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PHD COURSE IN TRASLATIONAL MEDICINE

PhD Thesis

SLEEP QUALITY AND REST-ACTIVITY CIRCADIAN RHYTHM IN PEOPLE WITH METABOLIC SYNDROME

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SUMMARY

ABSTRACT

Background. Alterations in sleep and Rest-Activity circadian Rhythm (RAR) increase the risk of developing many pathological conditions, including Metabolic Syndrome (MS). Moreover, people with MS have been shown to sleep poorly and exhibit impaired RAR. Recently, metformin, an insulin sensitizer drug widely used in various cardiometabolic diseases, was introduced as adjuvant agent for the management of sleep disorders. It is shown to have an opposite effect to sleep disorders on the same parameters.

The aim of my PhD project was to assess whether changes in daily habits are able to modify sleep, daily activity levels, sedentary habits and MS parameters in healthy people with MS, and also to investigate whether metformin treatment can modify sleep and MS parameters.

Methods. 133 subjects with MS were enrolled in the study. At baseline and after one year of intervention, all participants underwent a medical examination to evaluate anthropometric and MS parameters. Also, they carried out a continuous 7-day actigraphic monitoring to investigate the RAR and sleep. Participants received double randomization, based on drug (metformin or placebo) and dietary approach (cooking courses or general awareness). Randomization based on drug treatment, adjusted for dietary approach, age, gender and baseline values, was considered for the data analysis.

Results. At baseline, most subjects showed poor sleep quality and quantity. The comparison between the two sexes showed no differences in sleep parameters, and no difference was present between people with different severity of MS.

Regarding RAR, influences related to sex have been found. Females showed higher daily movement and a less fragmented RAR compared to males. After one year of intervention, an improvement of MS in 45.7% of the participants was found. In 37.5% of them it was no longer possible to diagnose the MS. Metformin and placebo groups showed an improvement of their syndrome condition, with higher effects in metformin-treated subjects. A statistically significant increase in actual sleep time was found in metformin group at follow-up compared to baseline. Moreover, metformin-treated subjects showed an improvement of actual sleep time and sleep efficiency, while people treated with placebo displayed no change in actual sleep time, and a worsening in sleep efficiency. Although not significant, metformin group also showed a good trend of improvement in almost all other sleep parameters, while opposite trends were found in people treated with placebo. Regarding the RAR, the intervention showed no effects. This result was rather predictable, as a change in daily activity levels is required to modify the RAR, for example by encouraging strategies to increase daily activity and decrease sedentary behaviors.

Conclusions. The results of this PhD research project support the hypothesis that metformin may be a new valid approach, together with a healthy eating style, for the secondary prevention not only of the metabolic syndrome condition but also of any related sleep disorders.

INTRODUCTION

Metabolic syndrome (MS) is defined as a cluster of metabolic risk factors, such as abdominal obesity, high blood pressure, high fasting hyperglycemia and dyslipidemia, that increases the risk of cardiovascular disease and type 2 diabetes mellitus. Genetic, metabolic and environmental factors, play an important role in the development of MS. The principal environmental factors studied are physical activity, nutrition and sleep.

This PhD research project focused on two main issues: the relationship between metabolic syndrome and circadian rhythms; and the investigation of the role of metformin treatment on sleep disorders in patients with MS. In particular, the first one focused on the alteration of *sleep* and *rest-activity circadian rhythms* in people with MS. The Rest-Activity Circadian Rhythm (RAR) is the expression of 24 hours spontaneous activity, due to that it is used to evaluate the daily activity movement of people.

Thanks to relationships that have been demonstrated over the years between the psycho-physical status and the rhythms, the latter can also be considered markers of a state of health or, conversely, of disease. In literature it is well documented that alterations of circadian rhythms expose people to health problems and diseases, such as hypertension, obesity, eating disorders, dyslipidemia, diabetes, cardiovascular diseases, and several cancers.

Although the alteration of the RAR has been shown to be associated with impaired health, few studies have investigated the relationship between these alterations and the MS. Among these, some studies supported the existence of a relationship between regular and robust RAR and lower risk of developing MS or its components, such as hypertension, diabetes, dyslipidemia, and obesity. By contrast, several prospective and cross-sectional studies have documented the relationship between sleep disturbances (sleep breathing disorders, sleep duration, snoring, short sleep duration, difficulties in initiating and maintaining sleep) and an increased risk of developing MS components. Indeed, sleep disorders are emerging as environmental factors that increase the risk of developing MS. The main relation between MS and altered sleep quality may be explained by several factors: (1) a bidirectional association between sleep disorders and obesity, glucose metabolism and insulin resistance, (2) alteration of cellular signaling associated with mitochondrial respiratory function and insulin signaling pathway, like AMP-activated protein kinase and insulin/insulin-like growth factor 1 signaling, (3) misalignment of circadian rhythms. Due to the aforementioned relationship between sleep disorders and MS, especially considering the link with metabolic factors, metformin was recently introduced as adjuvant agent for the management of sleep disorders. Therefore, the second issue of this research project was to investigate the possible role of metformin treatment on sleep disorders in patients with MS. Metformin is an insulin sensitizer drug and is widely used for the

reduction of insulin resistance and for the control of glucose metabolism. Moreover, it seems to act, with opposite effects, on the same MS parameters influenced by sleep disorders. In particular, one of the effects of metformin is to promote the activation of AMPK, which stimulates the oxidation of fatty acid, promotes glucose transport and glycolysis, and inhibits gluconeogenesis. It also affects insulin and IGF-1/Akt pathways, as well as testosterone and blood IGF-1 concentration. All of these processes are affected by sleep disorders. Data from animal experiments support a relationship between metformin treatment and management of sleep disorders, but the few preclinical data remain controversial. Although it has been shown that people with MS suffer from sleep disorders and that the use of metformin may be helpful in improving the quality and quantity of sleep, so far no studies have investigated the effects of metformin treatment on sleep disorders in patients with MS.

1. CHRONOBIOLOGY AND BIOLOGICAL RHYTHMS

In all living organisms, from the simplest unicellular organisms to the most complex ones, including human, temporal variations of biological functions can be found. Most biological phenomena are characterized by time-dependent modifications that occur at regular and rhythmic intervals. Chronobiology is the science that studies the temporal structure of organisms' functions, analyzing the periodic oscillations of these biological phenomena. Therefore, evaluating the changes in the temporal structure of organisms can be important when investigating the causes of the appearance of morbid phenomena. The recognition of changes in the parameters that characterize biological rhythms can be useful to prevent the onset or exacerbation of morbid phenomena. In fact, these modifications are often early warning signs of the next clinical evidence of disease. The knowledge of biological rhythms allows to define the time of greatest risk (chrono-risk) of disease development, and the temporal coincidence of the maximum values of periodic phenomena that can be the real cause of the development of diseases, in particular those with etiopathogenesis multifactorial.

Moreover, chronobiology aims at the temporally correct application of physical and pharmacological therapy of diseases, since the efficacy of a substance, its toxicity or the appearance of side effects can vary considerably in relation to the time of administration.

In nature there are rhythms with different frequencies: *ultradian* (less than 20 hours), *circadian* (almost 24 hours) and *infradian* (more than 24 hours) rhythms (Pittendrig 1960, Halberg 1964, Halberg et al. 1965), but most studies focused on the circadian rhythm. The study of the location of the rhythm generation and regulation centers took many years. Over the years, the researchers focused on various central neuronal system structures: the pineal gland, the suprachiasmatic nucleus, the hypophysis or part of it, and the hypothalamus. However, in 1977 the suprachiasmatic nucleus was the structure named biological clock, which is located on the top of the optic chasm where the optic nerve fibers cross. It processes signals coming from the eyes and releases hormones and other neuronal signals which trigger behavioral and physiological events. Halberg et al. (1977) showed that a laser ablation of the bilaterally symmetrical suprachiasmatic nuclei in rats modified the circadian rhythm of body temperature without interrupting the rhythm itself. In particular, the level of maximum and minimum temperature changed and sometimes also the average temperature over 24 hours, but the circadian temperature oscillation persisted (Halberg et al. 1979).

