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ACTIGRAPHIC ASSESSMENT OF SLEEP-ACTIVITY CYCLE IN PHYSIOPATHOLOGY: EXPERIMENTAL AND METHODOLOGICAL STUDIES

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"Ingenting er umulig, ikke gi opp"

Al mio essere resiliente

ed alla mia famiglia,

meravigliosa e sempre presente.

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PROLOGUE

The principal objective of my research during my PhD has been the investigation of the rest-activity circadian rhythms in physiopathology, dealing with both experimental and methodological issues. On the experimental side, the focus of my research program was centered on the investigation of the rest-activity circadian rhythms in patients with binge eating disorders. On the methodological side, my activity was aimed at exploring the relationships between the actigraphy-based assessment of circadian rhythmicity and the questionnaire-based assessment of circadian typology.

The thesis is organized in 11 Chapters. Chapter 1 provides a short introduction to chronobiology and to the components of a circadian rhythm. Chapter 2 describes the two most common methods used to evaluate the circadian rhythmicity, namely actigraphy and self-administered questionnaires. These two approaches have remarkable strengths and weaknesses. Actigraphy is a non-invasive method (usually based on a small, wearable actigraphic unit) that allows one to monitor the activity levels during the 24 hours, to detect the rest-activity circadian rhythm, to evaluate the activity levels during the nocturnal sleep and to assess the quality and quantity of sleep by specific sleep parameters. One alternative approach to assessment of the circadian typology of a subject is based on self-administered questionnaires. Questionnaires are obviously less objective than actigraphy-based assessments, but have the advantage of being simple and cost-effective. Chapter 3 provides a general overview of all the research projects I have taken part in throughout my PhD course. This chapter has been written with the reader in mind and aims to succinctly describe the structure and function of the subsequent chapters, 4 through 11. In Chapters 4 to 7, I will focus on the experimental core of my research activity during my PhD course, which is the chronobiological

investigation of obese patients suffering from binge eating disorder. First, I will provide an overview of the features characterizing this disorder. Then, I will describe three experimental studies that were carried out in these patients with the purpose of i): quantifying their rest-activity circadian rhythm (RARs); ii) describing their sleep behaviour; iii) evaluating the effectiveness of a physical activity program as an auxiliary therapeutic approach to the traditional treatment for BED.

In Chapters 8 to 10, I will illustrate the methodological core of my research activity during my PhD which aims to develop predictive formulas - based on linear regression - enabling investigators to use the questionnaire-based assessment of circadian typology (Morningness-Eveningness Questionnaire, MEQ) as a surrogate of the actigraphy-based assessment of circadian rhythmicity. A methodological project of this kind was successful is showing that both MEQ and its reduced version rMEQ are appropriate for the prediction of the actigraphy-based acrophase and this may prove useful when actigraphy-based measurements are not applicable, in so far as they result either too complex or time-consuming.

Chapter 11, the final chapter, is concerned with providing concise summaries of the other studies I have been involved in during my PhD course. Seven experimental studies are described in relation to: i) the influence of chronotype on circadian rhythm (RARs), on sleep, on physical activity and on cardiac autonomic function; ii) the effects of aerobic physical activity on sleep and on markers of insulin resistance in breast cancer women; iii) the effects of short and prolonged exposure to cave environments on human physiology.

The thesis also comprises an appendix containing the list of all the scientific papers that I co-authored in the course of my PhD thesis. The list reports both the published and the submitted articles.

1

CHRONOBIOLOGY

It has been firmly established that various functions recur periodically in living organisms and it is known that these rhythms are characterised by diverse frequencies, although only some of these frequencies, related to a restricted range of biological functions, have been (often inadequately) investigated by the growing field of chronobiology.

Chronobiology is the science that quantifies and investigates objectively mechanisms of biological time structure, including rhythmic manifestations of life. Among various subspecialties, chronobiology includes chronophysiology which describes temporal manifestations of physiological processes. It evaluates cyclical nervous, endocrinal, metabolic and other interactions within the organism which underlie biological temporal characteristics and their interactions with the environment (Halberg et al., 1977).

There are various approaches to studying periodic phenomena, and these have given rise to different schools of chronobiology. One set of researchers has been trying for years to locate a basic centre in the Central Nervous System (CNS) from which all periodic impulses originate. It is significant that most of these schools are interested almost exclusively in circadian rhythms, since it is difficult to imagine that such a controlling centre or biological clock (conceived as a discrete anatomical structure and not as a system or network of reciprocal influences) would be able to regulate a series of periodic phenomena characterised by markedly differing frequencies. However, when we look at nature there is just such a variety of rhythms: not only circadian but also weekly, monthly and annual rhythms with high frequency or very low frequency rhythms.

The researchers have concentrated their attention on various central neuronal system structures proposed at various times as the primary circadian rhythm generator: the pineal gland, the suprachiasmatic nucleus, the hypophysis or part of it, and the hypothalamus. All these structures are certainly involved, in fact they influence circadian rhythms, but the important point is that we are dealing with several structures and not one.

However, the structure most consistently proclaimed by physiologists as the biological clock is the suprachiasmatic nucleus, which is located on the top of the optic chasm where the optic nerve fibres cross; it processes signals coming from the eyes and releases hormones and other neuronal signals which trigger behavioural and physiological events. Numerous studies have been conducted on the topic but one of the most important is the study of Franz Halberg, the pioneer of chronobiology (Halberg et al., 1977). More than thirty years ago, Halberg showed that laser ablation of the bilaterally symmetrical suprachiasmatic nuclei in rats modifies the circadian rhythm of

body temperature but do not eliminate that rhythm. Specifically, the relationship between the environment and the body temperature rhythm is modified, at times, for example, of maximum and minimum temperature changes, and sometimes also the average temperature over 24 hours, but the circadian temperature oscillation persists (Halberg et al., 1979).

"Natura non facit saltus" (Nature does nothing in leaps). From the simplest unicellular organisms to the most complex mammals, the basic mechanisms that regulate biological functions adhere to a single set, organising principle.

If ablation of the suprachiasmatic nuclei modifies but does not eliminate periodic oscillations in body temperature, then those nuclei cannot be rhythm generators, but only one of the many nodes in the network of CNS structures concerned with maintaining body rhythms, and this is valid both in mice and in humans.

In conclusion, the CNS transmits its rhythmic information to cells in other brain regions and peripheral organs via a variety of outputs. These include neuronal connections, endocrine signals, body temperature rhythms, and indirect cues, provoked by oscillating behavior. Synchronization between central and peripheral clocks and synchronization of cellular clocks within the brain impact circadian timing, physiology, and behavior. Therefore, the main circadian oscillator, the suprachiasmatic nucleus, is located in the brain as well as secondary oscillators. In the periphery, circadian clocks are functional in most body cells (Dibner et al., 2010).

1.1 Rhythm

A rhythm is a regular periodic component in a time sequence, demonstrated by inferential statistical means, preferably with objectively quantified characteristics: frequency (f), acrophase (Ø), amplitude (A), MESOR (M), and waveform (W).

Rhythms thus include any set of biologic changes recurring systematically according to an algorithmic pattern or waveform validated in inferential statistical terms. Mathematically, most sinusoidal rhythms may be described by the use of approximating functions such as those of a form:

$$f(t) = M + A \cos(\omega t + \text{Ø})$$

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 provide a clearer indication that this function approximates the data better than 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 et al., 1972).

These are the characteristics of a rhythm (Figure 1):

- Acrophase, Ø: measure of timing. The lag from a defined reference time point 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 (Halberg and Reinberg, 1967; De Prins et al., 1977).

- 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 (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).

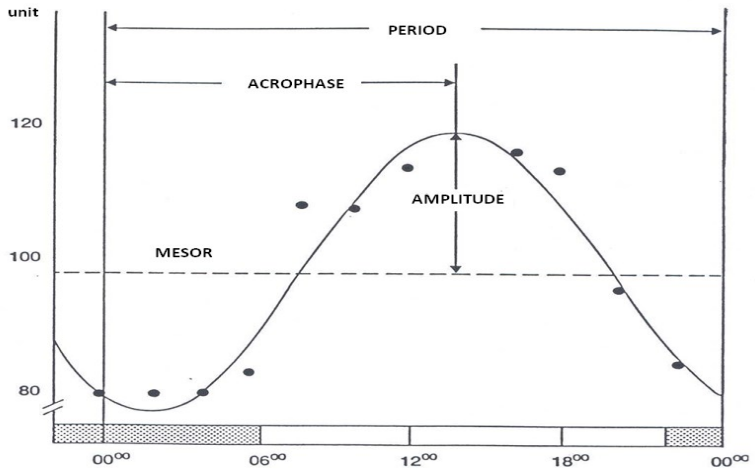

Figure 1. Example of a biological rhythm and its parameters: Acrophase (Ø), Amplitude (A) and MESOR (M).

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

- Ultradian: $\tau < 20$ hours, relating to biologic variations or rhythms with a frequency higher than circadian and, specifically, rhythms with frequencies greater than 1 cycle in

20 hours. It is permitted arbitrarily to set the low frequency limit of the ultradian range at 1 cycle in 20 hours (Halberg, 1964).

- Circadian: 20 hours $\leq \tau \leq$ 28 hours, relating to biologic variations or rhythms with a frequency of 1 cycle in 24 ± 4 hours. This term describes rhythms of a roughly 24 hour cycle length, whether they are frequency-synchronized with environmental schedules (Pittendrigh, 1960).

- Dian: 23.8 hours $\leq \tau \leq$ 24.2 hours, relating to biologic variations or rhythms with a frequency of 1 cycle in 23.8 to 24.2 hours, if not in precisely 24 hours. This is a special case of circadian period with an inferential statistical 95% confidence interval for period length within 23.8 and 24.2 hours (Halberg et al., 1965).

- Infradian: $\tau >$ 28 hours, 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 components 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).

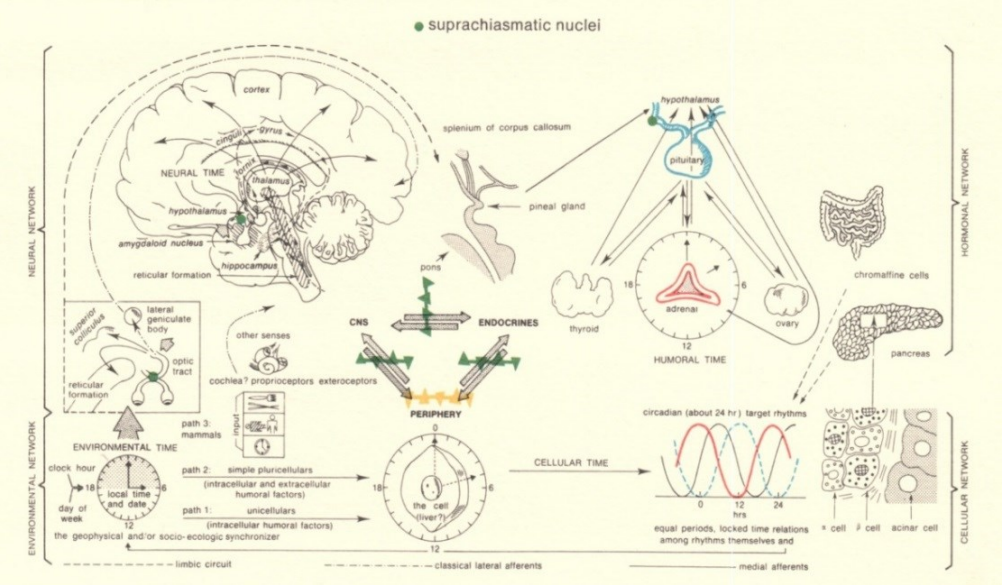

Figure 2. Schematic representation of the factors involved in circadian synchronization and environmental and/or organism desynchronization.

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 of their influence on a specific variable. For human beings the primary circadian synchronizers are mostly of socio-environmental origin: the best known are the alternation of light-dark cycles and rest-activity cycles. 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). Firstly, it was theorised that the temperature rhythm was the result of behavioural changes associated with the rest-activity cycle but this kind of explanation is not thorough enough; the standard method for demonstrating this is the effect of "constant routine": in this protocol, a subject had to remain awake, sedentary and relaxed for at least 24 hours. When this protocol is applied, 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:

- 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 and Waterhouse, 1982).
- The difference between the two rhythms is the exogenous component of core temperature rhythm, which is dominated by the rest-activity cycle (Reilly et al., 1997). These deductions are general, insofar as all rhythms show a mixture of endogenous and exogenous component when compared under normal living conditions and during a constant routine (Waterhouse et al., 2012).

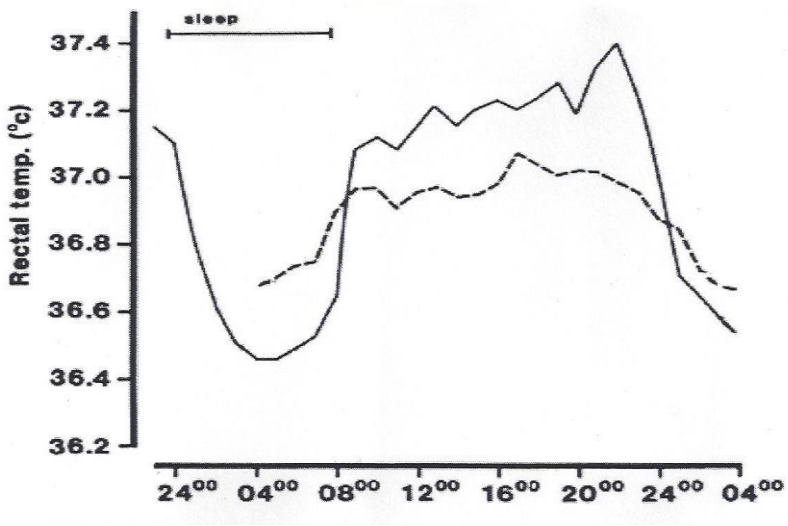

Figure 3. The daily rhythm of core temperature in a group of 8 young men; Full line: under normal condition (sleep from 12 a.m. to 7 a.m. indicated by bar); Dashed line: undergoing a 24 hours constant routine starting at 4 a.m.

1.3 Chronobiology and physical exercise

The relationships between the circadian rhythms and physical exercise have been studied since the '60s. There are numerous biological and behavioural functions, characterized by regular circadian rhythm that affects the physical exercise. These include pulmonary function (Spengler and Shea, 2000), body temperature (Waterhouse et al., 2005), mood (Boivin et al., 1997), reaction times (Wright et al., 2002), memory and readiness (Johnson et al., 1992) and cognitive functions (Dijk et al., 1992).

Defining the circadian rhythmicity of physical performance is not an easy task because the circadian rhythm of physical activity has, like the other biological functions, both exogenous and endogenous components and the latter are reflection of the circadian internal architecture (Rae et al., 2015). The approach to this type of investigation requires that the same physical test is repeated several times during the day (morning, afternoon, evening and in some cases even during the early night) and subsequently at the same times throughout the entire period of data collection.

Several studies are conducted on cycling and swimming, investigated in the expression of maximum power, technique, perception of fatigue and changes in mood during the daily increase of body temperature (Deschodt and Arsac, 2004; Kline et al., 2007).

Evidence suggests that the physical performances shows better results if practiced during the first part of the evening, a theory that has been highlighted numerous times (Winget et al., 1985; Chtourou and Souissi, 2012), but it has also been contradicted by other research (Dalton et al., 1997; Deschenes et al., 1998; Reilly and Garrett 1998; Bessot et al., 2006).

Better performances during the evening would be determined and associated with the peak of body temperature, which occurs in this part of the day. On the contrary, in the morning with lower temperature levels the performances would be worse. The results

illustrate, therefore, a parallelism between physical performance and body temperature, which, because of its increment, enhances the activation of muscles, nerves and their conductivity, respiratory system, cardiovascular system and metabolism (Atkinson et al., 2005). These findings are in line with a previous study (Atkinson and Reilly, 1999) which show how world records are broken by athletes in the evening hours.

This difference in performance throughout the day has often been found without methods that identify the real influence of circadian rhythms and, therefore, a worse morning performance could be due to a subject's nutritional state upon awakening, to the low level of joint stiffness after prolonged rest, to the lower environmental temperatures, to the inertia subsequent to a long period of sleep or lack of time for muscle warming (Youngstedt and O' Connor, 1999). Atkinson and colleagues (2005), however, have shown that even after a warm-up in the morning, the performances carried out in the first phase of the day remain of lower quality than the afternoon-evening.

Circadian variations are also present in lumbar flexion and extension, gleno-humeral lateral rotation and whole-body forward flexion (Gifford 1987) and in stiffness of the knee joint (Wright et al.,1969) with levels being higher in the early evening. Similar results are obtained for muscle strength which, independently of the muscle group measured or speed of contraction, reaches a peak between 2 p.m. and 7 p.m. (Reilly et al., 2000). The presence of circadian rhythms in short-term performances (1 minute or less) is less evident and related to the type of exercise performed and the muscle group tested (Bernard et al., 1998). Significant circadian rhythmicity is observed in length of jumps (Reilly and Down, 1986), in a stair run (Reilly and Down, 1992), in vertical jumps (Atkinson, 1994) and in short duration maximal ergometer tests (10-30 seconds) (Hill and Smith, 1991).

In conclusion, knowledge of the circadian variation in sports performance could be an important and practical consideration for both athletes and coaches taking part in competitions.

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2

METHODS

2.1 Actigraphy: a methodological and objective approach to the assessment of rest-activity circadian rhythm and sleep behaviour

Actigraphy is a technique to monitor the rest–activity circadian rhythm, daily physical activity, and the sleep-wake patterns (Ancoli-Israel et al., 2003; Littner et al., 2003; Lehnkering et al., 2006; Paquet et al., 2007; Montaruli et al., 2009; Roveda et al., 2011; Sadeh, 2011; Calogiuri et al., 2013; Roveda, et al., 2017a; Smith et al., 2018a; Smith et al., 2018b). Compared with the traditional polysomnography (PSG), actigraphy can conveniently record for days, weeks or even longer, providing important information in the assessment of a person's sleep-wake patterns in the long term (Ancoli-Israel, et al., 2003). These characteristics make monitoring by actigraph practical for extensive use. In fact, actigraphy is relatively simple to use: it allows subjects to manage their own

monitoring over several days thus obtaining highly reliable and reproducible data. Researchers have validated actigraphy against polysomnography, the gold standard in sleep studies (Kushida et al., 2001; So et al., 2005).

Although actigraphy is rarely used for clinical research (Sadeh et al., 1995), it is commonly utilized to diagnose insomnia, circadian rhythms disorders and excessive sleepiness (American Sleep Disorders Association, 1995). Under the auspices of the American Academy of Sleep Medicine (AASM), actigraphy has been shown as capable of providing specific information on the diagnosis of sleep disorders, circadian rhythms disorders and sleep variability in patients with insomnia, all data not obtainable in any other practical way (Ancoli-Israel, et al., 2003; Sadeh, 2011). Actigraphy enables us to evaluate levels of activity over 24 hours, to detect the rest-activity circadian rhythm and to evaluate levels of activity during nocturnal sleep to detect the quality and quantity of sleep by specific sleep parameters.

Sleep is an important component of our life, while sleep disorders have deleterious effects on health (Gay et al., 2004; Knutsson, 2003; Santhi et al., 2007). In fact, sleep deficiency adversely affects metabolic and endocrine functions (Spiegel et al., 1999) and the duration, timing and quality of sleep have an important effect on physical and mental health, performance and safety (Committee on Sleep Medicine Research Board on Health Sciences Policy, 2006).

Chronic insufficient sleep can result in several adverse physiological consequences, including increased appetite and food intake (Spiegel et al., 2004; Markwald et al., 2013), reduced insulin sensitivity and glucose tolerance (Spiegel et al., 1999; Van Cauter et al., 2007), disturbed mood (Dinges et al., 1997), impaired pancreatic β-cell responsiveness (Buxton et al., 2012), higher blood pressure, and an increase in inflammatory markers. Sleep disorders, such as insomnia, frequent night awakenings,

wandering at night, and unusual early morning awakenings, can also undermine the achievement of optimal amounts of sleep (Dowling et al., 2005). The stages of nocturnal sleep are controlled by different neurochemical systems connected with each other: the preoptic area and the suprachiasmatic nucleus which receive afferents directly from retinal fibers (Buijs et al., 2003; Dijk and Lockley, 2002; Sack, 2009; Sothern et al., 2009). The endogenous rhythm of the sleep-wake cycle is, in normal conditions, synchronized with the alternation of day-night cycle and other factors such as timing of meals and social routines. Such synchronization is important in order to maintain healthy sleep-wake patterns, as in fact its disruptions can lead to the emergence of different sleep problems (e.g., insomnia, sleep-disordered breathing, central disorders of hypersomnolence and insufficient sleep syndrome) (Sack et al., 2007a; Sack et al., 2007b). The quality and duration of people's nocturnal sleep is also sensitive to changes in the light-darkness periodicity associated with seasonality, which are complicated by the geographic latitude in which an individual resides (Ohayon, et al., 2017). Other factors that can affect sleep are related to school or work schedule times (Ohayon, et al., 2017) as well as psychological factors such as chronic stress and mental health problems (Boland and Ross, 2015).

2.2 Actigraph

Actigraph is the tool used to perform the actigraphic monitoring. It is a small device similar to a watch which contains an accelerometer that produces voltage when the device is in movement. A diary is often given to the subject to record important information about sleep behaviour patterns (i.e., bed time, get up time, hours of naps…). Most single-axis acceleration devices today use band-pass filters which eliminate very

slow (<0.25 Hz) and very fast movements (>2–3 Hz). Voluntary human movement rarely exceeds 3–4 Hz (Ancoli-Israel et al., 2003).

The analog signal from the actigraph may be digitized in different ways. The most common methods are time above threshold zero crossings, and digital integration. Time above threshold measures the amount of time per epoch produced by the signal in response to motion above a certain threshold (commonly 0.1–0.2 g). The zero-crossing method counts the number of times per epoch that the activity signal level crosses zero (or very near zero). Digital integration involves sampling the accelerometer output signal at a high rate, then calculating the area under the curve for each epoch. Digital integration is better for identifying movement amplitude compared with time above threshold, while zero crossing is the least efficient in identifying movement amplitude (Gorny and Spiro, 2001).

The best placement of the actigraph to obtain the most reliable data is still controversial. In most studies, investigators have placed the actigraph on the nondominant wrist. Studies comparing different actigraphy placements suggest that the wrist placement detects more movements compared with ankle and trunk placement and that the placement on the dominant arm detects more movements than the placement on the nondominant arm (Sadeh and Acebo, 2002).

The recommended minimum length for actigraphic monitoring is 3 days (Littner et al., 2003). This length is adequate when observing simple variables relative to sleep patterns or studying activity data using a parametric model of analysis of rhythm, such as the cosinor model. To obtain robust estimates in the study of rest–activity rhythm with a nonparametric approach, which quantifies processes of stability and instability, it may be necessary to collect actigraphic data for a longer period.

