on MEQ.

5. PUBLICATIONS

1. Conferences and congresses

1. *4th SISMES Congress. September 2012, Palermo. Italy.*

   Vitale JA, Formenti D, Alberti G, Carandente F. “Can the knowledge of chronotype be useful for the motivation and the training plans?” (oral presentation). In Sport Science for Health.

2. *27th Conference of International Society of Chronobiology, October 2012, Delhi, India.*

   Vitale JA, Calogiuri G, Weydahl A. “Influence of Chronotype on Responses To Physical Activity in the Morning vs. Evening” (oral presentation).

3. *18th annual congress of ECSS, June 2013, Barcelona, Spain.*

   1. **Vitale JA**, Roveda E, Montaruli A, Carandente F. “Evaluation of chronotypes in young Italian physically active adults.” (oral presentation). In Book of Abstracts of ECSS.

4. *18th annual congress of ECSS, June 2013, Barcelona, Spain.*

   Vitale JA, Scala L, Silvestri I, Scurati R, Michielon G, Alberti G, Venerando B, Tringali C. “Protective role of 17-beta-estradiol toward IL-6 leukocyte expression induced by intense training in female athletes” (oral presentation). In Book of Abstract of ECSS.

5. *5th SISMES Congress. September 2013, Pavia. Italy.*

   Rossi A, Calogiuri G, Formenti D, **Vitale JA**, Weydahl A. “The chronotype can influence the perceived exertion during self-paced exercise performed at different times of day.” (oral presentation). In Sport Science for Health.

6. *19th annual congress of ECSS, July 2014, Amsterdam, Netherlands.*

   Vitale JA, Roveda E, Montaruli A, Carandente F, La Torre A. “National Football League vs Italian Football League: comparison of body size/composition and performance characteristics” (oral presentation): In Book of Abstract of ECSS.

7. *6th SISMES Congress. September 2014, Napoli. Italy.*

   Vitale JA, Roveda E, Montaruli A, Galasso L, Caumo A, Carandente F. “Influence of chronotype on the circadian rhythm of activity levels” (poster presentation). In Sport Sciences for Health.

8. *20th annual congress of ECSS, June 2015, Malmo, Sweden.*

   Bruno E, Roveda E, **Vitale JA**, Montaruli A, Caumo A, Carandente F. “Circadian rhythm of activity levels: influence of chronotype.” (oral presentation). In Book of Abstracts of ECSS.

9. *20th annual congress of ECSS, June 2015, Malmo, Sweden.*

   Vitale JA, Roveda E, Bruno E, Montaruli A, Galasso L, Berrino F, Caumo A, Carandente F. “Aerobic 3-month physical activity program in breast cancer survivors: effects on sleep behavior, anthropometric indices of adiposity and fasting glucose metabolism.” (oral presentation). In Book of Abstracts of ECSS.

10. *69th SIAI Congress. September 2015, Ferrara, Italy*

    Vitale JA, Roveda E, Caumo A, Galasso L, Bruno E, Carandente F, Montaruli A. “Anthropometric indices of adiposity and fasting glucose metabolism in breast cancer survivors: effects of aerobic physical activity” (poster presentation). In Italian Journal of Anatomy and Embriology.

11. *7th SISMES Congress. October 2015, Padova. Italy.*

    Vitale JA, Roveda E, Caumo A, Montaruli A, Galasso L, LaTorre A, Carandente F. “A profile of Italian players of American football: anthropometric and physical performance differences among playing positions and comparison with non-drafted USA players” (oral presentation). In Sport Sciences for Health.

12. *7th SISMES Congress. October 2015, Padova. Italy.*

    2. Galasso L, **Vitale JA**, Parrello A, Roveda E, Montaruli A, Carandente F. “Chronotype

influences the perception of effort in relation to an aerobic physical test in different times of day" (poster presentation). In Sport Sciences for Health.

2. Scientific papers

1. **Vitale JA**, Calogiuri G, Weydahl A. (2013). "Influence of chronotype on responses to a standardized, self-paced walking task in the morning vs afternoon: a pilot study". Perceptual and Motor Skills 116(3): 1020-8. IF: 0.55.
2. Tringali C, Scala L, Silvestri I, **Vitale JA**, Scurati R, Michielon G, Alberti G, Venerando B. (2013). "Protective role of 17-β-estradiol towards IL-6 leukocyte expression induced by intense training in young female athletes". Journal of Sport Sciences 32(5): 452-61. IF: 2.09.
3. **Vitale JA**, Alberti G, Carandente F. (2013). "Possono i ritmi biologici ed il cronotipo influenzare la risposta all'attività fisica?" Scienza e Sport. Vol. 19. 2013.
4. **Vitale JA**, Roveda E, Montaruli A, Galasso L, Weydahl A, Caumo A, Carandente F. (2014). "Chronotype influences activity circadian rhythm and sleep: difference in sleep quality between weekdays and weekend". Chronobiol. International 32(3):405-15. IF 2.8.
5. Rossi A, Formenti F, Calogiuri G, **Vitale JA**, Weydahl A. (2015). "The effect of chronotype on psychophysiological responses during aerobic self-paced exercises ". Perceptual and Motor Skills 121(3): 840-55. IF 0.55.
6. **Vitale JA**, Caumo A, LaTorre A. (2016). "Il football americano: il modello fisiologico, le differenze tra ruoli ed il confronto tra Italia e USA." Scienza e Sport Vol. 30.
7. **Vitale JA,** Caumo A, Roveda E, Montaruli A, La Torre A, Battaglini CL, Carandente F. (2016). Physical Attributes and NFL Combine Performance Tests between Italian National League and American Football Players: A Comparative Study. Journal of Strength and Conditioning Research. IF: 2.07.