In summary, specialized suprachiasmatic nuclei neurons receive photic information from the retina via synaptic transmission by axons of the retino-hypothalamic tract. This electrical information is converted into chemical information that alters the phase of clock gene expression in a subset of central neuronal system neurons. The central neuronal system then 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 (Dibner et al. 2010).

1.1 Rhythms and synchronizers

The interaction between neural, hormonal and cellular systems determine the time structure of rhythms (Figure 1) (Halberg et al. 1977).

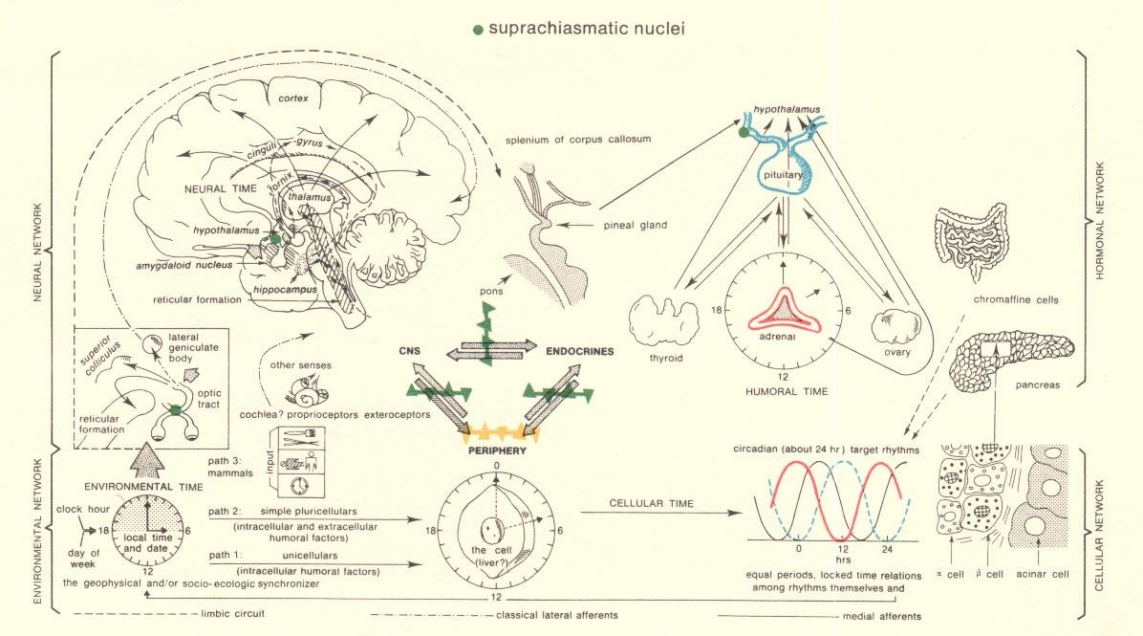

Figure 1. *Factors involved in circadian synchronization and environmental and/or organism desynchronization.*

The time structure shows periodic oscillations with different frequencies that could be influenced by environmental factors: the *synchronizers*. The rhythms of all organisms are subject to the influence of one or more synchronizers, which can be primary (dominant) or secondary, in relation to the role on the variable under consideration. The rhythm takes on the frequency (or an integer multiple or a submultiple of it) of the main ambient synchronizer. The main circadian synchronizers for humans are related to socio-environmental factors: the rest-activity alternation, the light-dark alternation and the times of food intake. The most important circannual synchronizers are the ambient temperature, the photoperiod, and the seasonal variability of food.

It is important to underline that the synchronizers do not create biological rhythms, but they are able to influence them. In conditions of isolation from the usual environmental synchronizers, the variables taken into consideration maintain a rhythmic trend that is not suppressed, but undergoes changes in the length of the period, assuming a trend called *free-running*. The synchronizers can influence biological rhythms, modifying the parameters that characterize them, but always acting on an irrepressible endogenous phenomenon.

The demonstration of the existence of a biological rhythm can be carried out with different procedures: one of the simplest is the *cosinor method* by Halberg et al. (1979), which involves the

application a classical statistical method (the least squares method) to identify the mathematical function of the sinusoidal type that best fits the data of a time series:

$$f(t) = M + A \cos(\omega t + \varphi)$$

where:

- M (MESOR): is the Midline Estimating Statistic of Rhythm, 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);
- A (Amplitude): is a measure of one half the extent of rhythmic change in a cycle estimated by the function (sinusoidal or other) used to approximate the rhythm, accordingly the difference between maximum and MESOR of a best fitting cosine. The units for Amplitude are original physiologic units (number of heart beats, mmHg in blood pressure) (Koukkari 1973, Koukkari et al. 1974);
- ω is the angular frequency corresponding to the ratio $2\pi/\tau$;
- t = is the time;
- φ (Acrophase): indicates the time interval within which the highest values of the variable are expected. The units of the Acrophase could be angular measures, degrees, radians, time units (seconds, minutes, hours, days, months, years), or physiologic episodic units (number of heart beats, respirations, etc). Angular measures are directly applicable to any cycle length and hence are proposed for general use because of greater familiarity; degrees are preferred over radians (Deprins et al. 1977, Halberg and Reinberg 1967).

Figure 2 displays an example of a biological rhythm and parameters derived by the cosinor analysis.

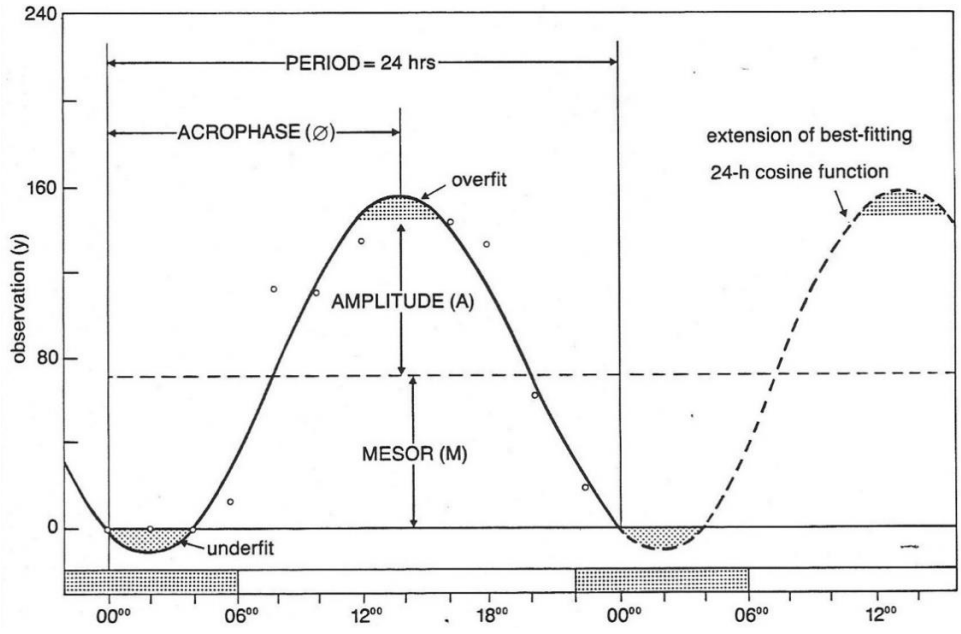

Figure 2. *Exemplification of a biological rhythm and its parameters: MESOR, Amplitude and Acrophase.*

2. CIRCADIAN RHYTHM ALTERATION AND HEALTH

Circadian rhythms influence daily behavior, as well as psychological and physiological functions, and they can change during the lifespan, both in physiological and pathological conditions (Cornelissen and Otsuka 2017, Montaruli et al. 2021).

Several studies investigated the aging effect on circadian rhythm parameters of different hormones. In particular, they noticed decreases in circadian Amplitude of urinary catecholamines associated with decrease in MESOR (Descovich et al. 1974) and modification in circadian Amplitude of circulating dehydroepiandrosterone sulfate, cortisol, prolactin, and aldosterone (Nelson et al. 1980, Cugini et al. 1982, Touitou and Haus 2000).

By contrast, recent studies have investigated the relationship between modification of circadian rhythms and health problems. Unfortunately, modern jobs or lifestyles (shift work, exposure to prolonged hours of artificial light, and bad nutrition) affect negatively the human circadian system (Mattson et al. 2014). Likewise, cardiovascular risk factors such as hypertension, obesity, eating disorders, dyslipidemia, and diabetes are associated to alteration in circadian rhythms (Karatsoreos et al. 2011, Buxton et al. 2012, Galasso et al. 2019).