2.3 Rest-activity circadian rhythm analysis in physiopathology

The rest-activity circadian rhythm is assessed non-invasively using an actigraph. Data are collected in 60-second epochs and transferred from actigraph to a personal computer using specific software to obtain the activity data, expressed in activity counts. In order to determine the rest-activity circadian rhythm, the activity data are analyzed using the single cosinor method (Halberg et al., 1977; Nelson et al., 1979). Hinging 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 the three rhythmometric parameters MESOR (M), amplitude (A) and acrophase (ϕ) which are indicated with the relevant 95% confidence intervals. The three parameters of activity levels are then processed using 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).

Furthermore, non-parametric statistics also exist for the analysis of activity rhythms when the data are non-sinusoidal in shape, more closely resembling a square wave pattern. In this circumstance it is necessary to use other techniques to describe the data (Van Someron et al., 1995; Marler et al., 2006; Calogiuri et al., 2013).

Rest-activity circadian rhythm and aging

Circadian rhythms are present in all physiological and behavioural phenomena and several studies have shown that they alter with age. The deterioration of circadian rhythms in elderly people is thought to be conducive to sleep problems and curtailed daytime function (Huang et al., 2002). Degeneration of suprachiasmatic nucleus in the elderly plays an important role in poor sleep and weakened circadian rhythm. In fact,

the suprachiasmatic nucleus is the main circadian pacemaker of the mammalian brain and manages hormonal and behavioural circadian rhythms (Rusak and Zucker, 1979).

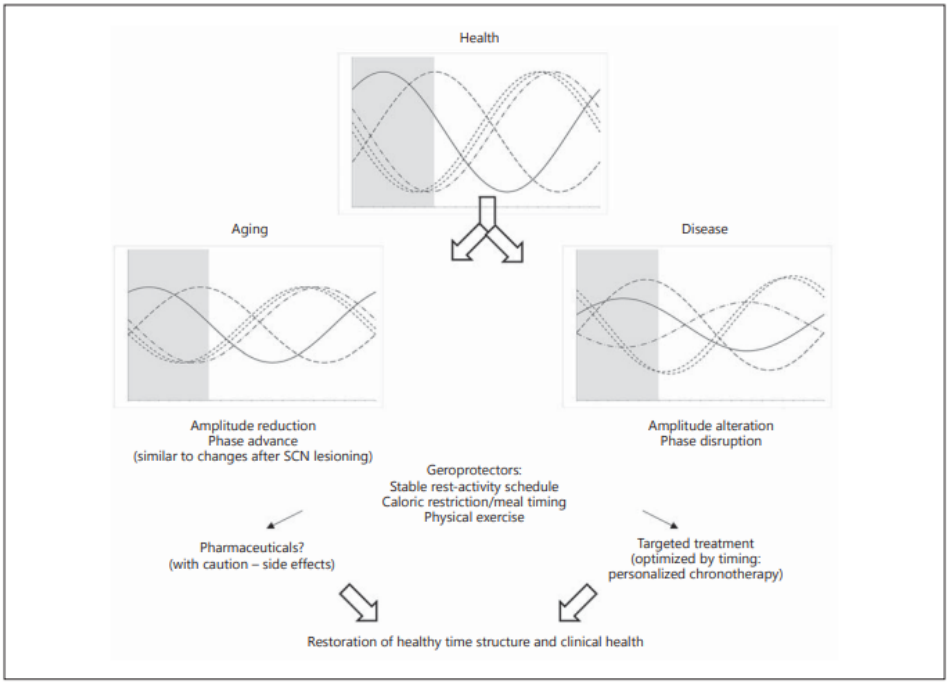

Figure 4. Comparison between a healthy circadian system and disrupted circadian systems during ageing process and in presence of disease (©Halberg Chronobiology Center).

Specifically (Figure 4), abnormalities in circadian rhythm, such as reduced circadian amplitude and labile circadian acrophase tending to occur in early stages of advancing age. Similar features have been observed in the experimental laboratory after bilateral lesioning of the suprachiasmatic nuclei (Halberg et al., 1979). These results suggest the involvement of clock genes during ageing process as in various disease conditions characterized by circadian rhythm disruptions (Cornelissen and Otsuka, 2017). Circadian disruption is deleterious to health and can lead to metabolic disease such as

diabetes and obesity (Tevy et al., 2013). To regain good health and salutary time arrangement, a regular routine and physical exercise are commendable in order to maintain a strong circadian system. Caloric restriction, characterized by an increased circadian amplitude, has also been correlated with a longer life span in several animal case studies. The merits of pharmacologic interventions, using geroprotectors, are being investigated in clinical applications with particular attention to the side effects. Furthermore, nonpharmacologic or pharmacologic treatment can be valorized by timing with personalized chronotherapy guided by marker rhythmometry. To evaluate circadian variations seems to be necessary to maintain the control and to generate health improvements (Cornelissen and Otsuka, 2017).

Rest-activity circadian rhythm and cancer

Rest-activity circadian rhythm abnormalities have been observed in several pathological conditions such as cancer (Mormont et al., 2002).

Compelling new animal, cell, and molecular research demonstrates the biology that connects circadian disruption with tumor incidence, growth and progression (Fu and Lee, 2003). An accumulation of evidence, including convincing epidemiologic data, authorizes the World Health Organization to conclude in 2007 that shift work is probably carcinogenic (Straif et al., 2007). Several independent clinical studies have demonstrated that circadian rhythms have prognostic value referring to mortality rates among patients with breast cancer (Sephton et al., 2000), colorectal cancer (Mormont et al., 2002; Levi et al., 2014), renal cell carcinoma (Cohen et al., 2012), and lung cancer (Sephton et al., 2013). Circadian disruption in cancer patients may have multiple etiologies: an important component is represented by physiological effects of the tumor (Mormont and Levi, 1997). However, psychological distress is strongly related to

disruptions of sleep and circadian rhythms (Sephton and Spiegel, 2003). The effects of stress and circadian disruption on cancer progression may be linked, and may be mediated by endocrine and immune factors (Antoni et al., 2006; Eismann et al., 2010). It is known that both early and advanced stage breast cancer patients show flattened cortisol rhythms (Abercrombie et al., 2004; Witek-Janusek et al., 2008). In particular, advanced breast cancer patients have display marked circadian disruption that affects the rest-activity, metabolic and immune cell rhythms (Eismann et al., 2010). Disrupted rest-activity rhythms occur simultaneously with poor functional and physical quality of life, and moreover sleep disorders and fatigue are already present before chemotherapy (Ancoli-Israel et al., 2006). In fact, the chemotherapy exacerbates the rhythm disruption and amplifies fatigue, generating depression and mood disorders (Roscoe et al., 2002; Savard et al., 2003).

The central circadian disruption can accelerate tumor progression (Eismann et al., 2010). Centrally mediated circadian rhythms control sleep-wake, rest-activity, metabolic, endocrine and immune function. Specifically, the centrally mediated glucocorticoid rhythm represents the main signal from the suprachiasmatic nucleus of the hypothalamus that regulates the circadian clocks present in the majority of peripheral tissues. Glucocorticoid rhythms control the circadian rhythms of proliferative cycles, cellular apoptosis, cell trafficking and cytokine secretion (Fu and Lee, 2003; Eismann et al., 2010).

Rest-activity circadian rhythm and neurodegenerative disease

Rest-activity circadian rhythm abnormalities have been observed also in neurodegenerative disease (Tranah et al., 2011).

Activity phase abnormalities in older adults with dementia establish a prediction of shorter survival (Gehrman et al., 2004) and increase the mortality risk (Paudel et al., 2010; Tranah et al., 2010). Disturbances of the sleep-wake cycle, which are characterized with poor activity rhythms, are particular pronounced in Alzheimer's disease (Satlin et al., 1995; Ancoli-Israel et al., 1997; Gehrman et al., 2005). Patients with diagnosis of Alzheimer show circadian disturbances, including reduced amplitudes and phase delay of circadian variation in core body temperature and activity (Van Someren et al., 1996).

Tranah and colleagues (2011), demonstrate that a delayed acrophase is associated with increased odds of developing of dementia or mild cognitive impairment (MCI) while an advanced acrophase is associated with elevated but non-significant odds to develop dementia or MCI. These results suggest that activity rhythm abnormalities are prognostic of increased risk of developing dementia and MCI in the elderly. It is not clear the mechanism behind this relationship but probably an altered circadian rhythm may affect numerous neurophysiological processes. In fact, disorders in the circadian timing system influence memory, cognitive function and behaviour through various neuroanatomical and neurophysiological mechanisms (Benca et al., 2009). The circadian contribution to cognition may also result from synchronized activities of an integrated network of clocks in the brain under the control of the suprachiasmatic nucleus pacemaker (Harmar et al., 2002). It is also possible that circadian activity rhythms are biomarkers of advanced physiological aging that generate additional risk but without a direct causal association with dementia or MCI.

Daily rhythms in sleep-wake behaviour and waking performance are produced by the interaction of multiple external and internal oscillators (Schmidt et al., 2007). Optimal cognitive performance depends on temporal alignment between sleep and clock-driven

mechanisms. The complexity of this relationship is related to the discovery that the suprachiasmatic nucleus is not the only brain pacemaker (Kyriacou et al., 2010) and that local semiautonomous clocks are present in the brain, including cerebral cortex, hippocampus and cerebellum (Hastings et al., 2008). The suprachiasmatic nucleus is important to determine the timing of sleep and wakefulness and simultaneously synchronizes the multitude of local brain clocks to a complementary circadian program (Kyriacou et al., 2010). Compromise of the local clock machinery with age may reduce the efficiency of temporal adaptation and impair cognitive function.

Rest-activity circadian rhythm and cardiovascular disease

Disruptions of circadian activity rhythms in the elderly are associated with adverse cardiovascular consequences such as an increased risk of cardiovascular disease (CVD)-related mortality (Tranah et al., 2010; Paudel et al., 2010) and stroke-related mortality (Tranah et al., 2010).

Paudel's study (2011) shows that in older men lower amplitude and higher minimum activity counts are significantly and independently associated with higher risk of incident cardiovascular (CVD) events and coronary heart disease (CHD) events. Delayed acrophase is moderately associated with increased risk of peripheral vascular disease (PVD) events. In addition, a previous Tranah's study (2010) reports greater risk of CHD-related mortality among women with reduced MESOR. Prior study also suggests that there is a circadian component in the timing of CVD and stroke events, with a higher frequency of CVD-related and stroke events occurring in the early morning hours (Muller et al., 1989).

The biological mechanisms underlying the associations between disrupted rest-activity circadian rhythms and increased risk of CVD are not known. Although, it is generally

perceived that circadian rhythm disruptions precede CVD-related events, it is plausible that prevalent CVD disease worsens circadian rhythm disruptions due to their debilitating impact on sleep-wake activity (Paudel et al., 2011).

Rest-activity circadian rhythm and healthy young subjects with different circadian typology

Circadian typology represents the expression of circadian rhythmicity in human being. In literature exist three different chronotypes: Morning-types, Neither-types and Evening-types. It is known that are present several differences in circadian rhythms of physiological parameters between Morning- and Evening-types. In particular, Morning-types wake up and go to bed early (Taillard et al., 2004) and have their best physical and cognitive performances in the first part of the day. On the contrary, Evening-types wake up and go to bed late and have their best physical and cognitive performances in the evening (Horne et al., 1980; Vitale et al., 2013). In fact, morningness is associated to difficulty in maintaining sleep whereas eveningness is associated to difficulty in initiating sleep (Taillard et al., 2001).

Furthermore, numerous studies have assessed the circadian rhythmicity of large number of biological markers, such as sleep-wake cycle, melatonin, cortisol or body temperature among chronotypes: Morning-types present earlier peaks of these parameters compared to Evening-types (Horne and Ostberg, 1976; Duffy et al., 1999; Gibertini et al., 1999; Kudielka et al., 2006). Morning-types have an earlier oral temperature peak approximately 2 hours before the Evening-types (Baehr et al., 2000) and also the acrophase of cortisol in serum is 55 minutes earlier in Morning-types than in Evening-types (Bailey and Heitkemper, 2001). As well, the best predictor of sleep

onset represented by the melatonin in blood and in saliva, occurs approximately 3 hours later in Evening-types than in Morning-types (Mongrain et al., 2004).

Our study (Vitale et al., 2015) shows that the circadian rhythm of activity levels is influenced by circadian typology: Morning-types have an early acrophase of their circadian rhythm of activity levels whereas Evening-types show an acrophase more than 2 hours later. Similar results are reported in Lee's study (2014) in which the mean activity acrophase of Evening-types is nearly 2 hours later in the morning compared to Morning-types.

2.4 Sleep analysis in physiopathology

Actigraphy has been shown to be a valid instrument for identifying rhythms as well as sleep disturbances (Ancoli-Israel et al., 2003; Morgenthaler et al., 2007).

One of the most important features of the rest–activity cycle, as measured by actigraphic monitoring, is an asymmetrical "square shape wave" (Barger et al., 2009; Dowling et al., 2005). In fact, a rest–activity cycle typically presents a phase of about 16 hours with high and relatively frequent activity episodes, followed by a phase of about 8 hours with lower and relatively infrequent activity episodes (Calogiuri, et al., 2013). The periods of activity and lack of activity assessed by the actigraph during the night phase are used to produce a series of parameters that represent the characteristics of a person's sleep. In particular, the sleep analysis allows for a night-by-night assessment of the sleep-wake patterns, generating parameters that provide a description of the phase, duration, and quality of the nocturnal sleep (Calogiuri, et al., 2013) (Figure 5).

The data are analyzed with the sleep detection algorithm provided by specific software.

The most relevant parameters that can be evaluated by sleep analysis with actigraphic data, which have also been validated versus PSG, are: bed time (BT), sleep start (Ss),

sleep end (Se), time in bed (TIB), total sleep time (TST), sleep latency (SL), mean activity (MA), number of awakening longer than 5 minutes (NA >5), wake after sleep onset (WASO), and sleep efficiency (SE%). These parameters and the terminology used to identify them might differ according to software manufacturer and version. The actigraphic data are collected over a predetermined period and sleep logs (i.e., reports of bed and wake times) and/or sleep diaries, used in addition of the automatic monitoring by actigraph, make the data analysis procedure easier and more reliable. For instance, researchers can macroscopically study actograms (i.e., graphs reporting the activity levels recorded during the monitoring) and compare them with the diary data to verify the absence of anomalies. The sleep log can be helpful in more precisely individualizing bed time and wake-up time. In fact, sleep diaries might be used to collect information like the number and timing of naps, the number of night awakenings, subjective perception of sleep, and the amount of sleepiness experienced during different times of the day.

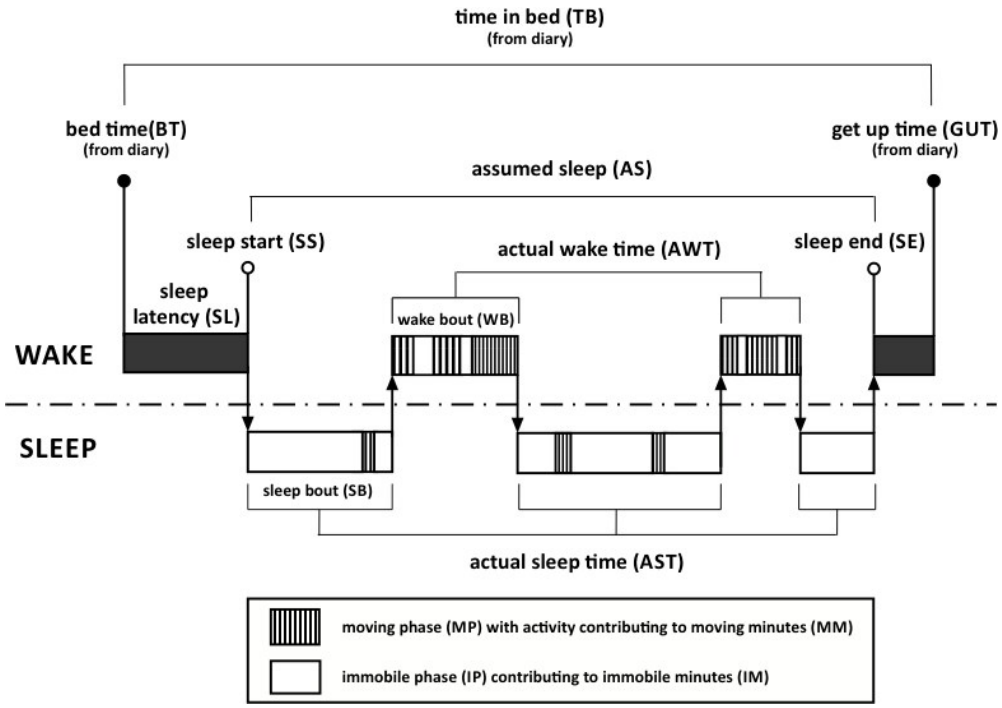

Figure 5. Night rest.

Figure 5 shows what happens during sleep and underlines the alternation both sleep-wake cycle and mobility-immobility phases. In fact, sleep is not a homogeneous and monolithic state but rather a composite and fragmented state, consisting in alternation of sleep and waking phases. In addition, within each phase there are periods of movement and immobility: the former prevalent during the phases of sleep while the latter during the waking phases. In particular, in this pattern there is a line that defines a superior zone indicated phase of waking and a lower zone indicated phase of sleeping. We distinguish the moments in white as Immobile Phase (IP) and those with black bars as Moving Phase (MP). IP increases the amounts of Immobile Minutes (IM) while MP increases the count of Moving Minutes (MM).

Sleep behaviour and aging

It is known that aging influences the sleep behaviour through modifications such as decrease in slow wave sleep, increase number and duration of night time awakening and shorter sleep duration (Carskadon et al., 1985; Monk et al., 1991; Kramer et al., 1999). Aging is also characterized by a decrease of the daytime activity level and increase of wakefulness during sleep episode (Renfrew et al., 1987; Dallosso et al., 1988; Elia et al., 2000).

Huang and colleagues (2002) assess the sleep and the rest-activity parameters using actigraphic monitoring in 65 healthy subjects. The volunteers were divided in 4 groups: young (21-34 years), middle-age (36-44 years), old (61-79 years) and oldest (80-91 years). The study shows that the old and oldest participants have a weakened and fragmented sleep compared to young and middle-age subjects. In fact, in the old and oldest categories the authors demonstrate increased numbers of nighttime awakening and daytime naps. In addition, in the same groups there is an evident decline in actual

sleep time and sleep efficiency as well as increased sleep latency and presence of fragmentation of sleep.

Sleep behaviour and cancer

Quantity and quality of sleep are important for the quality of life and have positive or negative influence on individual health (Atkinson and Davenne, 2007).

Evidence exists that sleep disorders are associated with an increased risk of cancer (Tamakoshi and Ohno, 2004; Hublin et al., 2007). For instance, shift workers show an increased risk of develop numerous cancers (Tatrow et al., 2004; Verkasalo et al., 2005; Pinheiro et al., 2006; Kakizaki et al., 2008; Thompson et al., 2011).

Numerous mechanisms could explain the association between sleep duration and cancer, including alterations in metabolism, circadian system, and immune function (Vijayalaxmi et al., 2002; Meier-Ewert et al., 2004; Vgontzas et al., 2004; Megdal et al., 2005; Dimitrov et al., 2007). For example, sleep deprivation generates metabolic changes that may predispose an individual to breast cancer, such as altered endocrine function (Spiegel et al., 1999), and increased inflammation (Meier-Ewert et al., 2004; Vgontzas et al., 2004). These aspects may directly contribute to obesity that is an important risk factor for postmenopausal breast cancer (Kosinski et al., 1999).

Sleep problems are common among cancer patients (Lee et al., 2004) and are associated with mechanisms that underlie also fatigue (Liu et al., 2012).

The influence of sleep on cancer prognosis is less clear. In fact, the diagnoses of cancer and/or cancer therapies are often related to fatigue and depression that can exacerbate a pre-existing sleep disorder or contribute to initiating one. So, many patients develop new sleep problems after their diagnosis of cancer (Costa et al., 2014). Factors contributing to the particularly high rates of sleep problems among cancer survivors

may include the occurrence or exacerbation of menopausal symptoms (e.g., hot flashes) caused by chemotherapy or endocrine therapy (Savard et al., 2004). Other factors contributing to sleep problems may stem from the increased stress or pain as a result of the cancer diagnosis and treatments (Savard et al., 2004). Psychological distress appears to increase shorten survival (Spiegel and Giese-Davis, 2003) and poor sleep efficiency is prognostic for early breast cancer mortality (Palesh et al., 2014).

Furthermore, is not clear the relationship between sleep problems and the adverse effects produced by chemotherapy treatment. Some studies show that sleep disturbances might be secondary to the pain and psychological distress experienced by the cancer patients (Plumb and Holland, 1977; Strang and Qvarner, 1990; Hu and Silberfarb, 1991). On the contrary, sleep problems were reported even when the pain and anxiety are low suggesting that sleep disorders may be independent of these psychological/physiological factors (Cimprich, 1999). Several studies report that women experience sleep disorders before chemotherapy treatment. Poor sleep, naps during day and complaints especially about the quality of sleep are present also 72 hours prior chemotherapy treatment (Ancoli-Israel et al., 2006). Sleep parameters in fact are already altered 48 hours prior chemotherapy (Berger et al., 2002) and sleep disturbances subscale reaches the highest score with all participants reporting to suffer of insomnia once or twice a week. In addition, is present a subjectively poor sleep correlated with higher levels of fatigue during the month prior chemotherapy (Berger et al., 2007). Moreover, lower daytime activity and increased nighttime restlessness are associated with higher fatigue levels during chemotherapy treatment and this relationship appears to exist also prior the beginning of chemotherapy (Berger et al., 1999). Lastly, it seems that the severity of symptoms such as fatigue, pain, dyspnoea,

insomnia, appetite loss and diarrhoea significantly increased with the rising number of chemotherapy cycles (Sultan et al., 2017).

Sleep behaviour and neurodegenerative disease

Sleep quality worsens with aging both in terms of decreased duration and in consolidation (Espiritu, 2008; Crowley, 2011). Sleep complaints are common among older adults and the most common being an inability to stay asleep at night (Foley et al., 1995). Other complaints associated with neurodegenerative diseases are insomnia, hypersomnia, parasomnia, excessive nocturnal motor activity, sleep apnea and sleep-wake rhythm disturbances (Chokroverty, 1999). Recent findings suggest that sleep quality play an important role in preserving cognitive function in elderly and reducing the risk of Alzheimer's disease, the most common cause of dementia (Lim et al., 2013). In fact, changes in sleep quality may contribute to cognitive decline among older adults (Landry and Liu-Ambrose, 2014) and disturbed sleep is observed in presence of mild cognitive impairment (Vitiello and Prinz, 1989; Beaulieu-Bonneau and Hudon, 2009) and increases with the severity of the neurodegeneration (Moe et al., 1995). In addition, sleep disturbances may contribute to neurodegeneration by exacerbating the aggregation of toxic proteins (Kang et al., 2009).