6. APPENDICES

1. INFLUENCE OF CHRONOTYPE ON RESPONSES TO A STANDARDIZED, SELF-PACED WALKING TASK IN THE MORNING VS AFTERNOON: A PILOT STUDY. (2013).

Vitale JA, Calogiuri G, Weydahl A.

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INFLUENCE OF CHRONOTYPE ON RESPONSES TO A STANDARDIZED, SELF-PACED WALKING TASK IN THE MORNING VS AFTERNOON: A PILOT STUDY[1]

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Summary.—The response to sub-maximal physical activity performed in the morning and late afternoon by individuals with different chronotypes was investigated. 22 participants filled out the Morningness-Eveningness Questionnaire and underwent a self-paced walking task that consisted of walking from the top to the bottom of a hill and back three times (1,836 meters, with a slope of 14.16%). The task was repeated twice: late afternoon (16:30) and early morning (08:30). Walking speed (time for completion in seconds), heart rate, and perceived exertion were measured during each task, with overall results given as a general descriptive analysis. Preliminary findings suggest that chronotype is likely to influence the responses to exercise, mostly with evening-types seeming at a disadvantage when performing a physical task in the morning. Individuals can be classified by circadian typology or chronotype, which is the propensity to be a morning-type, evening-type, or neither-type. The circadian typology, commonly referred to as being a morning person or an evening person or somewhere in between, is involved not only in the expression of physiological rhythms, but also in habits and lifestyles, such as sleeping patterns (Park, Matsumoto, Seo, & Shinkoda, 1999) that emerge especially during adolescence (Park, Matsumoto, Seo, Kang, & Nagashima, 2002) and remain throughout adult life (Koukkari & Sothern, 2006).

General knowledge of circadian variations of the physiological response to exercise may be inadequate, since it is potentially confounded with chronotype. Individuals' chronotype may affect the expression of their biological rhythms (Koukkari & Sothern, 2006), and therefore influence the individual's response to exercise stimuli and the benefit from physical performance. Some studies suggest that chronotype can influence not only performance but also the response to exercise at different times of the day in young adults (Brown, Neft, & LaJambe, 2008). A study using arithmetic subtraction as a stressor (Roeser, Obergfell, Meule, Vogele, Schlarb, & Kubler, 2012) found that chronotype had an effect upon heart rate (HR) response (Cohen's $d = 0.72$, comparing evening

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2. CHRONOTYPE INFLUENCES ACTIVITY CIRCADIAN RHYTHM AND SLEEP: DIFFERENCE IN SLEEP QUALITY BETWEEN WEEKDAYS AND WEEKEND. (2014).

Vitale JA, Roveda E, Montaruli A, Galasso L, Weydahl A, Caumo A, Carandente F.

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ORIGINAL ARTICLE

Chronotype influences activity circadian rhythm and sleep: Differences in sleep quality between weekdays and weekend