Figure 3 shows an example of the rhythm's changes during the life span in physiological and pathological conditions.

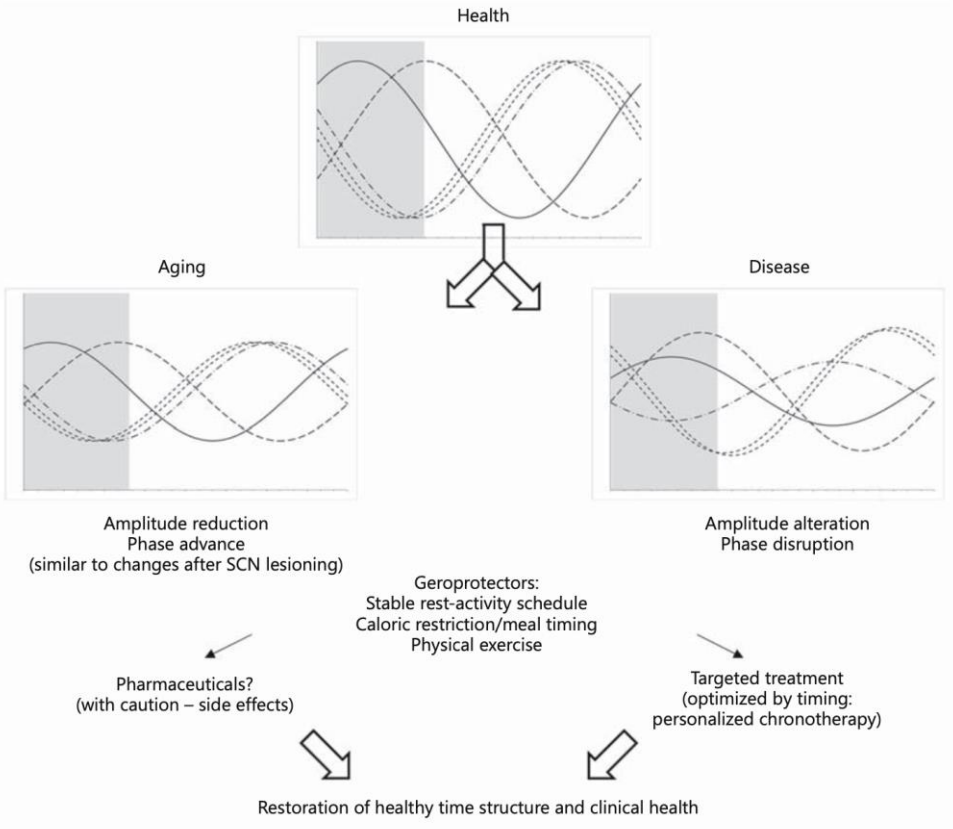

Figure 3. *Rhythm's changes during the life span in physiological and pathological conditions (Cornelissen and Otsuka 2017).*

2.1 Rest-activity circadian rhythm and sleep quality

Thanks to relationships that have been demonstrated over the years between the psycho-physical status and the rhythms, the latter can also be considered markers of a state of health or, otherwise, of disease. Indeed, the *Rest-Activity circadian Rhythm* (RAR), which is expression of 24 hours spontaneous activity, and the *sleep quality* can be considered reliable markers of the circadian timing system.

Like all rhythms, RAR and sleep quality can change during the life, both in physiological and pathological conditions. An elderly person tends to wake up and go to bed earlier (early Acrophase) and move less (lower MESOR and Amplitude values) than when he/she was younger. In particular, studies on aging showed a decrease of Amplitude and loss of RAR rhythm (Kripke et al. 2005), reduction of adaptability to phase resetting signals (Hofman and Swaab 2006), and advanced phase (Martinez-Nicolas et al. 2018). RAR alterations are also associated with impaired health. Psychiatric subjects show decreases of MESOR and Amplitude (Roveda et al. 2018), furthermore, people with lower Amplitude are exposed to greater cardiovascular risk (Paudel et al. 2011). Several studies also showed disrupted RAR in cancer patients (Sultan et al. 2017). Roveda et al. (2019) showed lower levels of MESOR and Amplitude in people with cancer, and Mormont and Waterhouse (2002) demonstrated that cancer progression tends to be accelerated in patients with alterations of RAR.

Studies concerning the signature electrical oscillations of sleep, measured with electroencephalography, and macro-level structure of sleep related to fragmentation, latency, efficiency and duration of sleep showed an alteration of these parameters with advancing age (Mander et al 2017). Moreover, Huang et al. (2002) showed weakened and fragmented sleep in the healthy elderly compared to young and middle-aged people, displaying lower quality and quantity of sleep. Other studies reported the relationship between sleep disturbance and diseases. Short sleep duration is associated with increased incidence of cardiovascular diseases, such as coronary artery disease, hypertension and obesity (Tobaldini et al. 2017, Cappuccio et al. 2010). Moreover, several studies showed the presence of altered inflammatory and immune response (Irwin et al. 2008) and a deregulation of leptin-ghrelin system and insulin sensitivity (Rafalson et al. 2010) in people with sleep disorders.

2.2 Rest-activity circadian rhythm and sleep quality in metabolic diseases

Several studies have investigated the relationship between irregular circadian rhythms and cardiovascular risk factors. Some of these risk factors are referred to hypertension, obesity, insulin resistance, and dyslipidemia. In particular, RAR and sleep alteration can play an important role in the development of these metabolic and cardiovascular health problems.

A recent study showed that a robust and stable RAR, higher wake-time activity and lower activity at night, is associated with lower blood pressure (Makarem et al. 2020a). Furthermore, it has been shown

that the regularity in sleep may indeed be associated with a lower risk of hypertension (Makarem et al. 2020b, St-Onge et al. 2016).

The impact of physical activity as well as the daily movement level on weight is well demonstrated in literature. A more fragmented RAR was associated with total and central adiposity. Obese adolescents had almost three times higher risk of developing high fragmentation of daytime activity compared to normal weight adolescents (Garaulet et al. 2017). Like daily activity, also sleep affects the risk to develop obesity. In fact, sleep disorders are related to obesity due to its effect on leptin and ghrelin (Wu et al. 2014, Taheri et al. 2004).

Xiao et al. (2020) showed that impaired RAR was associated with metabolic disorders. Particularly, lower level of Amplitude, MESOR, Amplitude to MESOR ratio, and overall rhythmicity were associated with elevated fasting insulin level and higher insulin resistance. Altered RAR exposes subject to higher risk to develop hyperglycemia and diabetes (Griggs et al. 2021). On the other hand, glucose intolerance and insulin resistance are associated with acute sleep deprivation (Tasali et al. 2009). Moreover, Jennings et al. (2007) showed that sleep quality is negatively correlated with higher levels of glucose, fasting insulin and insulin resistance.

Regarding dyslipidemia, on one hand the relationship with RAR is well demonstrated. Hoopes et al. (2021) and Sohail et al. (2015) showed an inverse relationship between robust and stable RAR and dyslipidemia. On the other hand, the influence of sleep is less clear; also because the few studies in the literature have not had the main objective of evaluating the lipid profile (de Azevedo Abreu et al. 2015, Jike et al. 2018, Barros and García-Río 2019). Bjorvatn et al. (2007) showed higher total cholesterol and lower HDL levels in people with short sleep durations. However, when they adjusted for gender, smoking and BMI, most of the relations between sleep duration and these metabolic measures were no longer significant. Instead, Qian et al. (2016) showed a significant positive association between sleep fragmentation and dyslipidemia in a sample of older man with type 2 diabetes. By contrast William et al. (2007) showed no differences in hypercholesterolemia between good and bed sleepers in a sample of women with type 2 diabetes.

Hypertension, dyslipidemia, hyperglycemia and obesity are factors related to the MS: a cluster of metabolic risk factors such as abdominal obesity, high blood pressure, high fasting glucose levels and dyslipidemia (Alberti et al. 2009). Although the above studies are not related to people with MS, we can assume that, due to these results, people with altered RAR and sleep disorders have higher risk to develop MS.