Sleep behaviour and cardiovascular disease

Interesting is that an irregular duration of sleep has been recently associated with vascular damage. It is known in fact that habitually short and long sleepers have an increased risk to develop incident calcification of the coronary arteries (King et al., 2008), incident coronary heart disease (Cappuccio et al., 2011), incident type 2 diabetes

(Yaggi et al., 2006; Cappuccio et al., 2010a; Holliday et al., 2013; Jackson et al., 2013), incident stroke (Cappuccio et al., 2010a) and death (Cappuccio et al., 2010b; Grandner et al., 2010). In addition, chronic insomnia is related to an increased risk of developing or dying of cardiovascular disease (Sofi et al., 2014). The Chicago cohort of the CARDIA study reports that short duration of sleep, assessed by actigraphy, is associated with a greater 5-year incidence of coronary artery calcifications (King et al., 2008). Another population study performed in Germany show that both short and long duration of sleep are related to an increased risk of atherosclerosis (Wolff et al., 2008). Finally, recent data from the Whitehall II study suggest that the effect of short sleep on coronary heart disease risk may be mediated by poor sleep quality (Chandola et al., 2010).

The mechanisms that underlie the association between an irregular sleep and cardiovascular events are not fully understood. One explanation could be the causative mechanisms that associate an inadequate duration of sleep with adverse health outcomes. These mechanisms include reciprocal changes in circulating levels of leptin and ghrelin (Spiegel et al., 2004; Gangwisch et al., 2008) that increase appetite, caloric intake, and reduce the energy expenditure (Spiegel et al., 2009). In this way, occurs a condition of obesity (Taheri et al., 2004) and impaired glycemic control (Spiegel et al., 2005) with consequent increase of cardiovascular risk. Low-grade inflammation is another factor that is activated during short sleep with possible implications not only for cardiovascular disease but also for other chronic conditions such as cancer (Miller and Cappuccio, 2007).

Lastly, there is no evidence that sleeping habitually between 6 and 8 hours per day in an adult is related to dangerous long-term health consequences. However, sleeping 9 hours or more per night may represent an important diagnostic tool for detecting subclinical or undiagnosed co-morbidity. People reporting consistently sleeping 5 hours or less per

night could have a higher risk for cardiovascular morbidity and mortality (Cappuccio et al., 2011).

Sleep behaviour and healthy young subjects with different circadian typology

In literature association between chronotype and sleep parameters exists. Numerous studies have assessed the sleep quality in different chronotypes using either self-assessment questionnaires or objectively method such as actigraphy. Studies that used self-assessment questionnaires report that Evening-types are more inclined to sleep complaints than Morning-types (Buysse et al., 1989; Johns, 1991) and the prevalence of nightmares and insomnia symptoms are higher in Evening-types compared to Morning-types (Merikanto et al., 2012). Studies that used actigraphy report differences in sleep efficiency between Morning-types and Evening-types (Lehnkering and Siegmund, 2007). One study (Martin et al., 2012) that assess the relationship between chronotype and sleep, reports differences in sleep onset and sleep offset among chronotypes. In fact, Evening-types generally go to bed and wake-up significantly later than Morning-types on both work and free days. So, eveningness is related to later bed time and wake up time and shorter time in bed during week (Giannotti et al., 2002; Roepke and Duffy, 2010; Kabrita et al., 2014; Lee et al., 2014). Our study (Vitale et al., 2015) examines the differences in sleep quality between weekdays and weekend in different circadian typology: Morning-types sleep better and spend more immobile time in bed compared to Evening-types during WD and that Evening-types have the same sleep quality as the Morning- and Neither-types during WE by increasing their sleep efficiency and immobile time. It seems that Evening-types accumulate a sleep debt during WD due to their social and academic commitments that oblige them to wake up earlier with respect

to their preferred sleeping times. Indeed, they recover during the WE in whom they sleep better and longer.

2.5 The Morningness-Eveningness Questionnaire: a subjective approach to assess the circadian rhythmicity

Actigraphy is the reference objective method to measure circadian rhythmicity (Ancoli-Israel et al., 2003) and generates the three main parameters which describe the rhythm: amplitude, MESOR and acrophase.

One alternative, simple and subjective approach to detect the circadian typology is based on self-assessment questionnaires. The most used and known is the Morningness–Eveningness Questionnaire (MEQ) by Horne and Ostberg (1976).

The MEQ consists of 19 items that investigate the sleep-wake cycle, the best moments of the day to perform physical and cognitive activities and subjective alert state. The answer options are presented in the multiple-choice modality. The total score is between 16 and 86 and determines five chronotypes: extreme Evening-types from 16 to 30, moderate Evening-types from 31 to 41, Neither-types from 42 to 58, moderate Morning-types from 59 to 69 and extreme Morning-types from 70 to 86.

Exists however a classification in three chronotypes more used in literature (Baehr et al., 2000; Bailey and Heitkemper, 2001; Mongrain et al., 2004): Evening-types from 16 to 41 with a delayed circadian phase, Neither-types from 42 to 58 with an intermediate circadian phase and Morning-types from 59 to 86 with an early circadian phase (Kerkhof et al., 1996). Another reduced version of the Morningness-Eveningness Questionnaire (rMEQ) is present in literature. The rMEQ is a self-assessment questionnaire to detect the circadian typology of a subject but consist of only 5 items (Adan and Almirall, 1991). The total score is between 4 and 25 and determines three

chronotypes: Evening-types from 4 to 11, Neither-types from 12 to 17 and Morning-types from 18 to 25.

The MEQ might be related with the circadian parametric portrait provided by actigraphy. In fact, our study (Vitale et al., 2015) reports that the chronotype obtained by the MEQ is associated to different mean values of the acrophase whereas amplitude and MESOR are not different among the three chronotypes. In another study (Lee et al., 2014), the mean acrophase of activity circadian rhythm is different among the three chronotypes and the study shows a moderate negative association between the MEQ score and the activity acrophase.

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3

CONCEPTUAL MAP OF SCIENTIFIC INVESTIGATIONS

In the following Chapters I will describe the several scientific studies in which I have been involved during my PhD course.

I decided to divide the scientific studies in two different groups: the core studies that will be illustrated in details in Chapters 4 to 10 and the other studies that will be illustrated summarily in Chapter 11.

In Chapters 4 to 7, I will focus on the experimental core of my research activity during my PhD and I will describe three experimental studies that were carried out in patients with binge eating disorder with the purpose of: i) quantifying their rest-activity circadian rhythm (RARs); ii) describing their sleep behaviour; iii) evaluating the effectiveness of a physical activity program as an addition to the traditional treatment for BED.

In Chapters 8 to 10, I will illustrate the methodological core of my research activity during my PhD and I will describe two studies about the relationship between the

actigraphy-based assessment of circadian rhythmicity and the questionnaire-based assessment of circadian typology (Morningness-Eveningness Questionnaire, MEQ).

In Chapter 11, the final chapter, I will describe summarily the other studies in which I have been involved during my PhD. Seven experimental studies are described that deal with: i) the influence of chronotype on activity circadian rhythm (RARs), on sleep, on physical activity and on cardiac autonomic function; ii) the effects of aerobic physical activity on sleep and on markers of insulin resistance in breast cancer women; iii) the effects of short and prolonged exposure to cave environments on human physiology.

Ten scientific studies illustrated in my PhD thesis have already been published and two scientific studies have been submitted to a scientific journal. The list of all scientific papers that I co-authored in the course of my PhD are also reported in Appendix.

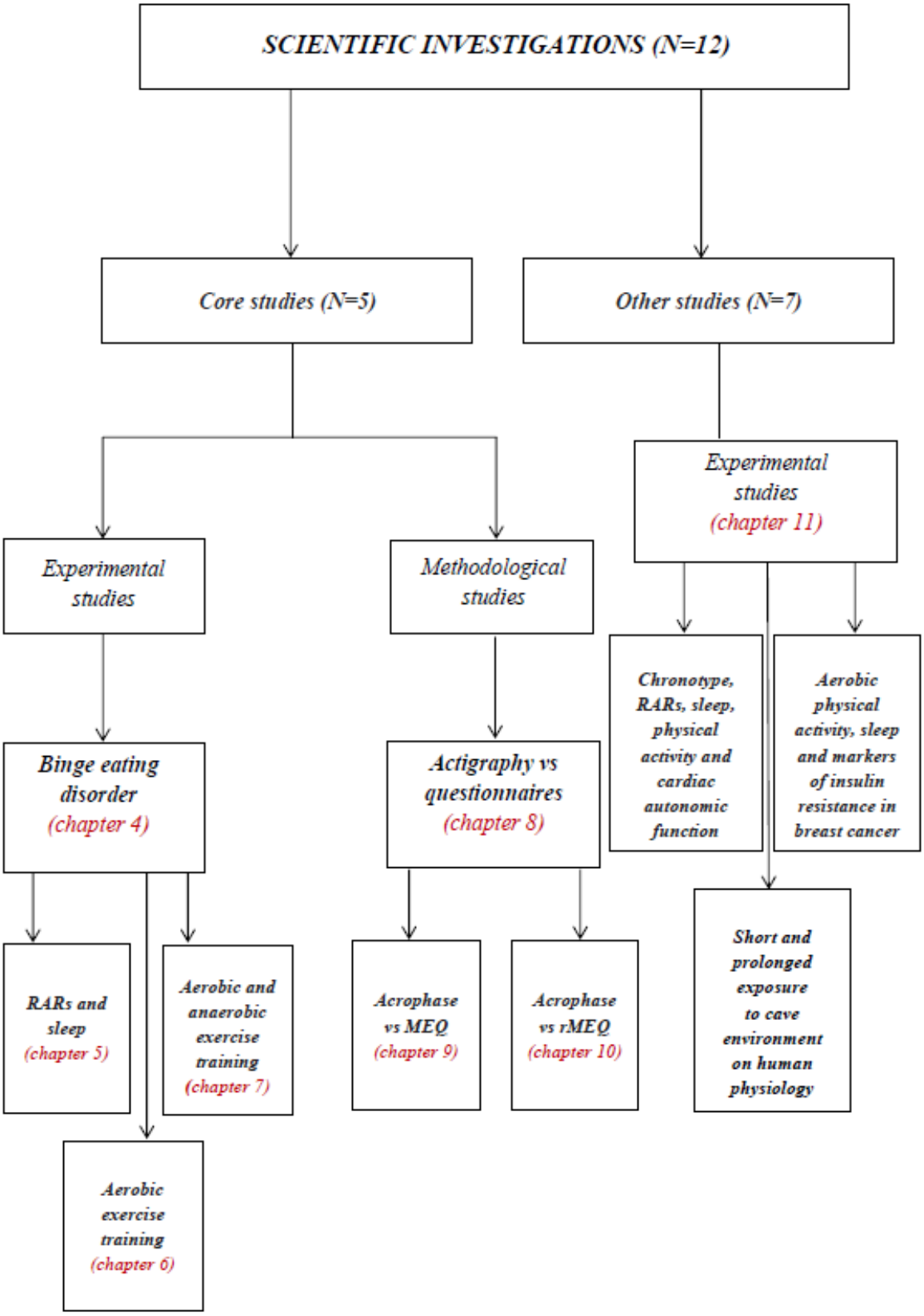

Figure 6. Conceptual map of 12 scientific investigations described in my PhD thesis.

4

ACTIGRAPHIC ASSESSMENT OF BINGE EATING DISORDER

One of the main topics in my PhD thesis is the investigation of the rest-activity circadian rhythms in patients with binge eating disorder (BED). This chapter is aimed to provide an overview of this pathology. Our main concerns were: rest-activity circadian rhythms, sleep and physical activity as an addition to the traditional treatment of BED.

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) in 2013 included the binge eating disorder (BED) as an independent diagnosis (American Psychiatric Association, 2013). BED is characterized by frequent and persistent episodes of eating large amount of food in a brief period followed by a loss of control and marked distress in absence of regular compensatory behaviours. The binge

episodes take place quickly without the physical necessity to eat and in solitude, due to the shame of own behaviour. These episodes are associated with feeling depressed, disgusted, or guilty after overeating (Javaras et al., 2008; American Psychiatric Association, 2013).

The binges seem to be induced by the difficulty in managing emotions and controlling impulses (Claes et al., 2006; Dingemans et al., 2006; Rosval et al., 2006) and represent an escape from an intolerable emotional state (Waller, 2002).

Elements that can trigger a binge are (Greeno et al., 2000):

- unpleasant feelings, such as a strong tension, anxiety and depression;
- feeling fat and a weight gain;
- the conduct of restriction and the consequent hunger: the deprivation of food, indeed, leads to justify an uncorrected behaviour that then results in binge;
- a strong and rigid dietary rules that exclude an increasing number of food;
- lack of adequate organization of the own time;
- loneliness and boredom;
- premenstrual tension;
- the use of alcohol that by loosening self-control makes it more vulnerable and affects the ability to judge with an underestimation of the consequences.

To make a correct diagnosis of BED the disorder must occur at least one day a week for 3 months. Unlike bulimia nervosa, in this disease to measure the frequency of binges is important to consider the days when the behaviour occurs rather than the number of individual episodes because the absence of compensation methods generates difficulty in recognizing the beginning and the end of overeating episodes.

Referring to binge episodes that are as well characteristic symptom of bulimia, a diagnosis of BED is made only if the subject does not constantly use it to obviate the

excessive introduction of food. Some binge eaters occasionally use compensatory behaviours but what differentiates them from bulimic patients is that they do not have the pervasive need to immediately cancel the effects of binge regaining absolute control of their body weight.

During the binge episodes these subjects generally appreciate the smell, taste and consistency of food. A retrospective analysis indeed shows that the patients eat different food depending on their emotional state: salty and fat food when they are anxious while sweet and chocolate food when they feel depressed (Mitchell et al., 1999).

4.1 DSM-5 diagnostic criteria for binge eating disorder (American Psychiatric Association, 2013)

A. Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following:

1. Eating, in a discrete period of time (e.g., within any 2-h period), an amount of food that is definitely than what most people would eat in a similar period of time under similar circumstances.

2. A sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much on is eating).

B. The binge eating episodes are associated with three (or more) of the following:

1. Eating much more rapidly than normal.

2. Eating until feeling uncomfortably full.

3. Eating large amounts of food when not feeling physically hungry.

4. Eating alone because of feeling embarrassed by how much one is eating.

5. Feeling disgusted with oneself, depressed, or very guilty afterward.

C. Marked distress regarding binge eating is present.

D. The binge eating occurs, on average, at least once a week for 3 months.

E. The binge eating is not associated with the recurrent use of inappropriate compensatory behaviours as in bulimia nervosa and does not occur exclusively during the course of bulimia nervosa.

The level of severity is based on the frequency of episodes of binge eating:

Mild: 1-3 binge eating episodes per week.

Moderate: 4-7 binge eating episodes per week.

Severe: 8-13 binge eating episodes per week.

Extreme: 14 or more binge eating episodes per week.

4.2 Epidemiology and risk factors for binge eating disorder

BED is the most common eating disorder and occurs predominantly in females than in males. The average age of onset for BED is 23.3 years (Kessler et al., 2013) and it is observed across gender, racial and sexual orientation groups (Jennings et al., 2015).

Several factors have been associated with the development of BED. Indeed, body dissatisfaction in comparison to the internalized ideal generates dietary restraint and binge episodes (Dakanalis et al., 2015). As well the emotional states (depression, sadness, nervousness, distress tolerance) are associated to a greater likelihood of developing binge eating (Meule and Platte, 2015; Pearson et al., 2015) and the self-critical perfectionism, related to high standards for behaviour and performance, is associated with self-criticism and could generate eating disorders when it is performed below the own standards (Boone et al., 2014).

4.3 Rest-activity circadian rhythm and binge eating disorder

BED patients, characterized by reduced daily physical activity, could be a population with a higher risk to develop rest-activity circadian rhythms abnormalities. Our study (Roveda et al., 2018) is the first study that investigates this aspect through a comparison between women with BED and BMI-matched control group of women without BED diagnosis. There is a positive association between body mass index (BMI) and obesity although it is not a criterion for BED diagnosis. In fact, 65% of BED patients are obese (De Zwaan, 2001; Hsu et al., 2002; Hudson et al., 2007). Due to the presence of overweight and obesity, patients with BED may have a higher risk of motor inactivity compared to healthy individuals. Vancampfort and colleagues in a recent review (2013) show that the level of physical activity reported by obese individuals with BED has been evaluated to be 15% less than the level of a BMI-matched control group. In our study, while acrophase is not different between BED and control group, MESOR and amplitude resulted significantly different with MESOR and amplitude significantly lower in BED patients compared to control group. Specifically, disruptions of rest-activity circadian rhythms in BED cannot be ascribed to obesity per se because the participants of the two groups are all obese with similar BMIs but probably is related to disease-specific neuropsychological factors of BED (Roveda et al., 2018). Increasing evidence suggests that the feeling of loss of control over food is an important marker of eating disorder neuro-psychopathology. This signifies that self-regulatory difficulties play a fundamental role in the maintenance of BED. Self-regulation seems to be connected to executive functions that are responsible to reach the set goal and to correct behaviour (Kittel et al., 2017). Executive functions moderate the intention-behaviour link between physical activity and eating disorders (Hall et al., 2008). Deficit in executive functions are present in BED patients and this situation could have also

consequences on rest-activity circadian rhythms in these subjects (Aloi et al., 2015; Manasse et al., 2015).

Another consideration is the relationship between rest-activity circadian rhythms and abnormal eating patterns. It has been shown that the circadian timing and frequency of meals could influence the circadian rhythms. Specifically, food restriction in mice amplifies their circadian rhythms with an increase of amplitude when food intake is restricted to a single daily meal (Cornelissen and Otsuka, 2017). Probably the overeating and the alteration of long-lived food behaviour in BED patients may have generate a circadian amplitude reduction (Roveda et al., 2018).

4.4 Sleep behaviour and binge eating disorder

Evidence demonstrates that short sleep has been related to obesity in many populations including older adults (Cappuccio et al., 2008; Patel et al., 2008). Specifically, one study shows that variability in night-to-night sleep duration and increased time napping during the day are strongly associated with obesity (Patel et al., 2014). The relationship between sleep behaviour and body mass index (BMI) is crucial and bidirectional: a restriction in sleep duration can affect metabolic and nutritional balance of the body. Likewise, the obesity could enhance the risk of sleep disorders (Roveda et al., 2018). In this way, irregularity of sleep-wake habits may also lead to irregularity in eating patterns. Indeed, Baron's study (2011) demonstrates that young adults with later bed time consume more calories and are heavier. Irregular eating behaviours are also associated with metabolic syndrome (Sierra-Johnson et al., 2008) and both snacking between meals and skipping breakfast are predictors of increased weight (Coakley et al., 1998; Van Der Heijden et al., 2007; Timlin et al., 2008; Woo et al., 2008).

Relatively little is known about the relationship between poor sleep and increased risk of eating disorders. Nevertheless, several studies examine specifically the connection between sleep and binge eating disorder. Our study (Roveda et al., 2018) demonstrates that sleep debt in patients with BED is more dependent on obesity rather than the eating disorder: in fact, no significant differences are observed between women with BED and BMI-matched control group for all sleep parameters. In this study, the presence of BED does not symbolize an additional risk factor for sleep disorders and it seems that sleep irregularities could be related to overweight/obesity and to the lower physical activity levels. Another study (Vardar et al., 2004) evaluates sleep in obese patients with BED compared to obese patients without BED and to healthy controls. The subjects with obesity and BED have higher total score for the Pittsburgh Sleep Quality Index (PSQI) than subjects without BED who are obese or normal weight. This result indicates a poor quality of sleep for the obese patients with BED that report also increased sleep latency. Similar results are present in Tzischinsky's study (2000) in which self-reported data suggest a difference in daytime sleepiness and mid-sleep awakenings when comparing healthy control group to obese patients with and without BED diagnosis. In another study (Trace et al., 2012) poor and/or short sleep is associated with an increased risk of binge eating in a subsample of women from the Swedish Twin Registry. Indeed, women report not getting enough sleep, sleeping poorly, having problems falling asleep, feeling sleepy during work or free time, having disturbed sleep, waking too early and not getting enough rest and all these aspects are likely related to the presence of binge episodes. Additionally, it seems that people with higher BMI may be more likely to eat while waiting to initiate sleep or it may be the act of eating may influence one's ability to sleep (Yeh and Brown, 2014). So, the digestive process may slow cognitive functioning and biological processes, promoting sleep. In this way, eating at night may

become an effective sleep aid. However, there are also individuals for whom eating causes reflux and other negative health consequences that disturb sleep.

Aspen's study (2014) reports interesting findings about the appetite-regulating hormones, ghrelin and leptin, which work in tandem to signal hunger and then promote satiety, and that may be involved in the relationship between disrupted sleep and eating disorders. Leptin, which is released by adipocytes, increases fat mass and reduces appetite (Ahima et al., 2000; Chin-Chance et al., 2000), whereas ghrelin, a peptide produced predominantly by the stomach, stimulates appetite and decreases energy expenditure (Nakazato et al., 2001; Wren et al., 2001; Muccioli et al., 2002). Sleep deprivation is associated with decreased leptin and increased ghrelin levels (Spiegel et al., 2004; Taheri et al., 2004; Chaput et al., 2007; Schmid et al., 2008) and this condition generates a hormonal imbalance that increasing appetite and decreasing energy expenditure with consequent weight gain (Ahima et al., 2000; Chin-Chance et al., 2000; Wren et al., 2001). Another important component is represented by the stress that may be a common factor underlying sleep problems and binge eating. The key hormonal pathway that regulates the endocrine response to stress is the hypothalamic-pituitary-adrenal (HPA) axis (Tsigos and Chrousos, 2002). Cortisol secretion is positively associated with food intake in women (Epel et al., 2001; Newman et al., 2007) and increases in cortisol and abnormalities of the HPA axis are also related to sleep curtailment (Balbo et al., 2010). So, inadequate sleep may result in increased cortisol levels, which may raise hunger and susceptibility to binge eating. In addition, cortisol dampens the appetite-suppressive effects of leptin (Zakrzewska et al., 1997) and increases plasma ghrelin levels (Rouach et al., 2007; Lutter et al., 2008).

4.5 Treatment for BED: the role of physical activity

The traditional therapy for the binge eating disorder is based on cognitive-behavioural therapy (CBT), interpersonal therapy (IT) and nutritional and weight loss interventions. There are few studies that underline the importance for these patients to introduce changes in physical activity as an addition to the traditional treatment. It is known that BED is generally associated with both obesity and physical inactivity (Sherwood et al., 1999; Hrabosky et al., 2007; Vancampfort et al., 2014). Moreover, BED obese patients are also more sedentary than age- and body mass-matched obese patients without BED (Levine et al., 1996; Hrabosky et al., 2007). BED patients present a lower level of weekly physical activity participation and a lower physical self-perception that could be associated with a lower functional exercise capacity; they refuse physical activity for several barriers as social physique anxiety, health problems, compulsive issues, reduced level of fitness, lack of social support and limited access to facilities (Vancampfort et al., 2015).