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Several studies have shown the differences among chronotypes in the circadian rhythm of different physiological variables. Individuals show variation in their preference for the daily timing of activity; additionally, there is an association between chronotype and sleep duration/sleep complaints. Few studies have investigated sleep quality during the week days and weekends in relation to the circadian typology using self-assessment questionnaires or actigraphy. The purpose of this study was to use actigraphy to assess the relationship between the three chronotypes and the circadian rhythm of activity levels and to determine whether sleep parameters respond differently with respect to time (weekdays versus the weekend) in Morning-types (M-types), Neither-types (N-types) and Evening-types (E-types). The morningness–eveningness questionnaire (MEQ) was administered to 502 college students to determine their chronotypes. Fifty subjects (16 M-types, 15 N-types and 19 E-types) were recruited to undergo a 7-days monitoring period with an actigraph (Actiwacth® actometers, CNT, Cambridge, UK) to evaluate their sleep parameters and the circadian rhythm of their activity levels. To compare the amplitude and the acrophase among the three chronotypes, we used a one-way ANOVA followed by the Tukey–Kramer *post-hoc* test. To compare the Midline Estimating Statistic of Rhythm (MESOR) among the three chronotypes, we used a Kruskal–Wallis non-parametric test followed by pairwise comparisons that were performed using Dunn's procedure with a Bonferroni correction for multiple comparisons. The analysis of each sleep parameter was conducted using the mixed ANOVA procedure. The results showed that the chronotype was influenced by sex (χ^2 with $p = 0.011$) and the photoperiod at birth (χ^2 with $p < 0.05$). Though the MESOR and amplitude of the activity levels were not different among the three chronotypes, the acrophases compared by the ANOVA *post-hoc* test were significantly different ($p < 0.001$). The ANOVA *post-hoc* test revealed the presence of a significant difference ($p < 0.001$) between the M-types (14:32 h) and E-types (16:53 h). There was also a significant interaction between the chronotype and four sleep parameters: Sleep end, Assumed Sleep, Immobility Time and Sleep Efficiency. Sleep Efficiency showed the same patterns as did Assumed Sleep and Immobility Time: the Sleep Efficiency of the E-types was poorer than that of the M- and N-types during weekdays (77.9% ± 7.0 versus 84.1% ± 4.9 and 84.1% ± 5.2) but was similar to that measured in the M- and N-types during the weekend. Sleep Latency and Movement and Fragmentation Index were not different among the three chronotypes and did not change on the weekend compared with weekdays. This study highlights two key findings: first, we observed that the circadian rhythm of activity levels was influenced by the chronotype; second, the chronotype had a significant effect on sleep parameters: the E-types had a reduced sleep quality and quantity compared with the M- and N-types during weekdays, whereas the E-types reached the same levels as the other chronotypes during the weekends. These findings suggest that E-types accumulate a sleep deficit during weekdays due to social and academic commitments and that they recover from this deficit during "free days" on the weekend.

Keywords: Activity, actigraphy, chronotype, circadian rhythm, morningness–eveningness, sleep

INTRODUCTION

Chronotype is the expression of circadian rhythmicity in an individual and can differ among individuals. There are three different chronotypes: Morning-types (M-types), Evening-types (E-types) and Neither-types (N-types). Several studies have shown differences between M-types and E-types in the circadian rhythms of different physiological variables. For instance, M-types have, under normal conditions, an earlier oral temperature peak approximately 2 h before the E-types (Baehr et al., 2000), and the acrophase of cortisol in serum is 55 min earlier in M-types than in E-types

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3. THE EFFECT OF CHRONOTYPE ON PSYCHOPHYSIOLOGICAL RESPONSES DURING AEROBIC SELF-PACED EXERCISES. (2015). Rossi A, Formenti D, Calogiuri G, Vitale JA, Weydahl A.

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THE EFFECT OF CHRONOTYPE ON PSYCHOPHYSIOLOGICAL RESPONSES DURING AEROBIC SELF-PACED EXERCISES[1]

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Summary.—It was hypothesized that an individual's chronotype might influence the response to physical activity at a given time of day. This study aimed to analyze the psychophysiological responses during a walking task at different times of day in individuals with different chronotypes. 46 students (M age = 24.8 yr., SD = 7.2) filled in the Morningness–Eveningness Questionnaire to determine chronotypes. Heart rate, walking time, and the rating of perceived exertion (RPE) were measured during two self-paced walking sessions: one in the morning (08:30) and one in the afternoon (15:30). A multivariate analysis of variance found a significant interaction between chronotype and time of day. The *post hoc* analysis showed a significant difference for RPE in the morning session, with evening types reporing a higher RPE compared with the morning types. The chronotype and the time of day when a physical task is undertaken can influence the RPE response, although it might not influence physiological or performance parameters. This has to be taken into account, because it can affect test reliability as well as possibly have a negative influence on the affective responses to a given task.

Under normal conditions, people's physiological functions change periodically over a daily cycle of about 24 hr. (approximately 22–26 hr.), with patterns that have been defined as *circadian rhythms* (Koukkari & Sothern, 2007). Circadian rhythms have been shown in basic physiological functions such as body temperature, heart rate (HR) at rest, oxygen consumption, metabolic rate, sweat rate, and cardiac output during exercise (Cohen, 1980; Reilly & Brooks, 1990; Cappaert, 1999; Drust, Waterhouse, Atkinson, Edwards, & Reilly, 2005; Waterhouse, Drust, Weinert, Edwards, Gregson, Atkinson, *et al.*, 2005; Reilly, Atkinson, Gregson, Drust, Forsyth, Edwards, *et al.*, 2006; Calogiuri, Weydahl, & Sothern, 2011). Differences in circadian rhythms were found between sexes, with a shorter intrinsic rhythm and larger fraction of sleep in women than in men (Wever, 1984).

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