3. METABOLIC SYNDROME

Metabolic syndrome is defined as a cluster of metabolic risk factors, such as abdominal obesity, high blood pressure, high levels of fasting plasma glucose and dyslipidemia (Alberti et al. 2009), that increases the risk of cardiovascular disease and type 2 diabetes mellitus (Hanson et al. 2002, Motillo et al. 2010). It is shown that people with MS are exposed at twice the risk of developing cardiovascular diseases over the next five to ten years compared to people without the syndrome and five-fold increase in risk for type 2 diabetes mellitus (Alberti et al. 2009).

In 1988 Reaven identified the MS, originally called Syndrome X, like a syndrome originated from insulin resistance. However, the first formalized definition of the MS was proposed in 1998 by a consultation group based on the definition of diabetes for the World Health Organization (Alberti and Zimmet 1998). After many years of discussions, in the first decade of XXI century, the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute tried to reconcile the different clinical definitions. Nevertheless, they did not come to an agreement on the definition of the limit values for the waist circumference. Indeed, until now the definition of cut-off for waist circumference was related to the countries. To date, the diagnosis of MS requires the presence at least three of the following five factors: *hypertension, abdominal obesity, low levels of High-Density-Lipoprotein* (HDL), *high levels of triglycerides and fasting glucose*. Table 1 shows the limit values of the parameters to be considered affected by the MS. In relation to the waist circumference parameter, Table 2 shows the cut-off for different countries.

MS PARAMETERS	CUT-OFF
Triglycerides	≥150 mg/dL
HDL	<40 mg/dL in males <50 mg/dL in females
Systolic/Diastolic blood pressure	≥130/85 mmHg
Fasting plasma glucose	≥100 mg/dL
Waist circumference	country specific definitions (Table 2)

Table 1. *Definition criteria for the diagnosis of metabolic syndrome.*

COUNTRIES	FEMALES CUT-OFF	MALES CUT-OFF
United States	≥102 cm	≥88 cm
Ethnic Central and South American	≥90 cm	≥80 cm
Canada	≥102 cm	≥88 cm
Europe	≥102 cm	≥88 cm
Asia (including Japanese)	≥90 cm	≥80 cm
China	≥85 cm	≥80 cm
Middle East, Mediterranean	≥94 cm	≥80 cm
Sub-Saharan African	≥94 cm	≥80 cm

Table 2. *Definition criteria for waist circumference in the metabolic syndrome related to different countries.*

3.1 Prevalence, incidence and etiology of metabolic syndrome

The prevalence and incidence of MS is difficult to evaluate due to heterogeneity of the involved factors. Saklayen (2018) estimated that MS is about three times more common than diabetes, the global prevalence can be estimated to be about one quarter of the world population. In 2017, about one third of US adults had MS. In China, in the same year, the incidence of MS was about 15.5% (Saklayen 2018). In a study of 2015 on 8468 subjects from different European countries, Scuteri et al. (2015) showed that the prevalence of MS in Greece and Spain was just under 40%, in Germany and Netherlands about 30%, while in Portugal, Belgium and Italy about 20%.

The assessment of prevalence and incidence of MS is also influenced by the same criteria used to define the syndrome. In a study of Tabatabaei-Malazy et al. (2021) the Iran prevalence rate of MS based on the National Cholesterol Education Program - Adult Treatment Panel III 2004 (ATP III) was 38.3%, 43.5% for the International Diabetes Federation (IDF), 40.9% for the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria and 47.6% for the Joint Interim Statement (JIS) classification.

Furthermore, the prevalence of MS has a direct association with age and smoking, besides it has reverse association with level of education (Masulli et al. 2006, Buckland et al. 2008, Han et al. 2020). Also sex would seem to play a role in the MS development, with males that are more at risk of developing MS. Several studies reported that MS was less common in females than males (Buckland et al. 2008, Roomi et al. 2019, Han et al. 2020). Although not yet confirmed, it has been hypothesized that the lower risk in female is related to the protective effects of oestrogen (Ng 2007). Moreover, it is shown a sex influence on each of the MS parameters. Mirmiran et al. (2008) showed that males had higher level of waist circumference, triglycerides, blood pressure, and lower levels of HDL compared to females. Miccoli et al. (2004) found that males had higher blood pressure, fasting

glucose, and triglycerides than females and Mulè et al. (2021) showed higher levels of waist circumference and fasting glucose in males compared to females.

The etiology of MS concerns a complex interaction between genetic (Janja 2007), metabolic (Ferrannini et al. 1991, Munoz Contreras et al. 2013), and environmental factors (Park et al. 2003, Mirmiran et al. 2008, Buckland et al. 2008).

Some studies investigated the major genetic loci related to the development of the MS. Kissebah et al. (2000), by genomewide scan, showed in more than 2000 Caucasian subjects an influence of different genes quantitative trait locus on chromosome 3q27 and 17p12. The first one was strongly linked to 6 traits: weight, waist circumference, leptin, insulin, insulin/glucose ratio, and hip circumference. The second one was strongly linked to plasma leptin levels. By contrast, McCarthy et al. (2003) investigated 110 candidate genes among coronary artery disease patients with metabolic abnormalities. They showed polymorphisms in 8 genes associated with MS. These alterations are related to different genes (Low-Density- Lipoprotein receptor, glycogen branching enzyme, interleukin 1 receptor type I, transforming growth factor beta-1, etc).

Different studies showed the relationship between metabolic factors and MS. Ferrannini et al. (1991), in a sample of just under 3000 subjects, showed a very high degree of relationship between the six conditions considered (obesity, Type 2 diabetes, hypertension, impaired glucose tolerance, hypertriglyceridemia, and hypercholesterolemia). They affirmed that a very different prevalence of one condition would probably affect the rate of occurrence of dual or multiple combinations of other conditions. Moreover, resistance to insulin-stimulated glucose uptake seems to modify biochemical responses in a way that predisposes to metabolic risk factors (Ferrannini et al. 1991). Also, Munoz Contreras et al. (2013) supposed that insulin resistance, associated to obesity, was considered the principal cause of MS. Vascular diseases and diabetes risk related to MS have been additionally associated to genetic susceptibility (over 20 susceptible loci) (Shu et al. 2010). When the β-cell functions correctly, the normal tolerance to glucose is maintained. In order for cell expansion to occur, an increase of the peptide signals like glucagon 1 (GLP-1) is required to promote proliferation, neogenesis and prevents apoptosis of β-cells (Levine et al. 2008). Prolonged hyperglycemia prevents these signals from performing effective cell regeneration, resulting in increased cell death by apoptosis (Munoz Contreras et al. 2013). Moreover, insulin plays an important role in the regulation of blood pressure, acts as a vasodilator by means of the liberation of nitric oxide from the vascular endothelium and facilitates tubular reabsorption of sodium through the endothelial action of the Angiotensin Converting Enzyme system (Chaudhary et al. 2011). In fact, patients with insulin resistance display hypertension and dyslipidemia comorbidities. The mechanisms by which insulin resistance induces atherogenic dyslipidemia are related to the hepatic accumulation of fatty acids, which promote triglyceride synthesis and production of low-density lipoprotein (Rader 2007).

By contrast, different environmental factors increase the risk to develop the MS. The principal environmental factors are bad nutrition and sedentary behaviors. The bidirectional association between obesity and high caloric intake, low physical activity levels or sedentary behaviors exposes people to develop MS. Park et al. (2003) showed that overweight or obese people were much more likely to have MS. Nevertheless, abdominal obesity and BMI play an important role in the development of MS (Buckland et al. 2008). Buckland et al. (2008) showed that obese people were almost twenty times more likely to develop MS compared to normal weight individuals. Referring to syndrome etiology, Hansen et al. (2006) showed that abdominal obesity and central body fat alter the processes of lipolysis, lipogenesis, fatty acid uptake and expression/secretion of inflammatory and hormonal factors. In this context, physical activity can play an important role as preventive or curative treatment for obesity and other problems related to a sedentary lifestyle. Due to its effect on the lipid profile, insulin resistance and overweight/obesity, it has been shown that high levels of physical activity are associated with a reduced risk of MS. Several studies have shown that active people, both male and female, had a lower risk to develop MS than inactive people (Zhu et al. 2004, Kim et al. 2011, Choi and Ainsworth 2016, Li et al. 2018).