Evidence shows that aerobic exercise and yoga have been proven to reduce BMI and binges (McIver et al., 2009; Vancampfort et al., 2013; Galasso et al., 2018). Unfortunately, debate exists about the amount (e.g., volume, frequency, intensity, and duration) and type (e.g., structured exercises vs lifestyle physical activity) of physical exercise needed to obtain physical and psychological benefits in BED (Vancampfort et al., 2013). However, specific physical therapy interventions in BED could assure broad beneficial results on the eating disorder, depression, and body mass index (BMI) (Pendleton et al., 2002).

It is known that the traditional treatment for BED constituted by CBT, combined with focused walking, allows a reduction in both binge episodes and weight in BED obese women (Levine et al., 1996). In fact, training exercise combined with CBT is more

efficient in reducing BED symptoms than CBT alone but it is still not clear how (Martinsen and Stephens, 1994; Vancampfort et al., 2013). For instance, our study (Galasso et al., 2018) shows that changes in BMI and aerobic capacity of BED patients induced by a combination of aerobic exercise training and traditional treatment are significantly greater than those with the traditional treatment alone.

In addition, the combination of diet and exercise in conjunction with CBT could likely be more beneficial for weight loss in BED patients (Jeffery et al., 1983), constituting the best way to ameliorate psychological state: depression, anxiety and eating disorders scores show an important decrease compared to results in people who follow the CBT alone or the CBT with only the addition of the nutritional program (Fossati et al., 2004). Levine and colleagues (Levine et al., 1996) show that patients following a 24-week walking program have lower depressive scores and are abstinent from binges. Another study (Pendleton et al., 2002) demonstrates that a 4-months program combining CBT with aerobic exercise results in less depressive symptoms and less binges per week than CBT alone. Adding physical activity may facilitate abstinence from binges through psychological pathways related to the recreational nature of the activity itself. Patients who exercise report indeed to experience less depressive symptoms. In a recent study (Deboer et al., 2012), physical activity seems to buffer the effects of anxiety sensitivity on binges; the relation between anxiety sensitivity and binges is significant among individuals with low levels of physical activity.

Final preamble

I will describe in the following Chapters three experimental studies performed in BED patients during my PhD course. Two of these studies have already been published while the third has been submitted to a scientific journal.

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5

ANALYSIS OF REST-ACTIVITY CIRCADIAN RHYTHM AND SLEEP BEHAVIOUR IN BINGE EATING DISORDER

1. INTRODUCTION

Rest-activity circadian rhythms (RARs) are involved in the control of the sleep-wake cycle and of other numerous pathophysiological functions (Mormont et al., 2000; Paudel et al., 2010). RARs generally worsen with age, including decreased amplitude, loss of rhythm (Kripke et al., 2005), decreased adaptability to phase resetting signals (Hofman and Swaab, 2006), and delayed phase (Chaudhury et al., 2005). Altered RARs increase the risk of adverse pathological events and its amelioration could provide a tool for health protection.

Although obesity is not a criterion for BED diagnosis, a positive association between body mass index (BMI) and BED can be observed (De Zwaan, 2001; Hsu et al., 2002;

Hudson et al., 2007). Due to the presence of overweight and obesity, patients with BED, characterized by reduced daily physical activity, may have a higher risk of motor inactivity compared to healthy individuals. The circadian RARs have never been specifically investigated in patients with BED and an actigraphy-based monitoring of daily physical activity may help to identify high-risk patients with disrupted RARs.

Another feature of BED is an altered sleep behaviour. Several studies investigated this relationship observing that the nature of this association is complex and probably related to the overweight/obesity (Cappuccio et al., 2008; Dixon et al., 2001; Di Iorio et al., 2012; Patel & Hu, 2008; Sally & Brown, 2014): high BMI in patients with BED has been shown to be related to shorter sleep duration and increased sleep latency (Vardar et al., 2004; Yeh and Brown, 2014).

2. AIM OF THE STUDY

The aim of the current study was to assess RARs and sleep parameters in patients with BED through actigraphy compared to a BMI-matched control group (Ctrl). The comparison of the outcomes with those from the BMI-matched Ctrl will allow us to recognize possible factors underpinning RARs disruption and sleep disorders other than those related to lower physical activity.

3. METHODS

Participants

A total of 16 women volunteered to be part of the study. Eight of them were patients with BED and other eight participants acted as sex-, age-, and BMI-matched Ctrl. After full explanation of the purpose and of the study methodologies, all participants signed an informed consent form.

Exclusion criteria for all participants included significant cardiovascular, neuromuscolar, endocrine, and psychiatric disorders (e.g., schizofrenia, major depression, past history of psychiatric disorders), any presence of pharmacological therapy, diagnosis of obstructive sleep apnea, pregnancy, and nursing. People with genetic obesity, based on an accurate family history inquiry by a physician, were also excluded (Herrera and Lindgren, 2010). Anthropometric, physiological and blood chemistry parameters are listed in Table 1.

	BED	***Ctrl***
Age (years)	55.7 ± 15.6	60 ± 2.4
BMI (kg/m²)	31.3 ± 1	31.6 ± 0.7
Systolic Blood Pressure (mmHg)	126.4 ± 9.9	124.2 ±6.2
Diastolic Blood Pressure (mmHg)	65.9 ± 11.5	64.8 ± 8.4
Heart Rate (bpm)	64 ± 4.1	66 ± 7.8
Glycemia (mg/dl)	82.5 ± 7.6	84.3 ± 6.8
Total Cholesterol (mg/dl)	217 ± 25.3	216.6 ± 24.1
HDL (mg/dl)	61 ± 9.1	61.2 ± 12.6
LDL (mg/dl)	135 ± 40.1	133.6 ± 36.6
Triglycerides (mg/dl)	105.3 ± 39.6	104.4 ± 36.8

Table 1. Anthropometric, physiological and blood chemistry parameters for the BED and Ctrl group (mean ± SD). The two groups resulted homogeneous for all parameters (Student's t-test)**.**

Binge Eating Disorder Group (BED)

The inclusion criteria were: females of age from 18 to 75 years, BMI ≥ 30, diagnosis of BED made by a psychiatrist using the DSM-5 criteria.

Participants were recruited from the outpatients clinic of the Department of Clinical Neuroscience, San Raffaele Scientific Institute Milan. Thirty women with BED diagnosis who partecipate in the weekly multidisciplinary program (a combination of psychological, nutritional and behavioural treatment) were evaluated. Twenty-two women were excluded in relation to the presence of, at least, one of the exclusion criteria. The other 8 women, responding to the inclusion criteria, were enrolled for the study. During the multidisciplinary treatment, all women were in day hospital care, with meals provided by the hospital. All BED patients kept a diary to record the number of binge eating episodes.

Control Group (Ctrl)

The inclusion criteria were: females aged from 18 to 75 years, BMI $\geq$ 30, lack of BED diagnosis. Participants were recruited from the San Raffaele Scientific Institute Milan.

Study design

In November 2015 all participants agreed to participate in the present study. All participants were requested to:

1. undergo a clinical visit to obtain: a) height and body mass to calculate BMI (kg/m^2); height and body mass were measured without shoes and heavy clothes; b) systolic, diastolic blood pressure (mmHg), and heart rate (bpm) using an electronic sphygmomanometer (DynaPulse 5000A Pulse Metric, Inc.); blood pressure was measured in triplicate (every ten minutes), using an arm cuff on the left arm after 10 minutes' rest in a supine position.
2. to allow a blood sample to be taken in order to measure glycemia (mg/dl), total cholesterol (mg/dl), high density lipoproteins (HDL, mg/dl), low density

lipoproteins (LDL, mg/dl) and triglycerides (mg/dl). Blood samples were collected after overnight fasting between 8 a.m. and 9:30 a.m.

3. undergo 5 consecutive days actigraphic monitoring (week days) to detected the RARs and sleep parameters. The actigraph (MotionWatch 8®, CamNtech, Cambridge, UK) was worn on the non-dominant wrist; the participants were instructed to remove them when swimming or bathing. A diary was given to record information about bed time, get up time, times and length of naps, periods when the actigraph was not worn and number of nocturnal awakenings. All participants completed the actigraph monitoring between November and December. Actigraphic monitoring started for all subjects during the first clinical visit.

The study was carried out in accordance with the 1964 declaration of Helsinki and was formally approved by the Ethics Committee of the S. Raffaele Hospital of Milan (Italy). The study protocol and procedures complied with the guidelines required by the journal (Portaluppi et al., 2010).

Experimental procedures

Assessment of RARs

The Actiwatch Software was used to obtain the activity data, expressed in activity counts and recorded every single minute over the entire length of monitoring (5 days). In order to determine the RARs, the activity data provided by the actigraph were analysed using the single cosinor method (Halberg et al., 1977; Nelson et al., 1979) identifying the three parameters characteristic of each statistically significant rhythm: MESOR (M), Amplitude (A) and Acrophase (ϕ). The rhythmometric parameters of activity levels were then processed with the average of population mean cosinor. This

method evaluates the rhythmometric characteristics of the activity levels of the population (Nelson et al., 1979).

In the current study, RARs were monitored non ivasively using an actigraph (MotionWatch 8®, CamNtech, Cambridge, UK). Rest-activity data were recorded during the weekdays for each participant.

Assessment of sleep parameters

In each participant, we considered seven sleep parameters for further analysis; all these parameters were calculated automatically by the Actiwatch Sleep Analysis Software.

1) *Sleep onset (S-on)*: the start of sleep derived automatically using the Actiwatch Sleep Analysis Software (expressed in hours and minutes).
2) *Sleep offset (S-off)*: the end of sleep derived automatically using the Actiwatch Sleep Analysis Software (expressed in hours and minutes).
3) *Sleep duration (SD):* the difference in hours and minutes between the Se and Ss.
4) *Sleep latency (SL)*: the period of time required for sleep onset after retiring to bed. SL is the period between bedtime and Ss and it is calculated by an algorithm, based on lack of movement following bedtime.
5) *Movement fragmentation index (MFI)*: the addition of the Movement Index (percentage time spent moving) and the Fragmentation Index (percentage of immobile phases of one minute). MFI was used as an index of restlessness.
6) *Immobility time (IT)*: the total time, expressed in hours, spent without recording any movement within the period of Ss and Se.
7) *Sleep efficiency (SE)*: the percentage of time in bed actually spent sleeping.

For each participants, the sleep parameters were calculated over a period of five nights.

Statistical Analysis

The normality of the distribution of each parameter was checked by graphical methods and by the Shapiro-Wilk's test. The variables under study were normally distributed. Student's unpaired t-test was used to compare anthropometric, blood chemistry, and sleep data between BED and Ctrl. The significance was set at $p < 0.05$.

The rhythmometric parameters of BED and Ctrl were compared using the Hotelling T^2 test. The Hotelling T^2 test, a generalization in the multivariate field of the Student's *t* test, allows the verification of the hypothesis that the distance between the mean vectors of two samples is nil. Statistical analysis was carried out with time series analysis-series cosinor 6.0 by expert soft technology.

We investigated the correlation between anthropometric, rhythmometric and sleep parameters with the Pearson Correlation Coefficient (r).

4. RESULTS

No significant differences were observed in anthropometric, cardiovascular and blood chemistry parameters of the two groups (Table 1). Women of both BED and Ctrl were obese, with normal resting cardiovascular and blood chemistry parameters. None of the women in the BED group reported episodes of binge eating in the week before and during the actigraphic monitoring. All subjects completed the 5-day monitoring.

Rest-activity circadian rhythms

The single cosinor method revealed statistically significant RARs ($p<0.001$) in all participants. The population mean cosinor applied to BED and Ctrl revealed the presence of a significant circadian rhythm in both groups ($p<0.001$). Table 2 reports the rhythmometric parameters measured in the two groups.

Group	*PR (%)*	*p-value*	*MESOR (a.c.) (mean ± 95% CL)*	*Amplitude (a.c.) (mean ± 95% CL)*	*Acrophase (hr:min) (mean ± 95% CL)*
BED n=8	48	<.001	170.00 [160.16 - 179.84]**	157.66 [121.79 - 195.14]*	14:54 [14:03 - 15:56]
Crtl n=8	47	<.001	301.60 [275.59 - 327.61]**	238.19 [156.03 - 320.35]*	15:06 [13:43 - 16:30]

Table 2. Population Mean Cosinor in BED and Ctrl group. The Hotellig T^2 test, comparison between the two groups, showed a significant difference in MESOR and amplitude (*p< .05; ** p < .01). PR: percentage of rhythm; MESOR (activity counts); Amplitude (activity counts); Acrophase (hour : minutes).

While acrophase was not different between BED and Ctrl group (14:54 *vs* 15:06 hr:min in BED and Ctrl, respectively), the MESOR and amplitude resulted significantly different. The Hotelling T^2 test revealed the presence of a significant difference between BED and Ctrl. As shown in Table 2 and Figure 1, BED had MESOR (170,00 *vs* 301.60 a.c. in BED and Ctrl, respectively; p<.01) and amplitude (157.66 *vs* 238.19 a.c.in BED and Ctrl, respectively; p<.05) significantly lower compared to Ctrl.

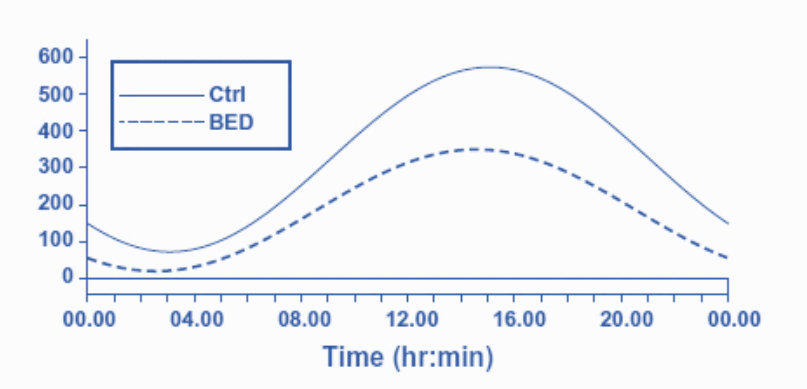

Figure 1. A comparison of rest-activity circadian rhythms in women with BED and BMI-matched control group (Ctrl) of women without BED diagnosis.

Sleep parameters

The results of the analysis of the sleep parameters are reported in Table 3. No significant differences were observed between the two groups for all parameters.

Sleep parameters	***BED***	***Ctrl***
S-on (hr:min)	23.22 ± 0.50	23.57 ± 0.49
S-off (hr:min)	07:36 ± 0.44	07:10 ± 0.30
SD (hr:min)	07:41 ± 0.40	07:04 ± 0.40
SL (hr:min)	00:20 ± 0.10	00:29 ± 0.20
MFI %	32.6 ± 5.3	33.4 ± 13.5
IT (hr:min)	6.58 ± 3.10	5.90 ± 7.90
SE %	80.7 ± 4.32	75.7 ± 8

Table 3. Sleep parameters for the BED and Ctrl group (mean ± SD). The two groups resulted homogeneous for all parameters (Student's t-test). Sleep onset (S-on), Sleep offset (S-off), Sleep Duration (SD), Sleep Latency (SL), Movement and Fragmentation Index (MFI), Immobile Time (IT), Sleep Efficiency (SE).

The SE, the most widely used parameter to summarize the sleep quality, was 80.7% and 75.7% in BED and Ctrl, respectively. Both these values were lower than the cut-off (SE=85%) for clinically significant sleep disruption (Shutte- Rodin et al., 2008).

No significant correlations were found among anthropometric parameters, rhythmometric and sleep parameters.

5. DISCUSSION

The main finding of this study was that RARs were different in BED as compared to Ctrl: patients with BED showed a lower MESOR and amplitude than Ctrl. These results provide the first experimental evidence of RARs disruption in patients with BED. These patients also displayed a sleep efficiency value below the normal cutoff, but they shared this abnormality with the obese Ctrl individuals. Thus, whereas sleep disorders could be reasonably ascribed to overweight/obesity, RARs disruption was a feature specific to BED pathology.

The acrophase was similar in BED and Ctrl (14:54 *vs* 15:06 hr:min in BED and Ctrl, respectively), thus indicating that, in this study, BED does not lead to an acrophase shift with respect to obese control group. Given that a delayed acrophase beyond 15:51 hr:min has been associated with a significant increase in neurodegenerative and cardiovascular diseases (Tranah et al., 2011), our findings suggest that both the BED and Ctrl obese participants did not have such risk factors. Referring to the other two rhythmometric parameters, patients with BED exhibited significantly reduced levels of MESOR and amplitude with respect to Ctrl (Table 2). Consequently, the dysfunctional RARs found in BED cannot be attributed to obesity *per se* because the participants of the two groups were all obese with similar BMIs (31.3 ± 1.0 *vs* 31.6 ± 0.7 Kg/m^2 in BED and Ctrl, respectively). Thus, we speculate that the RARs disruption in BED may be related to disease-specific neuropsychological factors. Few studies assessed the relationship between cognitive function and altered eating behaviour and several cognitive deficits have been described in patients with eating disorders (Duchesne et al., 2004; Jauregui-Lobera, 2013). Focusing on BED patients, increasing evidence suggests that the feeling of loss of control over eating is a salient marker of eating disorder neuropsychopathology (Kittel et al., 2017). This implies that self-regulatory difficulties

play an important role in the maintenance of BED. Self-regulation seems to be related to executive functions (such as inhibition, cognitive flexibility, and decision-making) that are capacity to reach the set goal and situationally to adjust behaviour (Kittel et al., 2017) and BED patients have a deficit of executive functions (Aloi et al., 2015; Manasse et al., 2015). In addition, obese patients with BED perceive themselves as less competent in undertaking physical activity than obese people without BED.

The detrimental effects of physical self-perception and the reduction of executive functions in BED could play a key role in determining a pathological reduction in diurnal physical activity, which was reflected in our study as a significant reduction in both MESOR and amplitude by actigraphy.

Another consideration concerns the relationship between RARs and abnormal eating behaviour. It has been shown that the circadian timing and frequency of meals could affect the circadian rhythms; in particular, food restriction in mice amplifies their circadian rhythms and the circadian amplitude is generally increased when food intake is restricted to a single daily meal (Cornelissen and Otsuka, 2017): we could speculate that overeating and the alteration of long-lived food behaviour in BED patients may have contributed to circadian amplitude reduction.

The presence of BED did not result in a sleep deterioration compared with obese Ctrl. Both groups, indeed, obtained sufficient sleep. Considering the SD and SL standard values (SD≥7 hours; SL≤30 min), both groups spent about 7 hours in bed (7:41±0.4 *vs* 7:04±0.4 h in BED and Ctrl, respectively) and presented a SL (20±0.1 *vs* 29±0.2 min in BED and Ctrl, respectively) within the reference values (Ohayon et al., 2017). Also sleep quality, as measured by sleep efficiency, was not different in the two groups (SE: 80.7±4.32 *vs* 75.7±8% in BED and Ctrl, respectively) but lower than 85%, that is the cut-off value for clinically significant sleep disruption (Shutte-Rodin et al., 2008).

Therefore, both BED and Ctrl exhibited almost a 5-10% drop in SE below the cut-off. The reduced sleep quality that BED share with the Ctrl group is probably related with obesity *per se*: the presence of BED does not represent an additional risk factor for sleep disorders.

Several studies have found clear evidence of a correlation between sleep duration and BMI (Lauderdale et al., 2009; Patel et al., 2006; Taheri et al., 2004). Furthermore, the relationship between sleep behaviour and BMI could be considered bidirectional: a restriction in sleep duration can interfere with metabolic and nutritional balance of the body. Likewise, the obesity could increase the risk of sleep disorders. Previous studies demonstrated that only one week of sleep curtailment increases appetite and food intake (Brondel et al., 2010; Omisade et al., 2010; Spiegel et al., 2004). Concurrently, epidemiologic studies revealed that among habitually short sleepers the prevalence of obesity is higher (Cappuccio et al., 2008). The results of our study did not shed more light on this relationship; we can therefore only assume that the shortage of sleep debt in patients with BED has more to do with obesity rather than the eating disorder.

6. CONCLUSIONS

The current study is the first actigraphy-based evidence of RARs disruption and sleep disorders in patients with BED. While sleep disorders could be reasonably ascribed to overweight/obesity and related to the lower daily physical activity, RARs disruption was specific to BED pathology. Actigraphy followed by analysis of both RARs and sleep quality qualifies as a novel, potential tool in the management of eating disorders.

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Rest-activity circadian rhythm and sleep quality in patients with binge eating disorder.

Roveda E., Montaruli A., **Galasso L.**, Pesenti C., Bruno E., Pasanisi P., Cortellini M., Rampichini S., Erzegovesi S., Caumo A. and Esposito F. (2018)

Chronobiology International, 35(2):198-207

This publication is also reported in Appendix (publication n° 9).

6

RELATIONSHIP BETWEEN AEROBIC EXERCISE TRAINING AND PHYSICAL PERFORMANCE IN BINGE EATING DISORDER

1. INTRODUCTION

Although obesity is not a criterion for BED diagnosis, a strong positive correlation between weight and BED has been observed (Hudson et al., 2007; Javaras et al., 2008; Yager, 2008) and in addition, the level of physical activity reported in BED is about half the level of obese individuals (Levine et al., 1996).

A recent review on physical activity in BED highlighted the fact that combined aerobic and yoga exercise training can reduce weight through physiological reactions to increased calorie expenditure and lead to a reduction of binge episodes (McIver et al., 2009). A specific exercise typology capable of inducing improvements in BED

symptomatology has not been found yet (Martinsen and Stephens, 1994; Di Iorio et al., 2012) but would ensure wide ranging benefits for the eating disorder, depression and body mass index (BMI) (Pendleton et al., 2002).

The traditional treatment for BED constituted by Cognitive-Behavioural Therapy (CBT), combined with focused walking, allows a reduction in both binge episodes and weight in BED obese women (Levine et al., 1996). On the other hand, the effectiveness of exercise programs has been shown to be hindered by the high rate of dropouts after six months (Fogelholm et al., 1999).

2. AIM OF THE STUDY

The aim of the current study was to investigate the effects of traditional BED treatment combined with aerobic exercise training, on weight and aerobic capacity compared to the effects of traditional treatment alone in patients with BED.

3. METHODS

Participants

Respecting the inclusion and exclusion criteria, we recruited fourteen patients diagnosed with BED for this study, three males and eleven females (American Psychiatric Association, 2013): six of them followed the traditional treatment combined with aerobic exercise training (Intervention Group, IG) and other eight participants followed the traditional treatment alone and served as the Control Group (CG). The inclusion criteria were: aged from 18 to 75 years, BMI ≥30, diagnosis of BED made by a psychiatrist using the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition criteria (DSM-5), treatment with a weekly multidisciplinary program and opportunity to exercise. The exclusion criteria were: pregnancy, nursing, genetic

obesity, presence of a severe current psychiatric condition necessitating residential psychiatry treatment in addition to the weekly multidisciplinary BED program.