4. METABOLIC SYNDROME AND REST-ACTIVITY CIRCADIAN RHYTHM

Although there are growing observational and experimental evidences that a good level of physical activity can decrease the risk of developing MS (Buscemi et al. 2014, Miglani et al. 2015), and reduce its parameters related to overweight, waist circumference, blood pressure, triglycerides and fasting plasma glucose (Zhu et al. 2004, Wang et al. 2018), few studies have taken into account the importance of the movement linked to daily activity evaluated by RAR (Sohail et al. 2015, Mulè et al. 2021).

4.1 Actigraphy

One of the most used method to objectively evaluate the daily activity movement during the 24 hours is actigraphy. The tool used is the actigraph, an accelerometer similar to a watch designed to record the intensity of movement as a function of time (Mirja et al. 2015). Different typologies, brands and models of actigraph exist, which use different hardware, software and set of algorithms with similar functionalities. Many of them record the daily activity movement, others the physical activity level, expenditure energy or the position of the body in the space. The actigraphs could be uniaxial, biaxial or triaxial (Corder et al. 2007). The most common methods used to digitize the actigraph signal 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. 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 to time above threshold (Gorny and Spiro 2001).

Regarding the part of the body where the actigraph could be worn, there are several theories. A typical positioning of the accelerometer to evaluate physical activity is the hip, as it allows to detect the acceleration and deceleration of the body during locomotion (Trost et al. 2005). Uncommon body parts were also used, such as the head or ear, which were immediately less used due to the poor acceptance and comfort of the participants. However, the use of wrist positioning for measuring physical activity has grown due to better acceptance and longer application time by the subject compared to other application points (application time to average skin of 99% on the wrist versus 65% in other districts) (Mirja et al. 2015).

4.2 Rest-activity circadian rhythm evaluation in metabolic syndrome

Optimal levels of daily movement and physical activity allow to maintain healthy status and are largely used as primary or secondary intervention in many pathologies. Several studies showed a positive effect on the metabolic and cardiovascular factors related to MS (Myers et al. 2019). Active

people, both male and female, were less prone to developing MS than inactive people (Zhu et al. 2004, Kim et al. 2011, Choi and Ainsworth 2016, Li et al. 2018). Moreover, Kim et al. (2013) showed that subjects who spent more time in light-intensity activity and less time in sedentary activity are less likely to develop MS. The positive effects of physical activity are mediated by improvements in related parameters, particularly through increased HDL cholesterol concentrations (Leon and Sanchez 2001, Skoumas et al. 2003, Mostafavi et al. 2015), decreased in triglyceride levels (Fahlman et al. 2002) and blood pressure (Katsmarzyk et al. 2003, Moreau et al. 2001), improved glucose tolerance and insulin sensitivity (Yates et al. 2007, Venkatasamy et al. 2013, Bird and Hawley 2017). Wewege et al. (2018) performed a meta-analysis to evaluate the effect of aerobic and resistance exercise among individuals with the MS. Aerobic exercise displayed higher effect compared to resistance interventions, improving all the parameters linked to MS: waist circumference, fasting glucose, HDL cholesterol, triglycerides and diastolic blood pressure. Additionally, vigorous intensity aerobic exercise, performed 3 days/week for ≥12 weeks, offered greater and more widespread improvements. Although the results seem to better support the use of aerobic exercise in patients with MS, they could be influenced by the fewer studies evaluating resistance interventions compared to aerobic activities. Another review displayed that a program of aerobic exercise performed in people with MS determined a decrease of BMI, waist circumference, systolic blood pressure and diastolic blood pressure, fasting plasma glucose, triglycerides compared to control group. Similar modifications were also observed using combined aerobic and resistance exercises (Ostman et al. 2017). Both reviews also reported improvement in cardiorespiratory fitness in participants after the physical activity training.

Although the positive effects on health of the daily physical activity are widely known, several studies have reported that a large portion of the world population spends most of the day in sedentary behaviors. In a review by Park et al. (2020), it was reported that approximately 31% of the global population aged ≥15 years practice inadequate level of physically activity, and the prevalence increases in older people (Harvey et al. 2013). In particular, the physical activity level of South Koreans was decreasing among adults aged ≥19 years. Therefore, in 2017, the rates of aerobic exercise, walking, and muscle training in the Korean adult population were 48.5%, 39.0%, and 21.6%, respectively, with most of the Korean population engaging in physical inactivity. Furthermore, Americans spend 55% of their time (7.7 hours a day) in sedentary behaviors, while Europeans spend 40% of their waking time (2.7 hours a day) watching television. Additionally, COVID-19 pandemic and the related limitation helped to increase the sedentary habits. A review of Stockwell et al. (2021) reported that most of the studies highlight overall decreases in the amount of physical activity post-COVID-19 versus pre-COVID-19 lockdown.

In relation to MS, another important point for wellness, which received little attention concerns the daily activity. However, it should not be considered as the practice of sporting activities, but the total amount of movement done during the day. As reported, the objective method used to detect the movement during the 24 hours is the evaluation of RAR by actigraphy.

Although altered RAR is associated with compromised health status in psychiatric subjects, people with greater cardiovascular risk and accelerated progression of cancer (Mormont and Waterhouse 2002, Paudel et al. 2011, Roveda et al. 2018, Roveda et al. 2019), few studies have investigated the relationship between RAR alteration and MS. Sohail et al. (2015) reported that subjects with a more regular activity rhythm showed a lower risk of developing MS or its components, such as hypertension, diabetes, dyslipidemia, and obesity. In particular, a higher interdaily stability was associated with an odds ratio of 0.69 of having the MS and a lower odd of having multiple components of the MS. Furthermore, the authors demonstrated that having a robust rhythm (high level of interdaily stability) was associated with lower risk to develop cardiovascular disease (odds ratio: 0.83). Sex also seems to play a role in the development of MS, in particular, males are the sex most at risk (Buckland et al. 2008, Roomi et al. 2019, Han et al. 2020). Mulè et al. (2021), who have investigated the sex differences in RAR in people with MS, showed differences between male and female groups in MESOR, Amplitude, Intradaily Variability and M10 (daily activity in people with normal sleep-wake cycles). Compared to males, females showed higher MESOR, Amplitude, and M10 values, indicating a greater daily activity, and lower IV value, suggesting a less fragmentated RAR. Also referring to MS parameters, the male participants displayed a worse condition than females, showing higher values of waist circumference and fasting glucose.

These preliminary evidences suggest the importance of considering the evaluation of RAR in subjects with MS. Not only a constant practice of physical activity is important in primary and secondary prevention of many pathologies, but also the role of daily movement should also be considered and not underestimated. It would be useful to encourage strategies aimed to increasing daily activity and decreasing sedentary behaviors, especially in people less inclined to engage in physical activity, such as using public and private means of transport as little as possible to move or prefer stairs to elevators. Practicing sports every day is certainly a good strategy to reduce the sedentariness, even if it is often not enough; especially if you spend most of the hours of the day doing sedentary activities. The daily activity may have particular importance in some pathological condition, such as MS in which the presence of overweight or obesity can produce a vicious circle that negatively affects the maintenance of an active life.

5. METABOLIC SYNDROME AND SLEEP QUALITY

Environmental factors, as well as genetic and metabolic factors, can play an important role in the development of MS. Along with physical inactivity and poor nutrition, sleep disorders are increasingly emerging as factors that can increase the risk to develop MS.

Several prospective studies have documented the relationship between sleep disturbances, including sleep breathing disorders and sleep duration, and an increased risk of developing MS components (Nilsson et al. 2004, Gangswich et al. 2007, O'Connor et al. 2009). Troxel et al. (2010), in a 3-year prospective study, showed that people who snore frequently and loudly tripled the risk of developing MS and in those who had difficulty falling asleep it increased by 80%. Moreover, they demonstrated in a population without MS at baseline, that loud snoring predicted the development of the MS. By analyzing each component of the MS individually, the study also showed the existence of a relationship between loud snoring and the development of hyperglycemia and HDL abnormalities.

In addition, a few cross-sectional studies have shown that a wider range of self-reported sleep disorders, including snoring, short sleep duration, difficulties in initiating and maintaining sleep, and poor sleep quality are associated with MS and its components (Leineweber et al. 2003, Gruber et al. 2006, Hall et al. 2008, Nock et al. 2009). In particular, self-reported bad sleep quality is related to high levels of waist circumference and fasting glucose concentration, as well as to high levels of BMI, fat percentage, fasting insulin concentration and estimated insulin resistance (Jennings et al. 2007). Hung et al. (2013) showed that subjects with MS had poor sleep, indicated by higher Pittsburgh Sleep Quality Index (PSQI) score; in addition, a higher prevalence of poor sleepers compared to subjects without the syndrome has been reported. They also showed that hyperglycemia and low HDL were positively associated with bad sleep score. Other studies have shown a positive correlation between obstructive sleep apnea and MS (Coughlin et al. 2004, Vgontzas et al. 2005, Xu et al. 2015). Kielbasa et al. (2016) showed that people with MS displayed higher prevalence of sleep disorders than people without the syndrome. Furthermore, the occurrence of MS exposed subjects to 1.7 times higher risk to develop the obstructive sleep apnea.

5.1 Sleep quality and metabolic syndrome development

Many studies regarding sleep used actigraphy as a valid substitute for the polysomnography, because it allows to easily record data for days or even longer periods (Kushida et al. 2001, Ancoli-Israel et al. 2003, So et al. 2005). Actigraphy is an objective method used for the sleep evaluation and it is commonly utilized to diagnose insomnia, circadian rhythms disorders and excessive sleepiness (American Sleep Disorders Association 1995, Sadeh et al. 1995). The actigraph position on the non-dominant wrist is usually used for sleep assessment to optimize the recording of small movements that occur at the distal extremities when the individual is supine (Ancoli-Israel et al., 2003).

Nevertheless, few studies investigated the relationship between MS and sleep disorders using an objective method like actigraphy. Matricciani et al. (2021) found a worse MS severity score in the late sleep adults. Huang and Redline (2019) showed that irregular sleep duration and timing were associated with higher incidence of MS and multiple metabolic disorders. In particular, higher variability in sleep duration and timing were associated with a greater number of altered metabolic factors. Moreover, sleep duration variability was associated with all metabolic components of the syndrome, except high blood pressure, and sleep timing variability was associated with central obesity, low HDL cholesterol, and high fasting glucose. In a study of Chin et al. (2010) it is shown an association between the increased prevalence of MS and the severity of obstructive sleep Apnea. However, the severity of obstructive sleep apnea had no significant effect on the onset of MS after adjustment for age and BMI. The authors also reported that the prevalence of severe obstructive sleep apnea in subjects with MS was higher than in healthy subjects, and that severe patients with obstructive sleep apnea and people with MS exhibited a shorter sleep duration in bed.

5.2 Sleep disorders and metabolic syndrome

5.2.1 Obesity, altered glucose metabolism and insulin resistance

The relation between short sleep duration and MS may be explained by a bidirectional association between sleep disorders and obesity, glucose metabolism and insulin resistance (Wu et al. 2014, Koren et al. 2015, Koren et al. 2016).

In people who display a short sleep duration, leptin levels decrease and ghrelin levels increase, supporting an effect on appetite regulation with an increase of hunger (Spiegel et al. 2004, Taheri et al. 2004, Chapman et al. 2012). These mechanisms underline the increased risk and prevalence of obesity in this population. Indeed, people that sleep less than 7 h/day showed high level of BMI (Kripke et al. 2002, Gupta et al. 2002, Hasler et al 2004). Chaptu et al. (2007) showed lower level of leptin in people that slept less than 6 hours compared to people that slept more. Furthermore, authors found an odds ratio of 1.69 to be overweight or obese in people that slept 5-6 hours; the odds ratio decreased with the increase of sleep hours (1.38 for 7-8 hours of sleep and 0.89 for 9-10 hours of sleep). Moreover, they showed lower body weight and adiposity indices (body fat mass, percentage of body fat, waist circumference, waist-to-hip ratio, abdomen thickness, etc.) in people who slept 7 hours or more than worse sleepers. In addition, it must also be considered that the increasing prevalence of obesity has led to an increase in the prevalence of sleep disorders due to respiratory problems in the general population. Poor sleep quality at night leads to excessive daytime sleepiness, encouraging sedentary habits (Akinnusi et al. 2012).

Sleep restriction was also associated to reduction of thyroid-stimulating hormone and testosterone levels, disrupt the pattern of Growth Hormone (GH) secretion, and elevate levels of proinflammatory cytokines. These complex endocrine changes might contribute to impaired insulin signaling in

peripheral tissues (Mesarwi et al. 2013). The onset of slow-wave sleep has been shown to be associated with decreased use of glucose by the brain, stimulation of GH release, inhibition of cortisol secretion, decreased sympathetic nervous system activity and increased vagal tone. All these factors affect glucose homeostasis throughout the body (Spiegel et al. 2009). Moreover, low sleep quality and high perceived sleep debt were associated with reduction in glucose control, as well as increase of insulin resistance (Tasali et al. 2009). Tasali et al. (2008), in healthy young adults, investigated the selectively suppressing slow-wave sleep (reducing it by 90%) on glucose tolerance, without reducing total sleep duration. Such low levels of slow-wave sleep are typical in the elderly and in people with sleep disorders like obstructive sleep apnea. The authors reported that after suppression of slow-wave sleep insulin sensitivity decreased by approximately 25%, not compensated by an increase in the insulin response to glucose. Also, glucose tolerance was reduced by approximatively 23%. As a result, reduced insulin sensitivity was strongly correlated with decreased slow-wave sleep.

Furthermore, sleep disorders alter cellular signaling associated with mitochondrial respiratory function and insulin signaling pathway, like AMP-activated protein kinase (AMPK) and insulin/insuline-like growth factor 1 (IGF-1) signaling (Saner et al. 2018). Phosphorylation of Akt, a crucial step of the phosphatidylinositol 3-kinase pathway that mediates most metabolic actions of insulin, was found markedly impaired after sleep restriction (Broussard et al. 2012).

Therefore, the reduced quality and quantity of sleep determines alterations in sympathovagal balance and hormonal response, affecting the hypothalamo-pituitary axes, mitochondrial respiratory function and insulin signaling pathway, as well as dysregulation of neuroendocrine control of appetite. All these factors cause development of impaired glucose metabolism, insulin resistance and obesity, resulting in an increased risk of developing metabolic diseases and health problems, including MS.

5.2.2 Misalignment of circadian rhythms

The circadian system controlled glucose and lipid homeostasis. Misalignments of circadian rhythms are associated with increased risk of developing obesity, diabetes and MS (Shanmugam et al. 2013). People that ate and slept 12 hours out of phase from their habitual time showed a circadian desynchronization that affects the daily cortisol rhythm and the level of leptin, determining hyperglycemia, hyperinsulinemia and hypertension (Scheer et al. 2009). Several enzymes involved in lipid homeostasis follow circadian rhythms, as lipin1 (involved in sugar and lipid metabolism), synaptojanin-2, disabled-2 (proteins involved in vesicular trafficking, cytoskeletal dynamics and exo-/endocytosis) and membrane-bound Niemann-Pick C as well as other enzymes involved in cholesterol biosynthetic pathway. Therefore, circadian disruption can lead to alteration of lipid homeostasis in the blood, resulting in an increased risk for metabolic and cardiovascular diseases (Shanmugam et al. 2013).

As known, in many cases modernization can lead to a misalignment between rhythms of biological functions and social commitments. The use of artificial light, exposure to night light, varying the length of the day and the use of electronic devices can contribute to develop sleep alteration that cause circadian disruptions by affecting the secretion of melatonin (Rodenbeck et al. 1999, Xie et al. 2017). Melatonin plays an important role in energy balance, as insulin secretion that appears to be coupled with melatonin secretion. Therefore, the alteration of the circadian rhythm of melatonin generates a cascade of events that expose subjects to a greater risk of developing alterations in the levels of triglycerides, HDL, weight, blood pressure and fasting glucose and consequently to the MS (Shanmugam et al. 2013, Hill et al. 2015).