The mean age of IG was 45 ± 16 years, with mean weight of 139 ± 22 kg and mean stature of 171 ± 10 cm (mean ± SD). The mean age of CG was 58 ± 13 years, with mean weight of 129 ± 28 kg and mean stature of 166 ± 9 cm (mean ± SD). All patients participated voluntarily in the study after being fully informed about the experimental protocol and procedures. A written informed consent was obtained before beginning the study, performed in accordance with the declaration of Helsinki and formally approved by the Ethics Committee of the S. Raffaele Hospital of Milan, Italy.

Experimental design

Anthropometric and exercise capacity evaluations

Stature and weight were measured to calculate body mass index (BMI, kg/m^2). Exercise capacity was measured using the Six-Minute Walk Test (6MWT, m).

All patients continued to participate into the weekly multidisciplinary program of S. Raffaele Hospital of Milan, constituted by Cognitive-Behavioural Therapy (CBT) and diet. In addition, the IG carried out aerobic exercise training for six months.

Six-Minute Walk Test (6MWT)

The 6MWT has been shown to be a reliable and valid test to assess the physical fitness of obese patients (Larsson and Reynisdottir, 2008; Beriault et al., 2009). It was performed according to the instructions by the American Thoracic Society (American Thoracic Society Statement, 2002).

Traditional BED treatment

The participants attended Cognitive-Behavioural Therapy (CBT) which was conducted by psychologist from the S. Raffaele Hospital and followed a diet prescribed by the nutritionist at S. Raffaele Hospital. The focus of traditional treatment procedure was to normalize the patient's incorrect eating behaviour (Pendleton et al., 2002).

Aerobic exercise training

IG attended aerobic exercise training over a period of six months. The exercise training programme was conducted by sport therapy experts and included four weekly sessions of 90-minute-long aerobic activity, such as brisk walking. Each training session also included 10 minutes of cool-down static stretches at the end of the session. The exercise practice session was conducted by the same trainer on set days and times during the six months.

Data Analysis

At baseline (PRE) and at the end (POST) of the six months of exercise training, all the participants underwent an anthropometric evaluation and an assessment of exercise capacity to evaluate if there were any differences between the traditional treatment alone and traditional treatment combined with aerobic exercise training.

Statistical analysis

The distribution of each parameter was tested for normality by Shapiro–Wilk's Test and all the variables under study were normally distributed.

Student's unpaired t-test was used to compare IG and CG in a PRE condition. The student's paired t-test was used to compare IG and CG anthropometric and exercise

capacity data between PRE and POST six months of aerobic exercise training (intragroup analysis). The significance was set at $p<0.05$. Finally, the student's unpaired t-test was used to compare the Δ values for IG and CG.

4. RESULTS

The results regarding BMI and exercise capacity in both two groups are summarized in Table 1 (mean ± SD).

	IG		***CG***	
	PRE	***POST***	***PRE***	***POST***
Weight (kg)	139 ± 22	125 ± 19 *	129 ± 28	122 ± 22 *
BMI (kg/m²)	47.2 ± 5.5	42.7 ± 5.1 *	46.6 ± 6.9	44.3 ± 6.1 *
6MWT (m)	435.9 ± 106.2	519.1 ± 151.5 *	455.5 ± 114.9	502.5 ± 110.7 *

Table 1. Anthropometric and exercise capacity parameters for the intervention (IG) and control (CG) group (mean ± SD) in two different time spans, before (PRE) and after (POST) six months of aerobic exercise training. * $p< 0.05$ vs PRE

BMI was calculated both in IG (n=6) and in CG (n=8) and in each group there were statistically significant differences (Table 1) (47.2 ± 5.5 kg/m² and 42.7 ± 5.1 kg/m² in IG PRE and IG POST, respectively; $p<0.01$; 46.6 ± 6.9 kg/m² and 44.3 ± 6.1 kg/m² in CG PRE and CG POST, respectively; $p<0.01$). The Student's paired t-test revealed statistically significant differences from PRE to POST condition. In fact, in each one of the fourteen participants, we found improvements at the physical test (Table 1): the distance covered by the IG had significantly increased after the training lasting six months (435.9 ± 106.2 m and 519.1 ± 151.5 m in IG PRE and IG POST, respectively; $p<0.01$).

The CG also showed an improvement in aerobic capacity from PRE to POST six months (455.5 ± 114.9 m and 502.5 ± 110.7 m in CG PRE and CG POST, respectively; $p<0.01$) probably because of the influence of diet and changes in body composition.

The Δ values relating to BMI of both two groups between baseline condition (PRE) and after six months of aerobic exercise training (POST) are shown in Figure 1.

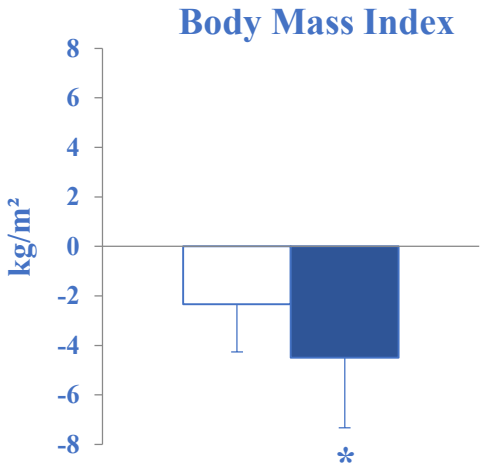

Figure 1. Δ values of body mass index (kg/m²) from PRE to POST conditions. Control Group, white column; Intervention Group, blue column. * $p< 0.05$

The IG, who followed the aerobic exercise training, obtained a greater reduction than CG (Figure 1): in fact there was a reduction of -4.50 kg/m² in IG and -2.30 kg/m² in CG; this reduction in BMI was statistically significant ($p<0.05$).

Figure 2 reports the Δ values regarding the 6MWT of both groups between baseline condition (PRE) and that after six months of aerobic exercise training (POST).

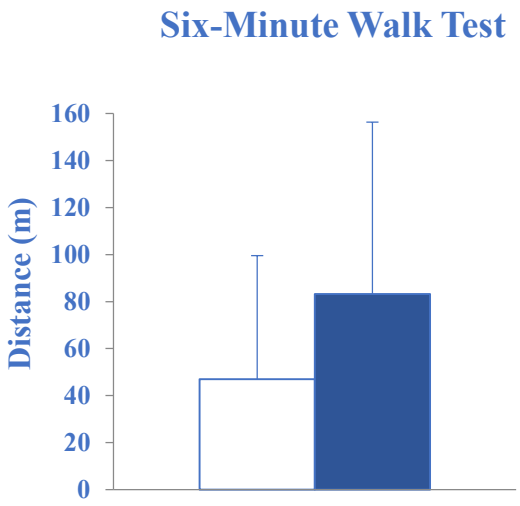

Figure 2. Δ values of Six-Minute Walk Test (m) from PRE to POST conditions. Control Group, white column; Intervention Group, blue column.

Referring to 6MWT (Figure 2), IG obtained greater results than CG: the improvement in IG was 83.2 m compared to 47 m in CG, who followed only the traditional treatment for BED, even though the difference didn't achieve statistical significance.

5. DISCUSSION

The focus of the current study was to investigate the combined effects of aerobic exercise training and traditional treatment on BMI and aerobic capacity compared to traditional treatment alone in patients with BED. The results showed that BMI decreased and aerobic capacity improved in both groups. However, BMI changes induced by the combined intervention were significantly greater than those induced by the traditional treatment alone, suggesting that the addition of physical exercise to the traditional BED treatment may elicit stronger effects on BMI via an increase in daily physical activity levels.

We observed an improvement in BMI in both groups. However, IG, which also experienced the aerobic exercise training programme, obtained a greater reduction than CG (Figure 1). The reduction in weight determined a change from serious third degree obesity to a moderate third degree obesity in CG. On the other hand, there was a shift from serious third degree obesity to a moderate third tending towards second degree obesity in IG, possibly because of the additional effect of aerobic exercise training (World Health Organization, 2000). When exercise is performed on a regular basis, loss in weight has been shown to occur on a long term basis and physical exercise generates very important physiological adaptations (Dovio et al., 2010; Roveda et al., 2011; Montaruli et al., 2012; Bruno et al., 2018), mainly involving the musculoskeletal system (Calogiuri et al., 2009; Montaruli et al., 2009; Gibala et al., 2012). Long-term, chronic adaptations occur also in the cardiovascular and respiratory systems. The physiological adaptations could possibly explain the significant improvement in aerobic capacity in the two groups examined. In fact, after six months of aerobic exercise training, the 6MWT increased in both IG and CG, probably because of the influence of diet and changes in body composition. As far as the 6MWT is concerned, IG obtained greater changes than CG who followed only the traditional treatment for BED, even though the difference between groups did not achieve statistical significance.

6. CONCLUSIONS

This is the first time that the capacity of exercise has been objectively measured in order to assess its value in the treatment of this kind of patient. In fact, the literature available shows the positive effects of physical exercise mostly in obese individuals. Only a few recent studies have evaluated the effects of physical exercise in binge eaters (Vancampfort et al., 2013), but the evaluation of physical activity was assessed only by

self-reported questionnaires and structured diagnostic interviews (Vancampfort et al., 2014). The addition of physical exercise to the traditional BED treatment may induce stronger effects on BMI by increasing daily physical activity levels in binge eaters and constitutes a novel and potential therapeutic approach to of eating disorders.

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Aerobic exercise training improves physical performance of patients with binge eating disorder.

Galasso L., Montaruli A., Bruno E., Pesenti C., Erzegovesi S., Cè E., Coratella G., Roveda E. and Esposito F. (2018)

Sport Sciences for Health, 14(1): 47-51

This publication is also reported in Appendix (publication n° 11).

7

RELATIONSHIP BETWEEN COMBINED AEROBIC AND ANAEROBIC EXERCISE TRAINING AND BEHAVIOURAL AND ANTHROPOMETRIC PARAMETERS IN BINGE EATING DISORDER

1. INTRODUCTION

BED obese reveals greater psychological problems (Grilo et al., 2013), increase in body mass, body dissatisfaction, lower self-esteem and an altered quality of life than obese-non-binge eaters (Javaras et al., 2008; Vancampfort et al., 2014). They develop depression, mood disorders, anxiety and substance abuse, and for these reasons, BED has been considered one of the most difficult psychiatric conditions to treat (Yager, 2008). The role of emotions and stress as a triggering cause is still under debate (Heatherton and Baumeister, 1991; Polivy and Herman, 1993; Striegel-Moore, 1993).

Cognitive-behavioural therapy (CBT) is the traditional BED treatment (Vocks et al., 2010), designed to identify and challenge maladaptive cognitions on eating and body mass thoughts. CBT focuses on stopping binges and not on weight loss (Vanderlinden et al., 2007; Vanderlinden et al., 2012).

Evidence suggests that training exercise combined with CBT is more efficient in reducing BED symptoms than CBT alone but is still not clear how (Martinsen and Stephens, 1994; Vancampfort et al., 2013). With some inconsistency (Hickson et al., 1988; Sale et al., 1990), aerobic and strength training is generally used to trigger health benefits and to increase physical performance (Wood et al., 2001; Izquierdo et al., 2004; Montaruli et al., 2012; Roveda et al., 2017). So far, the efficacy of the combination of aerobic and anaerobic training has never been assessed.

2. AIM OF THE STUDY

The aim of the current study was to investigate the effects of an addition of a combined aerobic and anaerobic exercise training (CAAET) to the conventional treatment in patients with binge eating disorder on anthropometric and psychological characteristics, aerobic capacity and muscle strength.

3. METHODS

Participants

Participants were recruited from Department of Clinical Neurosciences at IRCCS San Raffaele Turro in Milan, Italy. Based on the inclusion and exclusion criteria (Figure 1), the study sample involved 20 women with BED diagnosis using the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (American Psychiatric Association, 2013) and SCID-I (First et al., 1996). Ten of them carried out the

conventional treatment alone (CTRL), and other ten started with the combined aerobic and anaerobic exercise training (CAAET group). Inclusion criteria were: BED diagnosis, age 18-75 years, body mass index (BMI) ≥ 30 and capable to exercise. Exclusion criteria were: pregnancy, nursing, genetic obesity, and presence of a severe current psychiatric condition that required hospital recovery in addition to the weekly clinical BED program.

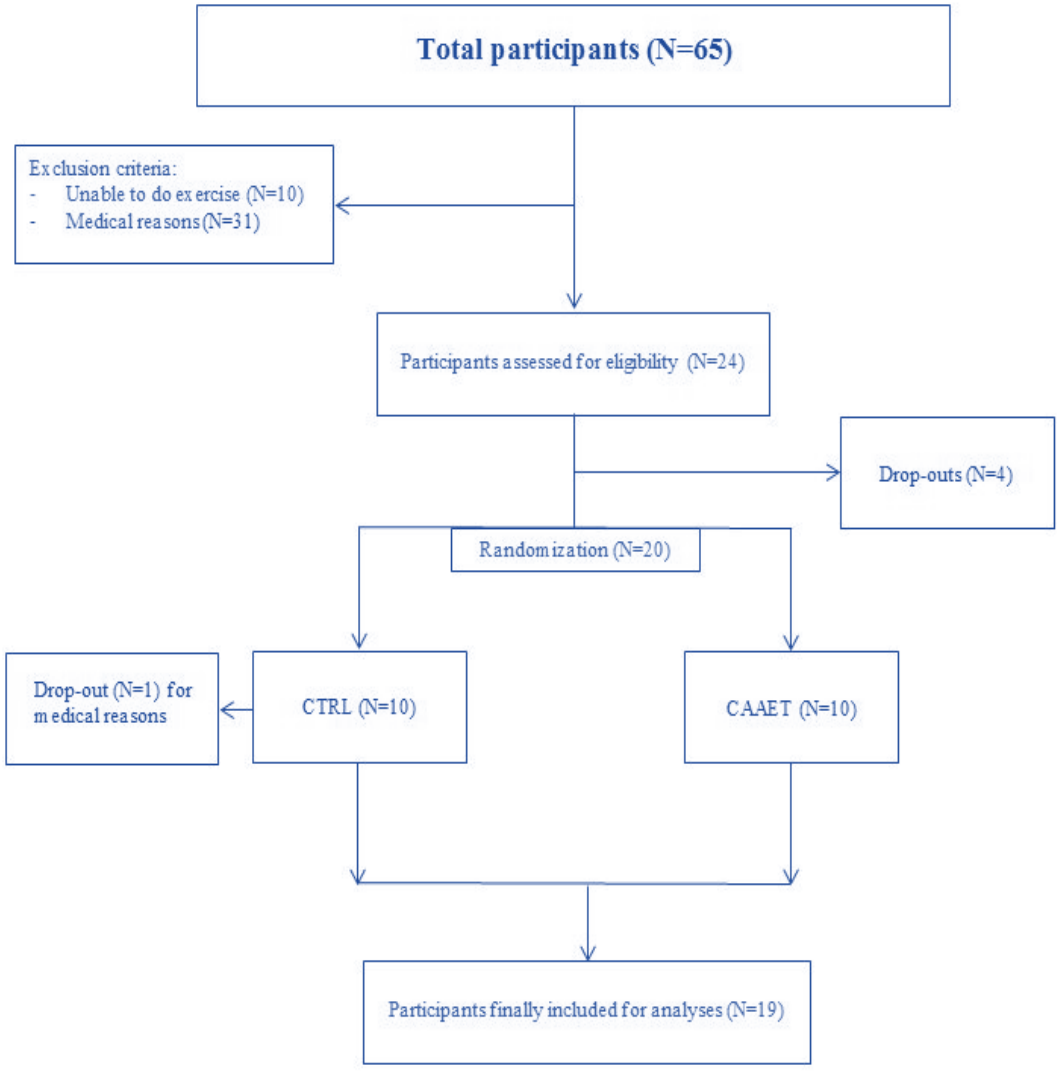

Figure 1. Flowchart for the study design and the participant's screening/selection according to the inclusion and exclusion criteria.

Table 1 shows the anthropometric characteristics of the patients who participated voluntarily in the study after being informed about the experimental protocol. A written informed consent was obtained before the beginning of the study, performed in accordance with the 1964 Declaration of Helsinki and approved by the Ethics Committee of the S. Raffaele Hospital, Milan, Italy, Protocol Number: TRDCA-01 (23/09/2014) (Galasso et al., 2018).

	PRE		***POST***	
	CTRL	***CAAET***	***CTRL***	***CAAET***
Age (years)	53 ± 13	54 ± 11	53 ± 13	54 ± 11
Body mass (kg)	107 ± 32	101 ± 21	102 ± 28*	87 ± 14*
Stature (cm)	168 ± 9	162 ± 6	168 ± 9	162 ± 6
BMI (kg/m²)	38 ± 10	38 ± 6	36 ± 9*	33 ± 3*

Table 1. Age and anthropometric characteristics (mean ± S.D.) in patients with BED in two different time spans, before (PRE) and after (POST) six months of combined aerobic and anaerobic exercise training.

CTRL, control group; CAAET, combined aerobic and anaerobic exercise training (intervention group). *p< 0.05 vs PRE.

Experimental design

All patients kept their individual weekly multidisciplinary therapy during the study, consisting of traditional BED treatment and a nutritional programme. Anthropometric values, psychological behaviour and exercise capacity were assessed before and after 6-month intervention, consisting of a combination of aerobic and anaerobic exercises (CAAET) in addition to the traditional therapy (CAAET group), or conventional therapy only (CTRL). Before engaging in the project, patients underwent three medical examinations. Firstly, anthropometric and psychological evaluations were made. During the second and third medical visits, patients performed the Six-Minute Walk Test (6MWT) and Squat Test (ST) on two different days, in random order, with 48 hours of rest in between. After 6-months of CAAET or CTRL, patients were evaluated again with the same procedures.

Experimental procedures

Anthropometric measurements

From stature and body mass measured in triplicate, BMI was calculated as the body mass in kilograms divided by the square of the stature in meters (kg/m^2).

Psychological evaluations

Binge Eating Scale (BES) is a psychometric test to evaluate eating behaviour, including emotional and cognitive symptoms. BES consists of 16 items meant to investigate the behaviour and mindset of the patient. Each answer is worth from 0 to 3 points and the total score is between 0 and 46: a score $\geq$ 17 indicates the possibility of suffering of BED (Gormally et al., 1982).

Bulimic Investigation Test Edinburgh (BITE) is a validated questionnaire which identifies compulsive eating behaviours and reveals possible compensatory conduct (vomit, abstinence from food, use of laxatives). BITE consists of 33 questions and is divided into two scales:

- Symptom Scale, which detects all kind of disorders;
- Severity Scale, which considers the danger of disorders.

As far as the Symptom Scale is concerned, a score ≥ 20 indicates the possibility of suffering from BED or Bulimia Nervosa; whereas a score between 10 and 19 means that the patient's eating behaviour is considered unusual, but not pathological; a score under 10, indicates that there are no eating problems.

As far as the Severity Scale is concerned, a score ≥ 10, signifies that the patient may be resorting to compensatory conduct; a score ≥ 5, indicates that the patient should be subjected to special medical examinations (Henderson and Freeman, 1987).

Exercise capacity assessment

At the beginning of Six-Minute Walk Test (6MWT), participants were asked to inform the trainers about pathological conditions that could interfere with physical performance. The 6MWT evaluate the physical fitness in obese patients (Larsson and Reynisdottir, 2008; Beriault et al., 2009) and followed the recommendations by the American Thoracic Society Statement (2002).

Squat Test (ST) was adopted to measure leg strength. The typical test pattern was requesting patients to bend and stretch their legs out beginning with a standing position where arms were folded across the chest and feet 20 cm apart. The objective was to reach a knee flexion of 90°. The score was calculated as the number of times that the

participant could rise from a "seated position" to a full stand within 30 seconds (Rikli and Jones, 1999; Morrow et al., 2000).

Intervention

Combined Aerobic and Anaerobic Exercise Training (CAAET)

The CAAET group attended the exercise programme for 6 months under the supervision of sport therapists; this included four weekly sessions of 90 minutes: 60 minutes of aerobic activity, such as brisk walking, and 20 minutes of exercises for muscle strength. Each training session included 10 minutes of cool-down static stretching at the end of the activity. The participants all followed the same exercise prescribed (CAAET) and training sessions were supervised by the same trainer throughout the 6months.

Traditional BED treatment

The aim of cognitive-behavioural therapy (CBT) was to normalize the patient's incorrect eating behaviour. It was performed by psychologists; in addition, the participants followed a nutritional programme prescribed by a professional nutritionist (Vanderlinden et al., 2012).

Data Analysis

In both groups the anthropometric, psychological and exercise capacity values were expressed as mean ± SD.

Statistical analysis

For each group, we carried out a power analysis that focused on the ability of the study to detect changes in BES, the most clinically meaningful parameter of eating disorders. To perform the power analysis, we needed an estimation of the Δ variability of BES. In our previous data, the subjects who underwent the BED traditional treatment showed a Δ of 8 ± 3 (mean ± SD), and thus we set SD= 3.

We decided that magnitude of the clinical difference in question as regards BES between the two groups was 5. By setting the power (1 - β) to 0.80 and the significance level α to .05 (2-tailed), we found that the sample size for each group had to be approximately 7 subjects. The power analysis was performed using the sample size calculator developed by Russel Lenth and available at http://www.stat.uiowa.edu/~rlenth/Power. In case there should be a significant departure of the data from the Gaussian distribution (thus leading to the use of a non-parametric test to establish the difference between the two groups), we added an addition 15% to the sample size calculated for the pooled t test (Lehmann, 1998). At the end, we obtained a sample size of 10 subjects per group. Since one of the recruited control subjects had to leave the experimental protocol due to unexpected medical reasons, the final sample size became 9 CTRL and 10 CAAET.

SPSS 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) was used to analyze all the variables. The distribution of each parameter was tested for normality by the Shapiro-Wilk's test. Because the investigated variables were not normally distributed, the Mann-Whitney non-parametric statistics test was used to compare CAAET and CTRL at baseline and after 6 months of training.

Effect sizes was calculated by dividing the difference between mean Δ scores by the average pooled standard deviation of the Δ. Effect sizes were interpreted according to

the criteria supplied by Cohen (1977) (d= .2 small; d= .5 medium; d= .8 large). The level of significance was set at $\alpha < 0.05$.

4. RESULTS

In PRE, the groups resulted homogeneous and there were no statistically significant differences for all variables.

Body mass and BMI were assessed both in CAAET and in CTRL in PRE and POST condition. In either groups the intra-group analysis showed in POST statistically significant differences ($p < 0.05$) (Table 1); inter-group analysis showed no statistically significant differences but an increased trend for improvement in CAAET when compared with CTRL (Table 1).

The Δ values of body mass and BMI of both two groups between PRE and POST are shown in Figure 2. The CAAET obtained a statistically significant reduction in body mass and BMI ($p < 0.05$) than CTRL (Figure 2).