5.3 Metformin treatment in the management of sleep disorders

Metformin is an insulin sensitizer drug, and it is widely used for the reduction of insulin resistance and the control of glucose metabolism, mediated by its effect on insulin, IGF-1 and Akt pathway, in various cardiometabolic diseases and in MS (Orchard et al. 2005, Tosca et al. 2010, Pierotti et al. 2013, Franciosi et al. 2013, Sanchez-Rangel and Inzucchi 2017, Foretz et al. 2019). Recently, metformin was also introduced as adjuvant agent for the management of sleep disorders. The wide use of this drug in medical approaches is based on the fact that metformin has not significant adverse effects. Metformin usually does not cause hypoglycemia, although low blood sugar may occur if this drug is used with other anti-diabetic drugs. Despite this product may contain inactive ingredients, which can cause allergic reactions or other problems, serious allergic reaction to this drug is rare. Another side effect of the use of metformin is on renal function. Due to this aspect, it is important modulate or avoid the use of the drug in subjects with or exposed to renal function problems. By contrast, gastrointestinal intolerance is one of the most frequently occurred, as well as myocardial infarction, although it is found less in metformin compared with other similar agents (Nasri and Rafieian-Kopaei 2014, Hsu et al. 2017, Corcoran and Jacobs 2021). Moreover, it can cause a condition called lactic acidosis, which usually occurs due to a drug overdose or in some contraindicated conditions (Nasri and Rafieian-Kopaei 2014). However, lactic acidosis associated with metformin is a rare condition with an estimated prevalence of on to five cases per 30 000 (Wang et al. 2017). Regarding the use of metformin in the management of sleep disorders, it is shown to have an opposite effect to sleep disorders on the same parameters. In particular, one of the metformin effects is to promote the activation of AMPK, which stimulates fatty acid oxidation, promotes glucose transport and glycolysis, and inhibits gluconeogenesis. Moreover, it affects insulin and IGF-1/Akt pathways, as well as testosterone and IGF-1 blood concentration, all processes affected by sleep disorders (Tasali et al. 2008, Spiegel et al. 2009, Tasali et al. 2009, Broussard et al. 2012, Mesarwi et al. 2013, Saner et al. 2018). Data from animal experiments support a relationship between metformin treatment and management of sleep disorders (Ramadan et al. 2006, Ramadan et al. 2007), but the

few preclinical data remain controversial (Wiwanitkit S. and Wiwanitkit. F. 2012). Kajbaf et al. (2013) showed that people with type 2 diabetes treated with metformin had higher levels of sleep quality and quantity compared to untreated people. Moreover, El-Sharkawy et al. (2014) reported that girls with polycystic ovarian syndrome treated with metformin have decreased the daytime sleepiness and sleep disorders.

Although it is shown that people with MS suffer from sleep disorders and that the use of metformin may improve the quality and quantity of sleep, so far no studies have investigated the effect of metformin treatment on sleep disorders in patients with MS.

Another point of interest concerns the role of physical activity on sleep and MS. Some studies have highlighted the positive effects of physical activity training on both sleep (Loprinzi et al. 2011, Kredlow et al. 2015) and MS (Lakka and Laaksonen 2007, Myers et al. 2019). Exercise, by inducing activation of AMPK and transcription of the g receptor (PGC-1α) activated by the peroxisome proliferator, leads to an increase in mitochondrial content and function by improving insulin sensitivity, and interacts closely with clock genes improving sleep (Saner et al. 2018). As reported above, some of its effects are similar in metformin treatment, with a positive effect on MS condition. Orchard et al. (2005) have investigated the effect of metformin and lifestyle intervention on MS condition both in people with and without MS. The lifestyle intervention consisted of following a healthy low-calorie and low-fat diet and engaging in physical activity of moderate intensity, such as brisk walking, for at least 150 minutes per week. The authors showed an improvement in MS condition both in people treated with metformin and in people following the lifestyle intervention. Therefore, the use of these two treatments together could have a double beneficial effect both on sleep and MS parameters. This represents a still unexplored field of research, but which could be of great interest.

6. AIM OF THE STUDY PROJECT

During my PhD course, in collaboration with Fondazione IRCCS Istituto Nazionale dei Tumori of Milan, I conducted a project which was part of a larger trial socalled Me.Me.Me (Metabolic syndrome, Metformine and Mediterranean diet).

The Me.Me.Me study starts from the hypothesis that an important part, about 25-33%, of the incidence and mortality caused by chronic age-related diseases could be prevented through an overall change in lifestyle. These changes include (1) a moderate calorie and protein restriction, obtainable with a traditional Mediterranean and macrobiotic diet, (2) an increase in physical activity level, and/or (3) preventive treatment with metformin, a drug whose mechanism of action involves the activation of the same genes activated by calorie restriction and physical activity (Richter EA et al. 2009). The main goal of the Me.Me.Me study is to reduce the total incidence of major age-related chronic diseases (malignant tumors, ischemic heart disease, stroke, diabetes), the second purpose includes a reduction in the prevalence of MS and its anthropometric incidence and metabolic components (abdominal obesity, hypertension, dyslipidemia, hyperglycemia).

The aim of my PhD project was to assess whether changes in daily habits are able to modify sleep, daily activity levels, sedentary habits and the MS parameters in healthy people with MS included in the MeMeMe study. Moreover, the project aimed to investigate whether metformin treatment can modify sleep and MS parameters. We also hypothesized to evaluate whether a physical activity protocol could provide an additive effect to that hypothesized by metformin on RAR, sleep and MS parameters.

7. MATHERIALS AND METHODS

7.1 Participants

The subjects recruited in my PhD project were a small part of the sample of the Me.Me.Me study (ERC-AdG-2012 n.322752). I recruited 133 healthy subjects with MS (74 females and 59 males). Before beginning the study, all risks and benefits were explained and informed consent was obtained from each participant. We then recorded anthropometric and MS parameters and checked for exclusion criteria.

The inclusion criteria were:

- presence of MS, based on at least three out of five of the following parameters (Alberti et al. 2009): fasting plasma glucose ≥100 mg/dL, blood pressure ≥130/85 mmHg, HDL <50 mg/dL for women and <40 mg/dL for men, triglycerides ≥150 mg/dL and waist circumference of ≥100 cm for men and ≥85 cm for women (Haftenberger et al. 2002);
- age ≥50 yars.

The exclusion criteria were:

- diagnosis of diabetes or fasting plasma glucose >126 mg/dL in two consecutive blood samples;
- concomitant treatment with metformin, an insulin sensitizer drug;
- serum creatinine >124 μL/L;
- proteinuria;
- renal, cardiac, or hepatic insufficiency;
- excessive alcohol consumption;
- diagnosis of cancer in the last 5 years.

7.2 Measurements

Participants were examined for:

- *anthropometric characteristics* (height, weight, BMI, waist circumference); the measurements were made with a body segmental impedance balance, BC-418 (Tanita); height and weight were measured without shoes and heavy clothes; waist circumference was measured with a tape in the mid-point between the lowest rib and the iliac crest during exhale;
- *systolic and diastolic blood pressure* that were measured in triplicate (every 10 min) using an electronic sphygmomanometer (DynaPulse 5000A Pulse Metric, Inc., Vista, CA, United States), with the cuff placed on the left arm and the subject supine, after 10 min of rest;
- *fasting glucose* (mg/dL), *total cholesterol* (mg/dL), *high density lipoproteins* (HDL, mg/dL), and *triglycerides* (mg/dL). They were assayed in blood samples collected between 8:00 and 9:30, after overnight fasting;

- *continuous 7-day actigraphic monitoring* to detect RAR and sleep quality. The actigraph Actiwatch (Cambridge Neurotechnology, Cambridge, UK) was worn on the non-dominant wrist, and participants were instructed to remove it when swimming or bathing. Subjects received a diary to record bed-time, getting up time, hours of naps, hours without wearing the actigraph, and the number of nocturnal awakenings. Actigraphic monitoring started at the first clinical visit and was completed for all subjects within 30 days. During monitoring, subjects continued their usual activities of teaching, office work, or housework.

All measurements were made at study enrollment (baseline) and after one year of intervention (follow-up).