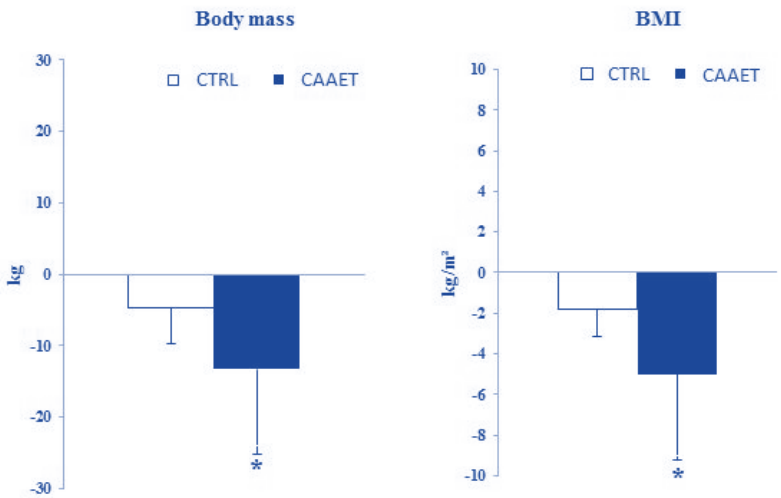

Figure 2. Δ values of anthropometric parameters in patients with BED before (PRE) and after (POST) six months of combined aerobic and anaerobic exercise training. CTRL, control group in white column; CAAET, combined aerobic and anaerobic exercise training (intervention group) in blue column. *p< 0.05.

Psychological evaluations were assessed both in CAAET and in CTRL in PRE and POST; in both groups the intra-group analysis showed in POST statistically significant differences ($p < 0.05$) (Table 2). Instead, inter-group analysis showed no statistically significant differences between the two groups (Table 2).

	PRE		***POST***	
	CTRL	***CAAET***	***CTRL***	***CAAET***
BES (score)	23 ± 9	23 ± 10	15 ± 7*	10 ± 8*
BITE Symptom (score)	14 ± 6	15 ± 7	9 ± 3*	7 ± 4*
BITE Severity (score)	9 ± 7	8 ± 7	6 ± 4*	3 ± 3*

Table 2. Psychological evaluation (mean ± S.D.) in patients with BED in two different time spans, before (PRE) and after (POST) six months of combined aerobic and anaerobic exercise training.

CTRL, control group; CAAET, combined aerobic and anaerobic exercise training (intervention group). *p< 0.05 vs PRE.

Figure 3 reports the Δ values of psychological questionnaires for both two groups in PRE and POST. Referring to BES, CAAET obtained greater results than CTRL, with a statistically significant difference ($p < 0.05$). Regarding the other psychological questionnaire BITE, there was an improvement in CAAET even though the difference did not achieve statistical significance.

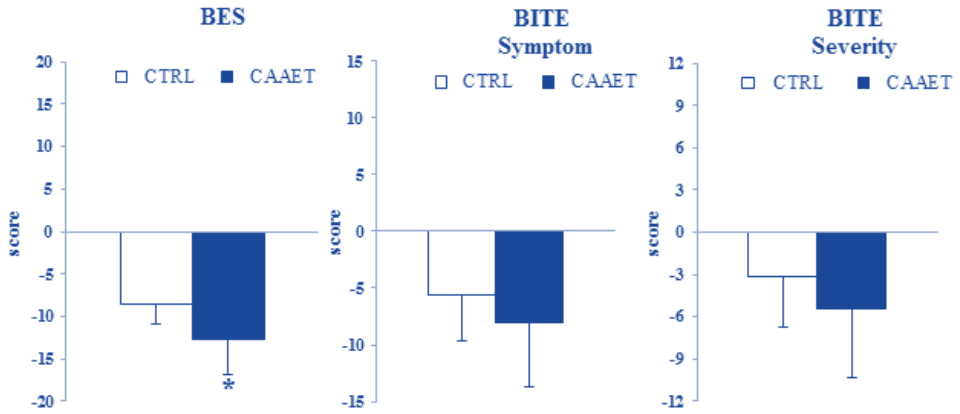

Figure 3. Δ values of psychological evaluation in patients with BED before (PRE) and after (POST) six months of combined aerobic and anaerobic exercise training.

CTRL, control group in white column; CAAET, combined aerobic and anaerobic exercise training (intervention group) in blue column. *p< 0.05.

Exercise capacity was assessed both in CAAET and in CTRL in PRE and POST; in both groups the intra-group analysis showed statistically significant differences ($p < 0.05$) in POST. The inter-group analysis showed improvements of aerobic capacity in CAAET: the distance covered increased significantly ($p < 0.05$) after the training intervention (Table 3).

	PRE		*POST*	
	CTRL	***CAAET***	***CTRL***	***CAAET***
6MWT (m)	450 ± 112	507 ± 74	520 ± 112*	612 ± 90* #
Squat Test (n°/30 s)	19 ± 6	22 ± 6	21 ± 7*	26 ± 5*

Table 3. Exercise capacity (mean ± SD) in patients with BED in two different time spans, before (PRE) and after (POST) six months of combined aerobic and anaerobic exercise training.

CTRL, control group; CAAET, combined aerobic and anaerobic exercise training (intervention group). *p< 0.05 vs PRE, #p< 0.05 vs CTRL.

Figure 4 reports the Δ values regarding exercise capacity of both two groups between PRE and POST. The CAAET obtained greater results in 6MWT and ST compared to CTRL but without reaching statistical significance.

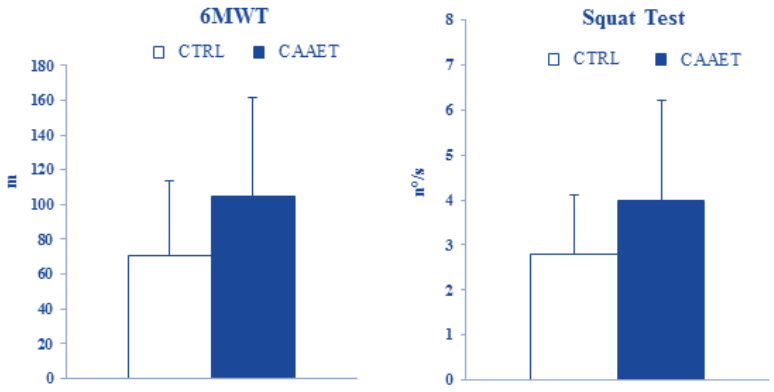

Figure 4. Δ values of exercise capacity in patients with BED before (PRE) and after (POST) six months of combined aerobic and anaerobic exercise training.

CTRL, control group in white column; CAAET, combined aerobic and anaerobic exercise training (intervention group) in blue column.

Effect sizes (ES) for body mass (d= 1.7), BMI (d= 1.5) and BES (d= 1.7) remained above the large range. ES for BITE Symptom (d= 0.6), BITE Severity (d= 0.6), 6MWT (d= 0.8), and ST (d= 0.7) remained in the large to medium-large range.

5. DISCUSSION

The current study analyzed the efficacy of an addition of a combined aerobic and anaerobic exercise training (CAAET) programme to the traditional treatment of anthropometric and psychological characteristics, aerobic capacity, and muscle strength in BED women. CAAET produced effects on anthropometric (body mass and BMI), psychological (BES) and exercise capacity (6MWT) although there were no changes in the CTRL. These findings suggest that adding an exercise training programme to the traditional BED treatment produces beneficial effects in BED women.

In POST, body mass and BMI, decreased in both groups (Table 1). These changes, which resulted from the inclusion of the physical exercise training intervention in the CAAET group, were greater when compared to those for the traditional treatment alone group, CTRL, even though the difference did not achieve statistical significance. In particular, the Δ analysis showed a significant reduction in body mass and BMI in CAAET women than CTRL (Figure 2).

The physical consequences of BED are due to a co-morbidity obesity and sedentary lifestyle. The weight loss in BED patients predisposes them to engage in a higher level of physical activity. In the literature available to date, this was associated with maintenance of weight loss over time in BED patients and with a reduction in binge eating (Mitchell and Perderson, 1995).

After the 6-month intervention, both groups showed improvements in psychological conditions (Table 2) obtaining greater scores in all questionnaires. Changes in

psychological response in CAAET achieved statistical significance only in terms of BES, nonetheless showing a trend towards greater change than CTRL as regards the other parameters. Referring to BES (Figure 3), the Δ analysis revealed that CAAET obtained greater results than CTRL in the reduction of binge episodes. Recent research has shown that abstinence from episodes of overeating is an important goal in the treatment of binge eating problems (Maddocks et al., 1992). Furthermore, the combination of diet and exercise in conjunction with CBT may likely be more beneficial for weight loss (Jeffery et al., 1983), in that it constitutes the best way to ameliorate psychological state. In agreement with our results, Pendleton and colleagues (2002) demonstrated that a physical activity program combined with CBT results in fewer depressive symptoms and binges per week than CBT alone. Physical activity seemed to buffer the effects of anxiety on binges (Deboer et al., 2012).

The exercise capacity (Table 3) improved in POST for both groups. A significant improvement in aerobic capacity in CAAET women was also observed: after 6 months of training, the distance covered during 6MWT was significantly longer (Table 3). Surprisingly, we didn't find the same result for muscle strength. The Δ analysis highlighted the fact that CAAET showed a tendency to higher scores than CTRL, even though the difference between the groups did not achieve statistical significance (Figure 4). Perhaps, physical activity programme was focused principally on brisk walking, the most natural and easiest activity to learn and perform, and only partly on strength exercises. This aspect could justify the significant improvement obtained only in aerobic capacity in CAAET women. In addition, BED patients presented a lower level of weekly participation in physical activities and lower esteem in physical perception of self, which could be correlated with a lower functional exercise capacity; they refused physical activity on the grounds of several barriers, such as physique-related anxiety,

health problems, compulsive issues, reduced level of fitness, lack of social support and limited access to facilities (Vancampfort et al., 2015). Overcoming these barriers is the first step toward participation in exercise training interventions.

Another feature of obese people is a relative reduction in skeletal muscle strength, reduced cardiorespiratory fitness (Hulens et al., 2003), gait inefficiency (Pataky et al., 2014) and an increased risk for physical co-morbidities (Ekman et al., 2013). On the other hand, exercise often represents the most accessible tool for weight management (Bond Brill et al., 2002) and for reduction of cardiovascular risk (Sandvik et al., 1993). The mechanisms by which aerobic exercise may provide cardio-protective benefits are multiple. One explanation could be the adaptations in autonomic control. As a consequence of aerobic training, indeed, sympathetic drive at rest is reduced and vagal tone is increased with potential effects on blood pressure, thrombosis, and other factors associated with coronary risk (Erikssen et al., 1998). Low levels of cardiorespiratory fitness, as expressed by low peak pulmonary oxygen uptake (VO_2), are associated with an increased risk of cardiovascular morbidity and mortality (Hood et al., 2011; Gibala et al., 2012; Venturelli et al., 2015). Strength training is able to generate peripheral adaptations leading to hypertrophy and other physiological adaptations (Zhang et al., 2014; Vitale et al., 2017a). However, a limited amount of data is available on the effectiveness of this training approach on the reduction of CVDs risk factors (Harber et al., 2009; Esposito et al., 2010). It is important to note that the physiological mechanisms activated by strength training are different to those involved in aerobic training in terms of central hemodynamics stimulation (Hautala et al., 2006; Vitale et al., 2017b). The limited heart rate (HR) and cardiac output responses are indeed counterbalanced by high stimulation of the peripheral circulation as during small muscle mass exercise (Coggan et al., 1992; Bonato et al., 2017).

Interestingly, aerobic training may lead to some gains in maximum strength (Hepple et al., 1997), while strength training may also improve aerobic capacity (Hikida et al., 2000), showing that some synergistic benefits of concurrent aerobic and strength training may also occur. Aerobic training, indeed, may also produce some stimuli for maximum strength improvements in healthy adults through muscle hypertrophy (Gibala et al., 2012) and remodeling of contractile properties of the muscle fibers (Hepple et al., 1997). Strength training has the capacity to improve peak VO_2 (Hikida et al., 2000) through increased capillarization (Hood et al., 2011; Venturelli et al., 2015).

6. CONCLUSIONS

The findings demonstrate that BED women belonging to the CAAET group obtain greater improvements in terms of quality of life and physical health, suggesting that adding combined exercise training to the conventional BED treatment may generate beneficial effects and is valuable in BED obesity. Future studies are required to further investigate the physical activity behaviour in BED patients, in particular, the role of strength training.

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This research project has led to the following publication:

Effect of combined aerobic and anaerobic exercise training on behavioral and anthropometric parameters in patients with binge eating disorder.

Galasso L., Montaruli A., Bruno E., Chiorazzo M., Ricceri A., Erzegovesi S., Caumo A., Roveda E. and Esposito F.

Submitted to *European Journal of Sport Science.*

This publication is also reported in Appendix (submitted paper n° 1).

8

RELATIONSHIP BETWEEN THE ACTIGRAPHY-BASED PARAMETERS OF CIRCADIAN RHYTHMICITY AND QUESTIONNAIRE-BASED PARAMETERS OF CIRCADIAN TYPOLOGY

The other main topic in my PhD thesis is the investigation of the relationship between the actigraphy-based assessment of circadian rhythmicity and the questionnaire-based assessment of circadian typology. This chapter is concerned with issues of a methodological nature. Our purpose was to establish whether a simple and low-cost assessment of circadian typology based on the use of validated self-administered questionnaires could substitute the expensive and labour-intensive assessment of circadian rhythmicity based on actigraphy monitoring.

The rationale underlying this research project was the observation that, although the estimation of circadian rhythms is primarily achieved by means of hand/wrist actigraphy, this method is expensive and labour-intensive. As a result, its widespread adoption is challenged. We wondered whether the assessment of circadian rhythmicity based on self-administered questionnaires could provide a simple and cost-effective predictor of actigraphic parameters. In this project we focused our attention on the Morningness-Evevningness Questionnaire (MEQ) by Horne and Ostberg (1976) and on its reduced version, rMEQ by Adan and Almirall (1991). We formulated the hypothesis that MEQ and rMEQ might be related to the circadian parametric profile provided by actigraphy. If this were the case, 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 or rMEQ score. We thus used both correlation and regression analysis with the objective of evaluating whether a linear regression formula using either the MEQ or its reduced version, rMEQ, would predict the actigraphy-based acrophase. As the reader will see in the two following chapters, our attempt was successful and we were able to work out a simple and practical prediction model of the actigraphy-based acrophase. The precision of the prediction of the acrophase yielded by the MEQ (or rMEQ) score was quantified by both the confidence and prediction limits surrounding the best-fit regression line. We found that the precision of the prediction was satisfactory.

The key message of this research project is that both MEQ and its reduced version rMEQ are suitable for the prediction of the actigraphy-based acrophase. Such a prediction method is convenient, simple to use and can be conducted quickly. It may prove useful when actigraphy-based measurement of the acrophase is not applicable in that it is too complex, costly or time-consuming. Other investigators can apply a similar,

general framework to their data and determine the specific regression parameters in order to yield accurate prediction for their chosen population of interest.

Final preamble

I will describe in the following Chapters the development of regression equations for MEQ and rMEQ, respectively.

These studies have already been published.

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9

THE MORNINGNESS-EVENINGNESS QUESTIONNAIRE SCORE CAN BE USED TO PREDICT THE ACTIGRAPHY-BASED ACROPHASE

1. INTRODUCTION

Hand-wrist actigraphy is being increasingly used to identify circadian rhythms (Ancoli-Israel et al., 2003). Actigraphy-based data yield a portrait of circadian rhythmicity entailing parameters such as the amplitude, the MESOR and the acrophase. Although the cost of actigraphy is progressively decreasing, its widespread adoption remains a challenge because actigraphy-based monitoring requires proper instruction and care, as well as the subject's compliance and collaboration. In addition, data recording has to be performed for at least seven consecutive days and data analysis has to be carried out by specialized software.

One alternative approach to assess the circadian typology of a subject is based on self-assessment questionnaires. The most used questionnaire is the Morningness-Eveningness Questionnaire (MEQ) by Horne and Ostberg (1976). 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). MEQ has been used extensively in the literature (Taillard et al., 2004; Jankowsky & Ciarkowska, 2008; Lee et al., 2014). It is not unreasonable to hypothesize that MEQ might be related with the circadian parametric portrait provided by actigraphy. In this case, one could use the easily measured MEQ score as a predictor of the expensive and labour-intensive actigraphic assessment.

2. AIM OF THE STUDY

The aim of the current study was to develop a simple linear model to predict the actigraphy-based parameters from the MEQ score.

3. METHODS

Participants

The data base consisted of the MEQ scores and the actigraphic parameters measured in 54 (27 males and 27 females) college students of the School of Sports Science of the University of Milan who were enrolled in the academic year 2013-2014. Their mean age was 22.1 ± 2.1 yrs. The study group consisted of 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). The available actigraphic parameters were the MESOR, the amplitude and 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. The

amplitude is the measure of one half the extent of the rhythmic variation in a cycle. The acrophase indicates the time when the highest value of the rhythm is expected.

Data analysis

The analysis of the data comprised three distinct phases that will be described below.

Correlation analysis

We considered the MEQ score and the three circadian parameters provided by actigraphy. The strength of the linear relationship between each pair of these four parameters was ascertained by calculating Pearson's correlation coefficient and displaying the data in a scatterplot matrix.

Development of a predictive model by linear regression

Since the correlation analysis showed a strong relationship between MEQ and the acrophase, we used simple linear regression (Kutner et al., 2004) to derive a linear expression yielding the acrophase as a function of MEQ. In this linear model, the MEQ score played the role of the predictor (i.e., independent) variable, whereas the acrophase played the role of the predicted (i.e., dependent) variable. The model assumed that, for a given value of MEQ, the acrophase was characterized by a gaussian distribution with a mean that was assumed to depend linearly on MEQ and constant variance σ^2.

$$acrophase = \mu(MEQ) + \epsilon = \alpha + \beta MEQ + \epsilon$$

where α and β are the intercept and the slope of the linear model, respectively, and ε is a random deviation around the mean having a normal distribution with zero mean and constant variance, σ^2. Parameters α and β, as well as the variance σ^2, were estimated from the experimental data by linear least squares and their estimates were denoted by

a, *b*, and mean square error, respectively. Precision of the estimated parameters was represented in terms of standard error, confidence interval and coefficient of variation for each parameter.

Statistical inferences concerning α and β were conducted testing whether or not $\alpha=0$ and $\beta=0$. In particular, by testing the slope of the regression line against 0, we were able to assess whether the MEQ score is a statistically significant predictor of the acrophase. Inferences concerning α and β were carried out using a t-test with n-2 degrees of freedom (the values of the t_{score} and the associated p-values were supplied by the statistical software).

Inference about parameter β was also performed using the analysis of variance (ANOVA). The analysis of variance approach to linear regression partitions the sum of squares and the degrees of freedom (df) associated to the Y variable (the acrophase). The breakdowns of the total sum of squares and associated degrees of freedom are displayed in the form of an ANOVA table supplied by the statistical software. The ANOVA table reports a test for the null hypothesis $\beta=0$ using a F ratio with (1, n-2) degrees of freedom, together with the associated p-value. This F-test is equivalent to the above-mentioned t-test (the F ratio is the square of the t_{score}). The ANOVA table also provides the mean square error (MSE), i.e., the sample estimate of the unknown variance (σ^2).

Provided that the null hypothesis $\beta=0$ is rejected, the estimated parameters *a* and *b* can be used to predict the acrophase for any particular subject for which the MEQ score is known. Such point estimate is accompanied by a confidence interval and a prediction interval (Kutner et al., 2004). The confidence interval measures the uncertainty about the mean value of the acrophase for a given value of the MEQ score. It has a confidence level (95% was used in the present study) and has a two-sided range with a lower and

upper bound. Like the confidence interval, the prediction interval has a confidence level and has a two-sided range. Unlike the confidence interval, the prediction interval predicts the spread for individual observations rather than the mean. Indeed, it measures the uncertainty associated to a new, individual observation of the acrophase for a given level of MEQ.

Diagnostics of the linear regression results

We evaluated the performance of the linear model and checked whether it provided an adequate fit of the sample data. Simple graphical methods based on the residuals, as well as on some formal statistical tests were employed. To visually determine how the linear model fitted the experimental data, the parameter estimates *a* and *b* were used to draw the regression line superimposed to the scatterplot diagram. Evaluation of the model performance was enhanced by plotting the residuals against the corresponding predicted acrophase values. Such residual plot provides an overall visual idea of whether the model adequately described the data and is useful to uncover systematic patters in the model predictions. If the model is appropriate for the data, the observed residuals should reflect the properties assumed for the random term, ε. In particular, we considered the use of residuals for graphically examining whether a nonlinear trend was appreciable, whether outliers or influential values were present, whether the error terms were normally distributed and with constant variance. The residuals were further evaluated by plotting their distribution by means of a histogram and a boxplot. These graphical approaches are particularly useful to detect asymmetries and outliers. In order to assess the normality of the residuals, the normal Quantile-Quantile (Q-Q) plot was derived.

To provide numerical indices of the model fit, we calculated the coefficient of determination, R^2, which coincides with the square of Pearson's correlation index and measures how close the data are to the fitted regression line. To ascertain how well the model could make new predictions on cases it has not already seen, we resorted to k-fold cross-validation (Maindonald & Braun, 2010). This technique uses the available data to mimic the process of generalizing to new data. It hinges on the idea of picking a small integer k (usually between 5 and 10) and divide the data at random into k equally-sized mutually-exclusive subsets (the subsets are commonly called *folds*). For each fold i (with i= 1, 2,…to k), the data belonging to the fold are removed from the data set, while the rest of the data are used as training set. Once the model has been identified from the training set, its performance is evaluated on the held-back data that act as the surrogate of a "new" testing set. Eventually, all the data in the dataset are used for both training and testing and the performances are averaged across the folds. In this study we subjected to cross-validation the acrophase vs. MEQ linear model with the purpose of checking whether the model was "stable", that is if the model could be applied to different samples from the same population without losing its predictive ability and without changing its best-fit parameters too much across samples. We used k=6 because this allowed to have a reasonably balanced amount of data in the training set (45) and in the testing set (9). The regression lines associated with the six folds were plotted on a single diagram that also displayed the data belonging to each fold. In addition, the results from the six folds were averaged to produce summary performance indices.

Software used for the data analyses

Pearson's correlation and linear regression analysis were performed using JMP®, Version 11 (SAS Institute Inc., Cary, NC, USA). The k-fold cross-validation approach

was performed using the R statistical software (freely available at *http://cran.r-project.org*). Specifically, we resorted to function *CVlm* (i.e., cross-validation for linear regression) belonging to package *DAAG* developed by John H. Maindonald and maintained by W. J. Braun (freely available at *http://cran.r-project.org/web/packages/DAAG/index.html*). A p-value less than 0.05 was considered statistically significant.

4. RESULTS

Table 1 shows the mean ± SD. of MEQ and of the three actigraphy-based circadian parameters (acrophase, MESOR and amplitude) stratified according to the chronotype are shown is Table 1. There were corresponding changes in MEQ and amplitude, but opposite (increasing) values for acrophase, as chronotype progresses from M- to N- to E-types.

Chronotype	*Sample size*	*Sex*	*MEQ score*	*acrophase (min)*	*MESOR*	*amplitude*
M	17	(8M, 9F)	66.2 ± 4.1	876 ± 56.6	266.3 ± 40.4	236 ± 57.4
N	18	(8M, 10F)	51.9 ± 3.3	948.4 ± 42.5	256.9 ± 40.1	232.9 ± 47.1
E	19	(9M, 10F)	34.1 ± 6.5	1056.2 ± 63.8	258.2 ± 49.5	213.8 ± 32.4

Table 1. MEQ score and actigraphy-based parameters stratified by chronotype: Morning-type (M), Evening-type (E), and Neither-type (N).

Figure 1 shows the scatterplot matrix of MEQ and the three circadian parameters.

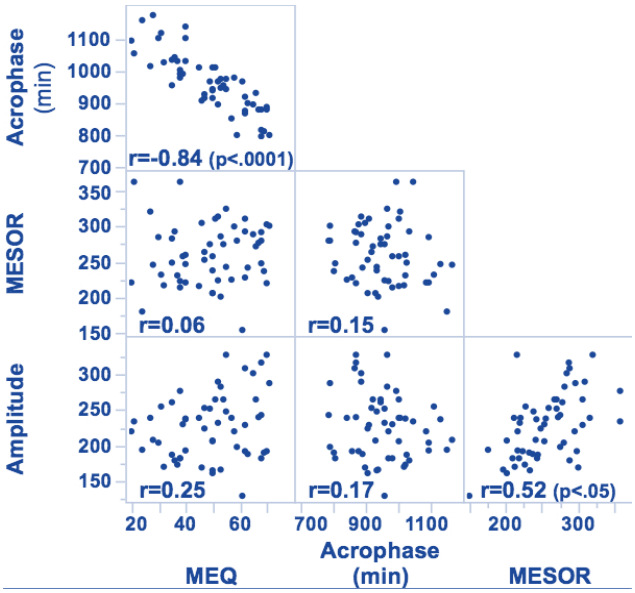

Figure 1. Scatterplot matrix, together with Pearsons's correlation coefficients, of the actigraphy-based parameters (Amplitude, MESOR, and Acrophase) and the MEQ score.

MEQ was inversely, strongly and significantly correlated with the acrophase (r=-0.84, p<0.0001).

This result motivated the subsequent linear regression analysis aimed to verify the possibility of using MEQ as a predictor of the acrophase. Of note is that MEQ was not correlated with MESOR and the amplitude.

The estimates of the slope and intercept of the regression line are reported below in Table 2.

Parameter	*Estimate*	*SE*	*Lower 95% CI*	*Upper 95% CI*	*CV*	t_{score}	*p-value*
Intercept (a)	1238.7	25.8	1186.9	1290.5	2.1	48	< 0.0001
Slope (b)	- 5.49	0.50	- 6.48	- 4.49	9.1	- 11.1	< 0.0001

Table 2. Linear regression results and regression coefficients together with measures of precision such as the standard error (SE), the lower and upper 95% confidence intervals (CI) and percent coefficient of variation (CV). The t_{scores} for testing the significance of the intercept and slope of the regression line against 0 are reported in conjunction with the corresponding p-values.

Both the regression parameters were precisely estimated and were significantly different from 0 ($p<0.0001$). The relationship between the acrophase and the MEQ score was represented pictorially by the scatterplot of the data together with the estimated regression line in the upper panel of Figure 2.

The equation of the regression line was:

Acrophase(min)= 1238.7 - 5.49* MEQ

This formula can be used to predict the acrophase of new subjects if their MEQ scores are known.

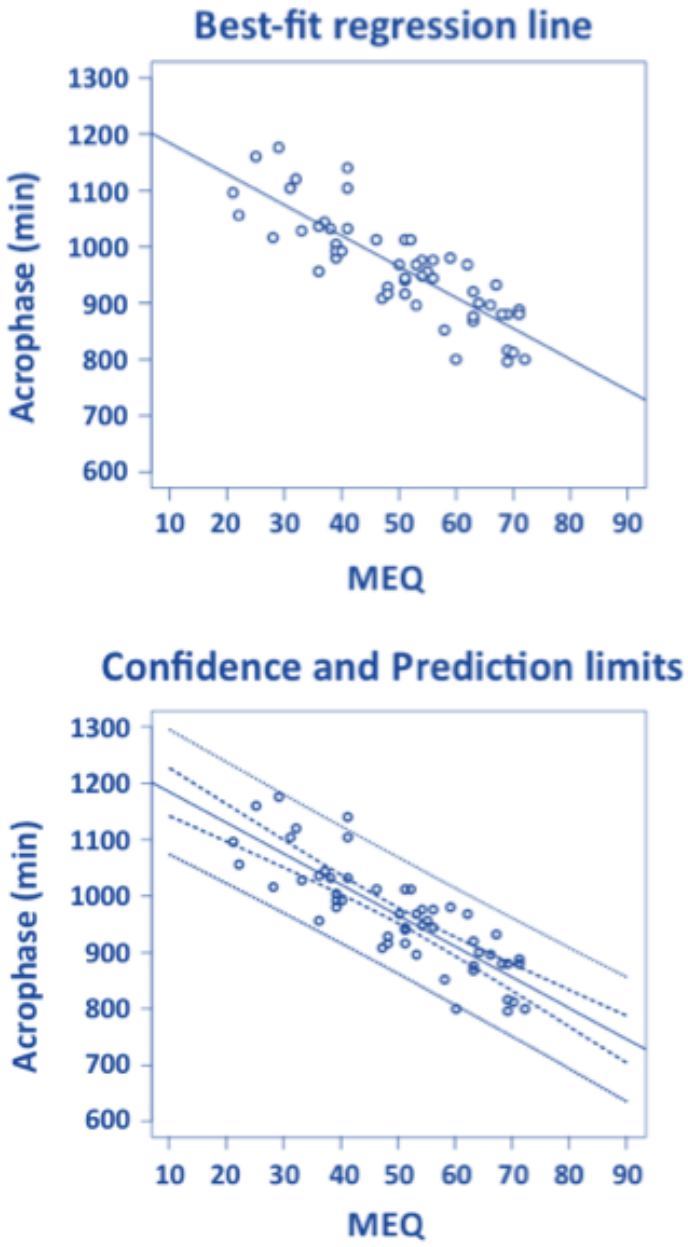

Figure 2. The upper panel shows the regression line superimposed to the experimental data. The lower panel shows the confidence/prediction limits (narrow bands: confidence limits; wide bands: prediction limits).

Also the F-test reported in the ANOVA table associated to regression confirmed that the slope was significantly different from 0 (Table 3). Of note that the MSE reported in the table was 2598 min^2.

Source	*DF*	*Sum of Squares*	*Mean Sum of Squares*	*F ratio*	*p-value*
Linear model	1	317783.1	317783.1	122.3	< 0.0001
Error	52	135102.2	2598		
Total	53	452885.3			

Table 3. Analysis of variance associated to linear regression and the decomposition of the variability within the regression model. The mean sum of squares relative to the error is the mean square error (MSE). The F-ratio for testing the significance of the slope of the regression line is reported in conjunction with the corresponding p-value.

The model fit was good. Indeed, the coefficient of determination R^2 was 0.70, indicating that 70% of the variance in the acrophase was explained by MEQ.

The bottom panel of Figure 2 reports the confidence and prediction limits given a MEQ score. As expected, the prediction limits are wider than the confidence limits. Of note is that the width of the prediction interval is not constant, being smaller near the middle of the MEQ range and wider as one proceeds to the extremes. In the central region of the diagram, the uncertainty affecting the prediction of a new individual acrophase value given the MEQ score was approximately ± 100 min.

The graphical analyses of the residuals are shown in Figure 3. The residuals were well-distributed around the zero line and did not show any systematic pattern. In addition, the

distribution of the residuals was congruent with the hypothesis of homoscedastic variation around the population line. This was confirmed by the lack of significance of the Breusch-Pagan test (p=0.29). Both the histogram and the boxplot of the residuals were symmetrical around zero and no outliers were detected. The Q-Q plot (not shown) indicated that the experimental quantiles matched the theoretical line well, except in the extreme left tail. Overall, the agreement with the normal distribution was good. The Shapiro-Wilk test confirmed that residuals showed no significant departure from normality (p>0.5).

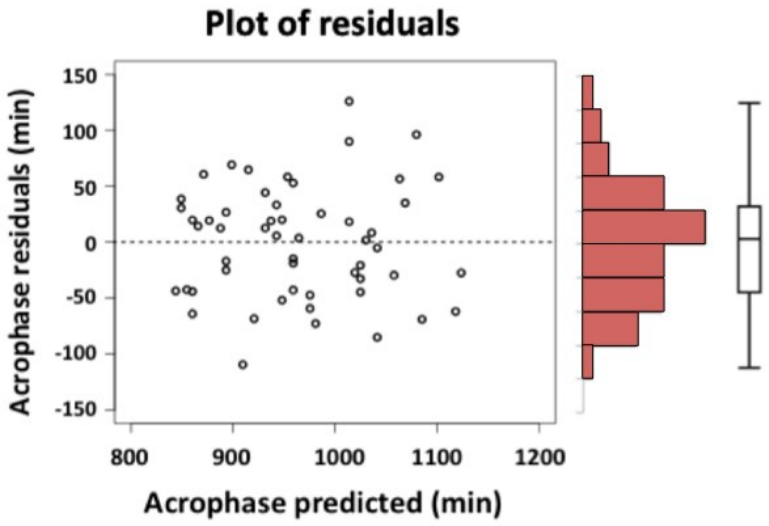

Figure 3. Best-fit line residuals plotted against the predicted acrophase. On the right side, the histogtram and the boxplot of the residuals are reported. Of note is that residuals do not show any polarization or systematic pattern.

Figure 4 reports the results of the k-fold cross-validation. This procedure provided MSE=2661 min^2 (mean value over 6 folds), corresponding to RMSE=52 min. Of note is that the cross-validation estimate of the RMSE was virtually identical to the RMSE value obtained using the experimental data both as training and testing set (that is, 51 min). In addition, the parameters of the regression lines estimated over 6 folds showed a very low variability: the y-intercept (parameter a) ranged from 1230.3 to 1242.8 min,

while the slope (parameter b) from ranged from -5.09 to -5.55 min per unit of MEQ score. As a result, the best-fit lines were almost parallel and very close to each other. All in all, k-fold validation indicated that the model performed well when was tested with data sampled from the same population used for model identification.

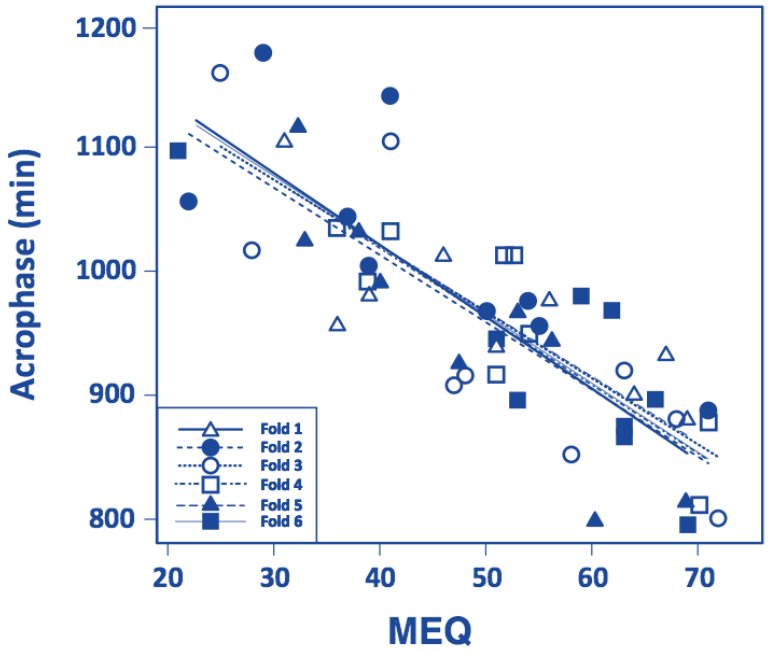

Figure 4. Results of the k-fold cross-validation procedure. Each testing set shows approximately the same degree of scatter as the others and the best-fit regression lines are virtually superimposed.

5. DISCUSSION

In this study we worked out a linear prediction model of the acrophase based on the questionnaire-based MEQ. The linear model was significant and able to provide a good description of the relationship between MEQ and the acrophase. Specifically, any additional point in the MEQ score corresponds to a shortening of the acrophase of approximately 5 minutes. The statistical significance of the MEQ score as a predictor of the acrophase was confirmed by the t-test that ruled out the null hypothesis ($\beta=0$), as

well as by the F-test in the ANOVA table associated with the regression. The reliability of the MEQ-based prediction of the acrophase was supported by the analysis of the residuals that showed a random, non-systematic pattern with a constant variance. The precision of the prediction of the acrophase yielded by the MEQ score was quantified by both the confidence and prediction limits surrounding the best-fit regression line. Given a certain MEQ score, such prediction limits defines a range within which the acrophase value is likely to lie. Thus, the point estimate of the acrophase should be accompanied by visual inspection of the prediction in order to have a realistic picture of the uncertainty of the prediction.

Further studies will be needed to ascertain whether the precision of the MEQ-based prediction can be enhanced by incorporating in the model other predictors. In this study we made an attempt to use a richer model incorporating also the sex of the subjects and their photoperiod at birth. However, the resulting multiple regression model failed to improve over the simple single-predictor model based on the MEQ score.

We would like to emphasize that prediction models, by their very nature, do not universally apply to all populations and experimental conditions. Thus a model can be valid and useful under certain conditions, but useless in another. Our model was capable to adequately predict the model-development data set and the results of k-fold cross-validation suggest that the model should maintain a good performance when confronted with new subjects drawn from the same population. However, it is presently unknown the model's degree of accuracy and precision when confronted with a separate, independent data set. The key message of our study is that the MEQ score is suitable to predict the actigraphy-based acrophase.

6. CONCLUSIONS

In this study we showed that it is possible to use the questionnaire-derived MEQ scores to predict the acrophase of the circadian rhythm by means of a linear regression model. This method is simple and cost-effective. It may prove useful when actigraphy-based measurement of the acrophase is not applicable because too complex, costly or time-consuming. Other investigators can apply such general framework to their data and determine the specific regression parameters yielding the best prediction for their population of interest.

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This research project has led to the following publication:

Predicting the actigraphy-based acrophase using the Morningness-Eveningness Questionnaire (MEQ) in college students of North Italy.

Roveda E., Vitale J.A., Montaruli A., **Galasso L.**, Carandente F. and Caumo A. (2017) *Chronobiology International*, 34(5):551-562

This publication is also reported in Appendix (publication n° 5).

10

ASSESSMENT OF THE PREDICTIVE ABILITY OF THE REDUCED MORNINGNESS-EVENINGNESS QUESTIONNAIRE SCORE

1. INTRODUCTION

In the previous chapter, we have shown that the MEQ score (Horne & Ostberg, 1976) can be used to predict the acrophase measured by actigraphy. This success motivated us go further and ask the following questions. What is the performance of the shorter version of the Morning-Evening-Questionnaire that is the so-called rMEQ (Adan and Almirall, 1991), which is based on 5 of the 19 original items? Does the predictive ability of rMEQ remains good enough or is degraded by the loss of information inherent to the item reduction? Answering these questions is relevant for those potential users of this equation who do not have at their disposal the full MEQ score, but only its reduced

version. This research project is thus the natural extension and integration of the work detailed in the previous chapter.

2. AIM OF THE STUDY

The aim of the current study was to develop a simple linear model to predict the actigraphy-based acrophase from the reduced MEQ score.

3. METHODS

Participants

We used the same data base of the study described in the previous chapter, namely the actigraphy and MEQ data collected in a sample of 54 college students of North Italy.

Data analysis

We used exactly the same data analysis techniques outlined in the previous chapter. The only difference is that the answers to Morning-Evening-Questionnaire were used to calculate the reduced MEQ. As a result, we developed a new linear equation enabling the rMEQ to predict the acrophase.

4. *RESULTS*

Figure 1 shows the scattergram of the data together with the best-fit regression line. The equation of the regression line was: acrophase =1183.4-15.94*rMEQ. The slope of the regression line (-15.94 min per unit of rMEQ) was significantly different from 0 ($p<0.001$). This means that a one-point increase in the rMEQ score is accompanied, on

average, by a decrease of almost 16 min in the acrophase. Pearson's r was 0.78 ($p<0.001$), and thus slightly lower than the value r=0.84 that measured the strength of the correlation between the acrophase and the full MEQ. If we make reference to the coefficient of determination (i.e., R^2), the full MEQ is able to explain 71% of the variability present in the acrophase, while rMEQ explains 61%.

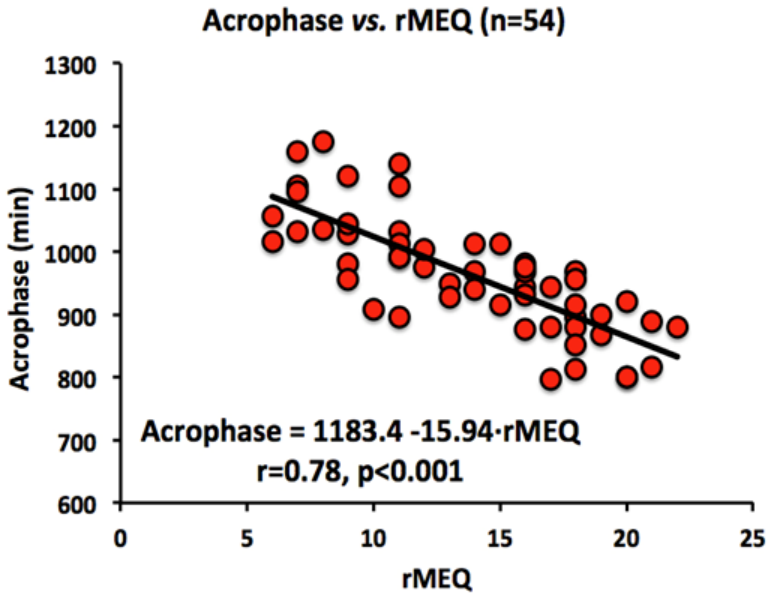

Figure 1. Scatter diagram of the relationship between the acrophase and rMEQ. The best-fit regression line has been superimposed to the experimental data.

5. DISCUSSION

In this study we addressed the following question: moving from 19 items of the full MEQ to the 5 items of the reduced version would jeopardize its predictive ability?

To our surprise, we found that the performance of the rMEQ was not exceedingly worse than that yielded by the full MEQ. Indeed, we found a weaker, but still reasonably good association between the rMEQ and the acrophase as compared to the results based on the full MEQ. This is probably related with the fact that rMEQ has been proven to be an

excellent surrogate of the full MEQ. Indeed, many authors have shown an elevated degree of agreement between MEQ and rMEQ, thus indicating that the rMEQ has considerable validity (Chelminski et al. 2000; Natale et al. 2006; Adan et al., 2012). We too, in the current study, found an excellent correlation between the full MEQ and rMEQ ($r=0.95$, $p<0.001$). Of course, such encouraging results hold in our specific population of college students of North Italy and cannot be safely extrapolated to older people or to other ethnicities.

6. CONCLUSIONS

In this study we have shown that not only the full MEQ score, but also its simpler and more easily administered short version, rMEQ, is a good candidate for the development of a linear equation capable to predict the actigraphy-based acrophase. This finding is good news because rMEQ can be more easily administered than the full MEQ.

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This research project has led to the following publication:

If the Morning-Evening Questionnaire (MEQ) is able to predict the actigraphy-based acrophase, how does its reduced, five-item version (rMEQ) perform?

Montaruli A., **Galasso L.**, Carandente F., Vitale J.A., Roveda E. and Caumo A. (2017)

Chronobiology International, 34(4):443-444

This publication is also reported in Appendix (publication n° 6).

11

OTHER ARTICLES

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

Vitale J.A., Roveda E., Montaruli A., **Galasso L.**, Weydahl A., Caumo A. and Carandente F. (2015)

Chronobiology International, 32(3):405-415

This publication is also reported in Appendix (publication n° 1).

Aim: To assess the relationship between Morning-types (M-types), Neither-types (N-types) and Evening-types (E-types) and the circadian rhythm of activity levels and to determine if there are differences between sleep parameters with respect to time, weekdays vs weekend, in the three chronotypes.

Methods: The Mornigness-Eveningness Questionnaire (MEQ), to detect the circadian typology, was filled out by 502 college students. The sleep parameters and the circadian rhythm of activity levels were evaluated through a 7-days monitoring period with an actigraph in 50 subjects (16 M-types, 15 N-types and 19 E-types).

Results: The chronotypes was affected by sex and the photoperiod at birth.

The MESOR and amplitude of the activity levels were not different among the three chronotypes while the acrophase was different between M-types (14:32 h) and E-types (16:53 h). N-types, the central circadian typology, show an intermediate acrophase between the two extreme chronotypes (Figure 1).

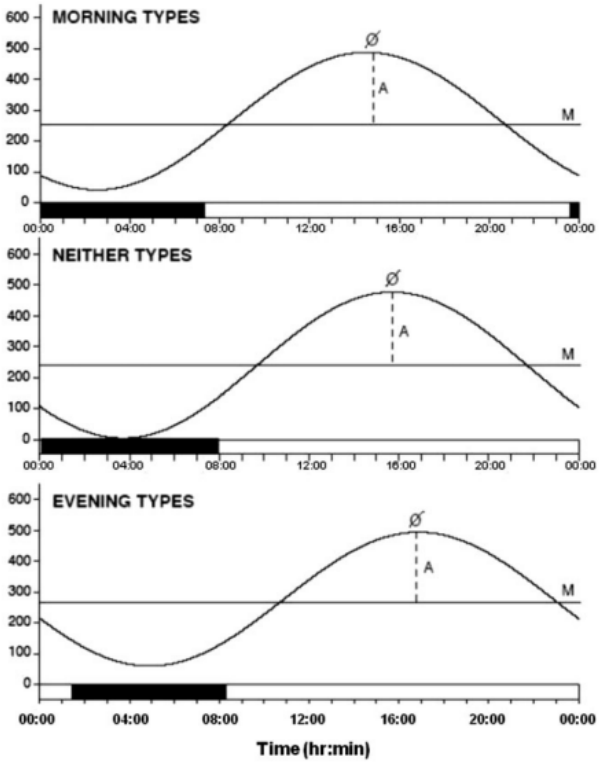

Figure 1. A comparison of rest-activity circadian rhythms among M-, N- and E-types. Φ: acrophase; A: amplitude; M: MESOR.

E-types report lower sleep quality and quantity than M- and N-types during weekdays, while during weekend E-types obtain the same levels as the other circadian typology. Specifically (Figure 2), the chronotype affects the sleep timing and duration during weekdays (WD) and the weekend (WE): M-types go to bed approximately 2 hours earlier compared to E-types both during WD and the WE. E-types wake up significantly later compared to M-types during WD and the WE and they present longer assumed sleep during the WE compared to WD.

E-types show shorter sleep duration during WD but they sleep longer during WE compared to other circadian typology: they accumulate a sleep debt during WD that they recover during the free days on the weekend. Regarding to the sleep quality, the M- and N-types present the same sleep efficiency during WD and the WE. On the contrary, E-types improve their sleep efficiency during the WE. Similar findings are present also for the assumed sleep and immobile time parameters: E-types spend less immobile minutes compared to M- and N-types during WD and they enhance their immobile time during the WE. Lastly, movement fragmentation index and sleep latency are similar for all chronotypes during both WD and the WE.

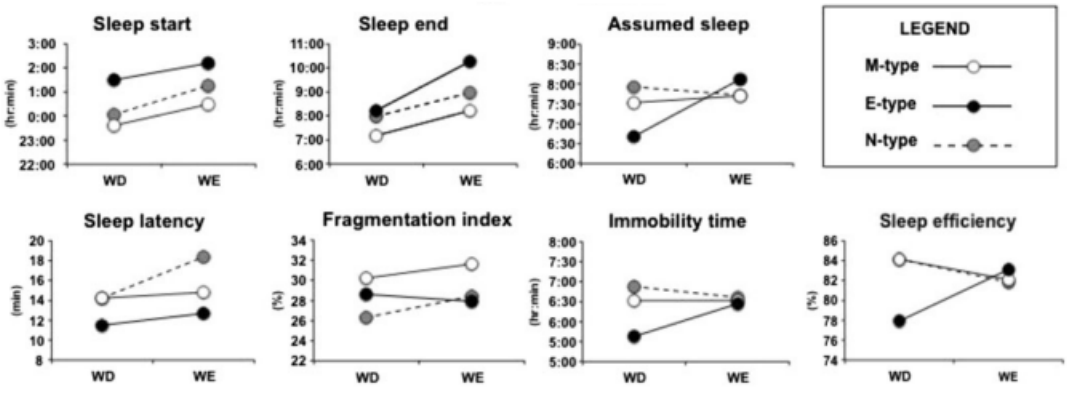

Figure 2. The actigraphic sleep parameters (mean values) assessed in M-, N- and E-types in different time spans, weekdays (WD) and the weekend (WE).

Conclusions: It seems that M-types sleep better and spend more immobile time in bed compared to E-types during WD and that E-types have the same sleep quality as the M- and N-types during WE by increasing their sleep efficiency and immobile time. E-types accumulate a sleep debt during WD due to their social and academic schedules that oblige them to wake up earlier with respect to their preferred sleeping times. As a solution, they recover during the WE in whom they sleep better and longer.

Sleep quality and high intensity interval training at two different time of day: A crossover study on the influence of the chronotype in male collegiate soccer players.

Vitale J.A., Bonato M., **Galasso L.**, La Torre A., Merati G., Montaruli A., Roveda E. and Carandente F. (2017)

Chronobiology International, 34(2): 260-268

This publication is also reported in Appendix (publication n° 3).

Aim: To investigate the relationship between sleep quality, chronotype and high intensity interval training (HIIT) at two different times of day (i.e., 08.00 a.m. and 08.00 p.m.).

Methods: The Mornigness-Eveningness Questionnaire (MEQ), to detect the circadian typology, was filled out by 547 college students. The sleep parameters were evaluated during the first and second HIIT sessions through a 4-days monitoring period with an actigraph in 23 subjects then randomly assigned to one of two groups: group 1 (n:11, 6 M-types and 5 E-types), group 2 (n:12, 6 M-types and 6 E-types).

Results: There is an important effect of the chronotype on sleep parameters after the evening HIIT session (Figure 1): Actual Sleep Time (AST), Actual Wake Time (AWT), Sleep Efficiency (SE), Immobile Time (IT) and Moving Time (MT) indicate a poorer sleep quality in M-types whereas no changes are present in sleep behaviour of E-types. On the contrary, there are no decreases in sleep parameters between M- and E-types after the morning HIIT session.

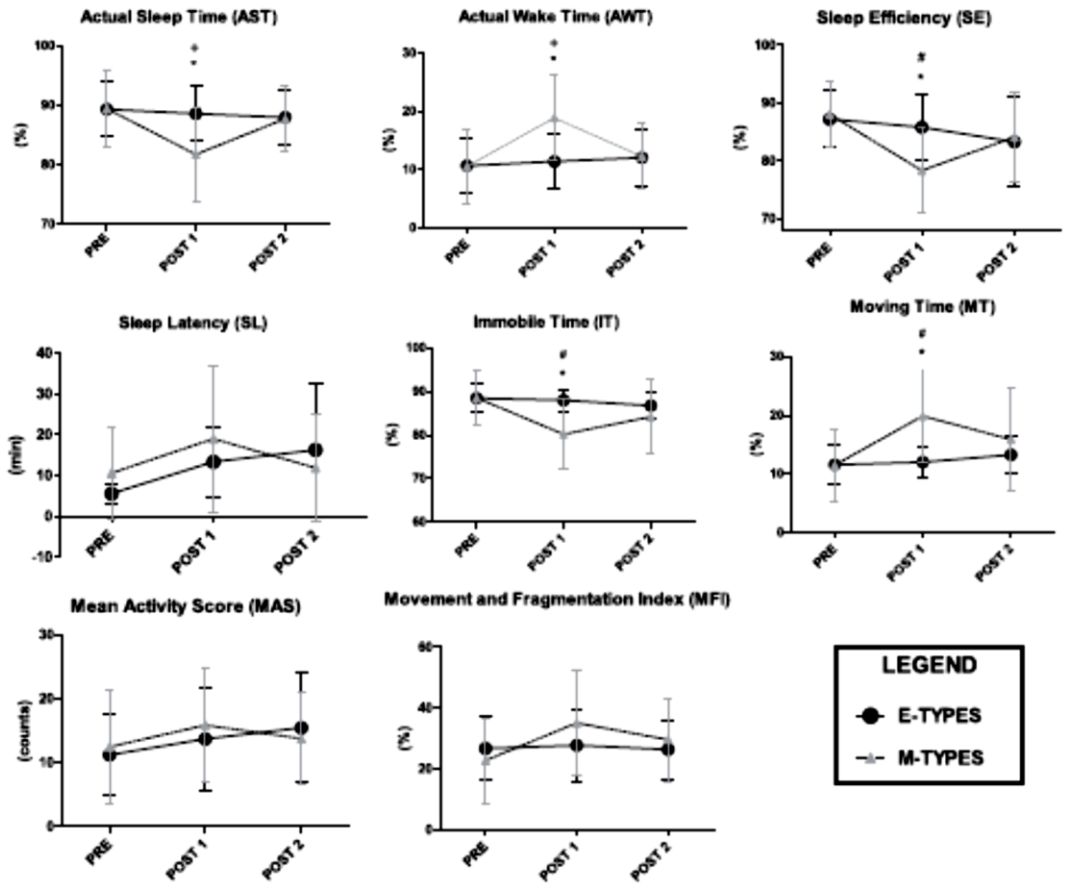

Figure 1: The actigraphic sleep parameters (mean values ± SD) assessed in M- and E-types in three different time spans, the night before (PRE), the first night after (POST1) and the second night after (POST2) the evening HIIT session.

Conclusions: The circadian typology is not able to influence sleep quality in response to a morning HIIT session but only after the evening HIIT session.

Protective effect of aerobic physical activity on sleep behavior in breast cancer survivors.

Roveda E., Vitale J.A., Bruno E., Montaruli A., Pasanisi P., Villarini A., Gargano G., **Galasso L.**, Berrino F., Caumo A. and Carandente F. (2017)

Int Cancer Ther., 16(1):21–31

This publication is also reported in Appendix (publication n° 4).

Aim: To evaluate the effect of 3 months of aerobic physical activity on sleep and circadian rhythm activity levels in breast cancer women.

Methods: 40 women with breast cancer were randomized into an intervention (n:19) and control group (n:21). Women of intervention group followed an aerobic physical activity program lasting 3 months. The sleep parameters and the circadian rhythm of activity levels were evaluated throughout a 7-days monitoring period with an actigraph. In addition, anthropometric and body composition values were evaluated through a body segment impedance balance and also energy expenditure and motion level throughout a 7-days monitoring period by means of an arm band.

Results: The control group reported a worsening of sleep: Sleep Efficiency (SE), Actual Sleep Time (AST) and Immobile Time (IT) decreased while Actual Wake Time (AWT), Sleep Latency (SL), Mean Activity Score (MAS) and Movement Fragmentation Index (MFI) increased. The intervention group maintained stable sleep parameters (Figure 1). Women who followed the physical activity program reduced significantly the percentage of fat mass with a tendency to increase the lean mass.

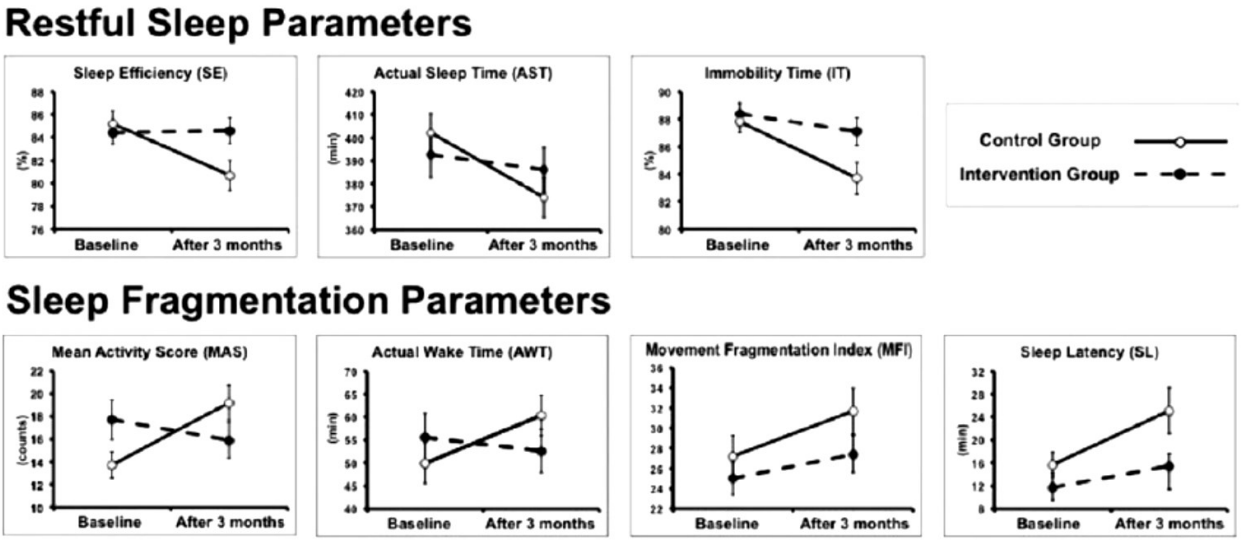

Figure 1. The actigraphic sleep parameters (mean values ± SD) assessed in the intervention and control groups in two different time spans, at baseline and after 3 months.

Conclusions: It seems that physical activity may have a protective effect on sleep in women who have had breast cancer.

Acute Modification of Cardiac Autonomic Function of High-Intensity Interval Training in Collegiate Male Soccer Players with Different Chronotype: A Cross-Over Study.

Bonato M., Agnello L., **Galasso L.**, Montaruli A., Roveda E., Merati G., La Torre A. and Vitale J.A. (2017)

Journal of Sports Science and Medicine, 16(2): 286-294

This publication is also reported in Appendix (publication n° 7).

Aim: To evaluate if the time of day (08.00 a.m. vs 08.00 p.m.) and the circadian typology could affect autonomic cardiac control in relation to a session of high intensity interval training (HIIT).

Methods: The Morningness-Eveningness Questionnaire (MEQ), to detect the circadian typology, was filled out by 547 collegiate male soccer players. 24 subjects (12 M-types and 12 E-types) were randomly assigned to either morning or evening training.

The Heart Rate Variability assessment were performed at rest, before (TO), after 12 (T12) and 24 (T24) hours of HIIT sessions.

Results: E-types showed a higher heart rate which indicated higher vagal indices with lower parasympathetic tone at rest and before (T0) the morning HIIT session compared to M-types. On the other hand, there are no differences between M-types and E-types during evening HIIT sessions (T0) (Figure 1).

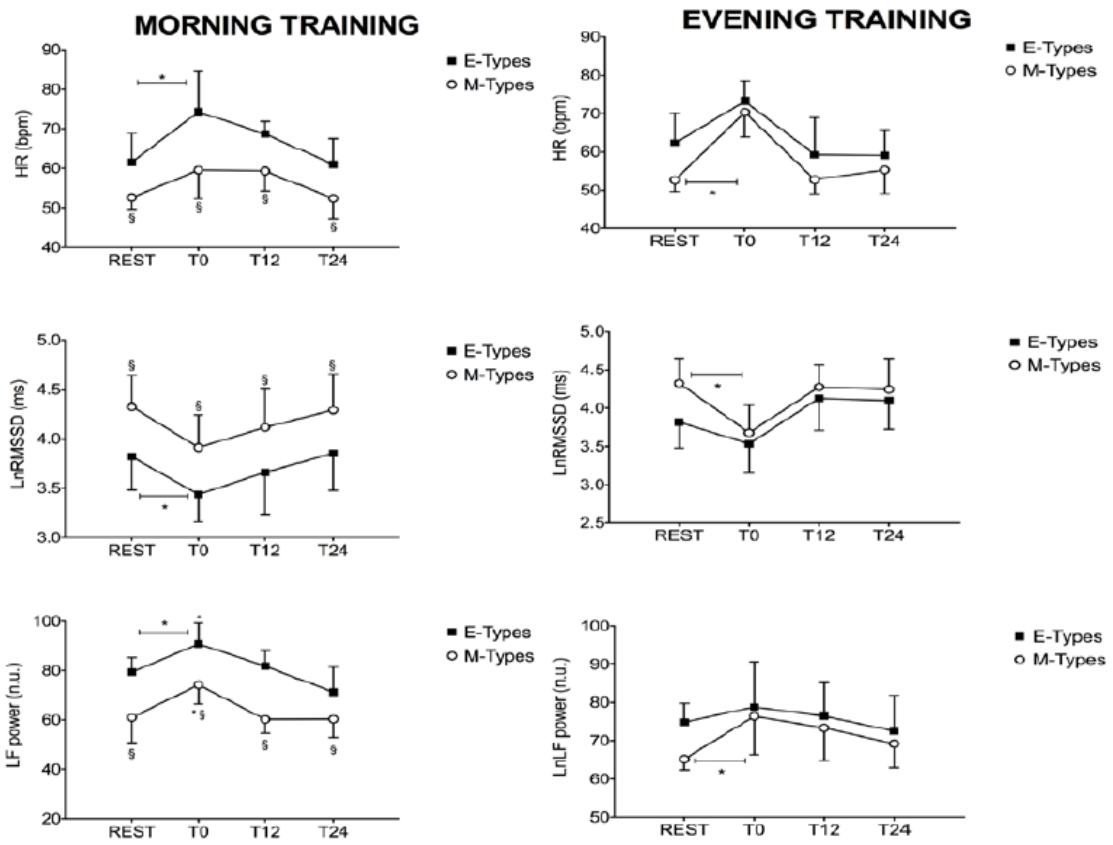

Figure 1. The heart rate variability parameters (mean values ± SD) assessed in M-types and E-types in four different time spans, at rest, before (TO), after 12 (T12) and 24 (T24) hours of HIIT sessions.

Conclusions: The stress response of HIIT is affected by both the time of the day and by the chronotype.

Effect of aerobic exercise intervention on markers of insulin resistance in breast cancer women.

Bruno E., Roveda E., Vitale J.A., Montaruli A., Berrino F., Villarini A., Venturelli E., Gargano G., **Galasso L.**, Caumo A., Carandente F. and Pasanisi P. (2018)

European Journal of Cancer Care, 27(2):e12617

This publication is also reported in Appendix (publication n° 10).

Aim: To evaluate the effect of 3 months of aerobic physical activity on insulin parameters and body composition in non-obese breast cancer women without insulin resistance.

Methods: 38 women with breast cancer were randomized into an intervention (n:18) or control group (n:20). The women in the intervention group followed an aerobic physical activity programme lasting 3 months whereas the women in the control group followed only the World Cancer Research Fund & American Institute for Cancer Research recommendation (WCRF/AICR) to be active. At baseline and after 3 months, the anthropometric and body composition parameters were evaluated through a body segment impedance balance. In addition, metabolic and hormonal parameters were evaluated and so was energy expenditure over a 7-day period of monitoring by means of an arm band.

Results: The women suffering from breast cancer who performed 3 months of aerobic activity, reduced their insulin levels and HOMA-IR index by 15% and 14%, respectively, while increased the insulin levels and HOMA- IR index by +12% and +16%, respectively the women belonging to the control group. Furthermore, the women

in intervention group enhanced lower limb muscle mass with respect to the control group.

Conclusions: The effects of 3 months of aerobic physical activity are evident on fasting insulin levels, HOMA-IR index and body composition parameters in breast cancer sufferers without insulin resistance.

The circadian typology: the role of physical activity and melatonin.

Montaruli A., **Galasso L.**, Caumo A., Cè E., Pesenti C., Roveda E. and Esposito F. (2017)

Sport Science for Health, 13(3): 469-476

This publication is also reported in Appendix (publication n° 8).

Aim: The review analyzes the interaction between the chronotypes, melatonin and physical activity.

Results: The pertinent literature presents 3 chronotypes: M-types, N-types and E-types and they are different in timing of sleep, peak physical and cognitive performances and personality. In addition, the circadian typology is influenced by sex and age: in fact, children, old people and women are more morning oriented while younger adults and men are more evening oriented. It seems that during the adolescence the sleep-wake cycle reports a shift toward eveningness with a return to morningness in the adulthood. Referring to the relationship between melatonin and physical activity, it is not clear if human melatonin concentrations change or remain unaffected by the exercise. The findings connected to this relationship emphasize the importance of taking light conditions into consideration, as well as the time of day when the exercise is done, the intensity of the exercise, age and fitness levels of the subjects.

Conclusions: Additional studies are necessary to clarify the relationships between chronotypes, physical activity and melatonin.

Human physiology during exposure to the cave environment: a systematic review with implications for aerospace medicine.

Zuccarelli L., **Galasso L.**, Turner R., Coffey E.J.B., Bessone L. and Strapazzon G.

Submitted to *Frontiers in Physiology*

This publication is also reported in Appendix (submitted paper n° 2).

Aim: The review analyzes the effects of short and prolonged exposure to cave environment on human physiology, with a view to extending the findings to implications for human planetary exploration missions and space medicine considerations.

Results: Cardiovascular, endocrine-metabolic and immunologic-hematological parameters, muscular responses and visual dysfunction were assessed in short term exposure cave studies while persistence or desynchronization of biological rhythms was analyzed in prolonged exposure studies. Studies report a reasonable interference based on specific subterranean protocols. Both acute response to cave progression and prolonged underground isolation generate adaptive mechanisms (e.g., changes in circadian system because of the absence of environmental synchronizers related to the 24 hours).

Conclusions: The environmental effects of cave progression have been identified as important for practical considerations when defining implications for astronaut expeditionary training courses in space analogues expedition in subterranean environments.

"Tracking physiological changes as a function of time helps health maintenance and even health improvement, adding life to years and not just years to life".

Germaine Cornelissen

APPENDIX

Published papers

1. Vitale J.A., Roveda E., Montaruli A., **Galasso L.**, Weydahl A., Caumo A. and Carandente F. (2015). Chronotype influences activity circadian rhythm and sleep: Differences in sleep quality between weekdays and weekend. *Chronobiology International,* 32(3):405-415.
2. Roveda E., Vitale J.A., **Galasso L.** and Carandente F. (April-June 2016). Riadattamento degli atleti dopo volo transcontinentale. *Sport & Medicina*-ISSN: 0392-9647 (2): 20-25.
3. Vitale J.A., Bonato M., **Galasso L.**, La Torre A., Merati G., Montaruli A., Roveda E. and Carandente F. (2017). Sleep quality and high intensity interval training at two different time of day: A crossover study on the influence of the chronotype in male collegiate soccer players. *Chronobiology International,* 34(2):260-268.
4. Roveda E., Vitale J.A., Bruno E., Montaruli A., Pasanisi P., Villarini A., Gargano G., **Galasso L.**, Berrino F., Caumo A. and Carandente F. (2017). Protective effect of aerobic physical activity on sleep behavior in breast cancer survivors. *Int Cancer Ther.*, 16(1):21-31.
5. Roveda E., Vitale J.A., Montaruli A., **Galasso L.**, Carandente F. and Caumo A. (2017). Predicting the actigraphy-based acrophase using the Morningness-Eveningness Questionnaire (MEQ) in college students of North Italy. *Chronobiology International,* 34(5):551-562.
6. Montaruli A., **Galasso L.**, Carandente F., Vitale J.A., Roveda E. and Caumo A. (2017). If the Morning-Evening Questionnaire (MEQ) is able to predict the actigraphy-based acrophase, how does its reduced, five-item version (rMEQ)

perform? *Chronobiology International,* 34(4):443-444.

7. Bonato M., Agnello L., **Galasso L.**, Montaruli A., Roveda E., Merati G., La Torre A. and Vitae J.A. (2017). Acute Modification of Cardiac Autonomic Function of High-Intensity Interval Training in Collegiate Male Soccer Players with Different Chronotype: A Cross-Over Study. *Journal of Sports Science and Medicine,* 16(2):286-294.

8. Montaruli A., **Galasso L.**, Caumo A., Cè E., Pesenti C., Roveda E. and Esposito F. (2017). The circadian typology: the role of physical activity and melatonin. *Sport Science for Health*, 13(3):469-476.

9. Roveda E., Montaruli A., **Galasso L.**, Pesenti C., Bruno E., Pasanisi P., Cortellini M., Rampichini S., Erzegovesi S., Caumo A. and Esposito F. (2018). Rest-activity circadian rhythm and sleep quality in patients with binge eating disorder. *Chronobiology International,*35(2):198-207.

10. Bruno E., Roveda E., Vitale J.A., Montaruli A., Berrino F., Villarini A., Venturelli E., Gargano G., **Galasso L.**, Caumo A., Carandente F. and Pasanisi P. (2018). Effect of aerobic exercise intervention on markers of insulin resistance in breast cancer women. *European Journal of Cancer Care,* 27(2):e12617.

11. **Galasso L.**, Montaruli A., Bruno E., Pesenti C., Erzegovesi S., Cè E., Coratella G., Roveda E. and Esposito F. (2018). Aerobic exercise training improves physical performance of patients with binge eating disorder. *Sport Sciences for Health*, 14(1):47-51.

1. **Galasso L.,** Montaruli A., Bruno E., Chiorazzo M., Ricceri A., Erzegovesi S., Caumo A., Roveda E. and Esposito F.

   Effect of combined aerobic and anaerobic exercise training on behavioral and anthropometric parameters in patients with binge eating disorder.

   Submitted to *European Journal of Sport Science.*

2. Zuccarelli L., **Galasso L.**, Turner R., Coffey E.J.B., Bessone L. and Strapazzon G.

   Human physiology during exposure to the cave environment: a systematic review with implications for aerospace medicine.

   Submitted to *Frontiers in Physiology.*