7.3 Study design

The Me.Me.Me study provided that participants enrolled in the study were randomized by means of a balanced computerized system that stratifies candidates by gender and age. People received double randomization based on drug (metformin or placebo) and dietary approach (cooking courses organized by the researchers or general awareness). A software double-blind randomized the assignment of placebo or metformin to the participants and also sent the instructions for preparing the tablet bottles to the pharmacy. The Me.Me.Me study provided, for the participants already randomized, a treatment with metformin or placebo 850 mg twice/day (1700 mg total/day). In case of intestinal disorders after randomization, participants were asked to continue the study by taking only one tablet. Instead, the dietary approach randomization consisted in participating either in all the cooking courses organized by the researchers or in general awareness courses (about 4 times). These courses consisted of raising people's awareness of a healthy lifestyle, following the guidelines established by the WHO for recommended physical activity levels and a healthy nutrition.

Therefore, based on pharmacological and dietary approach I divided the sample in four groups:

- Metformin group
- Placebo group
- Metformin + diet group
- Placebo + diet group

7.3.1 Physical activity intervention

The research project initially also involved the application of a physical activity protocol as a complementary strategy to improve RAR, sleep and MS parameters, thus modulating the main risk factors for the health in people with MS. I planned to divide the participants into an intervention group that should have attended a structured aerobic exercise (AEI) and a control group. So, considering both the physical activity program and the two treatments, I should have had four equal groups:

- Metformin group
- Placebo group
- Metformin + diet + AEI group
- Placebo + diet + AEI group

Unfortunately, due to the COVID-19 pandemic and related restrictions, the implementation of this part of the project, which provided for the participants to carry out a structured program of physical activity, was not possible to execute.

7.4 Data analysis

Shapiro-Wilk test was used to check the normality of the data for all variables (anthropometric, cardiovascular, metabolic, RAR and sleep quality). Moreover, Levene test was performed to evaluate the equality of variances between groups. Based on their results I performed:

- unpaired parametric or non-parametric test was used to investigate differences at baseline;
- paired parametric or non-parametric test was performed in the after-before analysis;
- analysis of variance (ANOVA) or Kruskal-Wallis test was used to compare people with different number of altered MS parameters;
- general linear model was used to investigate the effect of the two treatments on MS, sleep and RAR parameters. Due to the sample size, I only considered the randomization based on drug treatment. Then the sample was divided in two groups, adjusting the analyzes for dietary approach, age, sex and values at baseline. Post-hoc analyses were performed if necessary, adjusting the p-values with Bonferroni adjustment.

The subjects enrolled in the present study were a small part of the sample of the Me.Me.Me study; for this reason no sample size and power calculation were made.

7.4.1 Actigraphic data: rest-activity circadian rhythm

The Actiwatch Software (Actiwatch Sleep Analysis Software) was used to obtain the activity levels, expressed in activity counts (a.c.) and recorded every 60 s over the duration of monitoring (7 days). I used a low actigraphic sensitivity threshold (20 counts per epoch).

According to diary entries, subjects removed the actigraph, on average, once a day for about 30 min. To avoid bias, this period was not included in the data analysis. To determine the RAR characteristics, the activity data were analyzed using parametric and non-parametric analysis.

The *single cosinor method* was used for the parametric analysis. It describes the time course of activity using an oscillatory function $f(t) = M + A \cos(\omega t + \varphi)$ (Halberg et al. 1977, Nelson et al. 1979). This function returns three parameters (M is the MESOR – Midline Estimating Statistic of Rhythm; A is the Amplitude; φ is the Acrophase) that depict a parametric portrait of the activity rhythm for each subject. Briefly, the MESOR is the rhythm-adjusted mean that approximates the

arithmetical mean of the data for a given period. The Amplitude measures half the extent of the rhythmic variation in a cycle. The angular frequency of oscillation is ω and corresponds to the ratio $2\pi/t$, where t is the period of oscillation. The Acrophase indicates the time interval within which the highest activity values are expected. Instead, the population-mean cosinor method was used to investigate the presence of RAR in each group (Nelson et al. 1979).

By contrast, the magnitude of stability and fragmentation of RAR for each subject were analyzed with a non-parametric statistical method (Blume et al. 2016) to study non-parametric indices, including Interdaily Stability (IS), Intradaily Variability (IV), the average activity during the least active 5-h period (L5), L5 start time ($L5_{st}$), the average activity during the most active 10-h period (M10), and M10 start time ($M10_{st}$). IS indicates the day-to-day variability, providing information on the RAR synchronization with environmental stimuli; it ranges from 0, lack of synchronization, to 1 for perfect synchronization. IV provides information about RAR fragmentation, for example, due to a daytime nap or waking up in the night, which leads to a failure to maintain adequate activity levels over the 24-h profile. It ranges from 0 to 2, with higher values indicating a more fragmented rhythm. L5 indicates the nocturnal activity in people with normal sleep-wake cycles, but in nocturnal workers, it represents the diurnal activity. $L5_{st}$ gives information about the start time of L5 parameters. M10 is the daily activity in people with normal sleep-wake cycles, and in nocturnal workers, it represents the nocturnal activity. $M10_{st}$ gives information about the start time of M10 parameters.

7.4.2 Actigraphic data: sleep quality

Actigraphic monitoring also allowed us to investigate the sleep quality, evaluating seven sleep parameters calculated automatically by the Actiwatch Sleep Analysis Software.

- *Actual sleep time*: the amount of time, expressed in hours and minutes, between sleep start and sleep end. It is determined by adding up the number of epochs below the sensitivity threshold and then multiplying that value by the epoch length in minutes.
- *Actual wake time*: the amount of time, expressed in hours and minutes, between sleep start and sleep end. It is determined by adding up the number of epochs below the sensitivity threshold and then multiplying that value by the epoch length in minutes.
- *Sleep efficiency*: the percentage of time in bed actually spent sleeping.
- *Sleep latency*: the amount of time, expressed in minutes, between sleep onset and retiring to bed. An algorithm, based on lack of movement after bedtime, automatically calculates this.
- *Mean activity score*: the average activity score recorded between sleep start and sleep end.
- *Immobile time*: total time, expressed in percentage of no movement recorded between sleep start and sleep end.
- *Fragmentation index*: the sum of the "mobile time" (percentage of time spent in movement) and the "immobile bouts" (percentage of immobile <1-min periods).

8. RESULTS

8.1 Baseline conditions

The total sample of 133 healthy subjects with MS (mean age 63.9 ± 0.5) was composed by 55.6% of females (N=74; mean age 63.6 ± 0.7) and 44.4% of males (N=59; mean age 64.3 ± 0.8).

8.1.1 Anthropometric and metabolic syndrome characteristics

Table 3 shows the anthropometric and MS characteristics of the total sample, and of female and male groups.

ANTHROPOMETRIC AND MS PARAMETERS	**TOTAL SAMPLE** *(N=133)*	**FEMALES** *(N=74)*	**MALES** *(N=59)*
Height *(cm)*	165.7 ± 0.8	161.2 ± 0.9*	171.3 ± 0.9*
Weight *(Kg)*	87.3 ± 1.4	82.3 ± 1.6**	93.8 ± 2.3**
BMI *(Kg/m^2)*	31.7 ± 0.4	31.7 ± 0.6	31.8 ± 0.6
Waist circumference *(cm)*	101.8 ± 1.1	97.5 ± 1.3^{+}	107.3 ± 1.5^{+}
Systolic blood pressure *(mmHg)*	147.5 ± 1.4	145.3 ± 1.9	150.3 ± 2.2
Diastolic blood pressure *(mmHg)*	89.4 ± 0.8	88.6 ± 1.0	90.4 ± 1.3
Triglycerides *(mg/dL)*	121.4 ± 4.5	119.6 ± 6.1	123.7 ± 6.8
Fasting glucose *(mg/dL)*	102.3 ± 0.9	99.3 ± 1.2§	106.1 ± 1.3§
HDL *(mg/dL)*	54.6 ± 1.3	57.6 ± 1.8°	50.7 ± 1.7°
Altered MS parameters			
3	39.1%	47.3%	28.8%
4	46.6%	39.2%	55.9%
5	14.3%	13.5%	15.3%

Table 3. *Anthropometric and metabolic syndrome characteristics in the total sample and in female and male groups. Data are reported as mean ± se.* *, **, $^{+}$, $^{§}p<0.001$ *and* $°p<0.008$.

In the total sample the mean value of BMI was 31.7 ± 0.4 that corresponds to being obese. As reported in table 3, 46.6% (N=62) of subjects showed four altered parameters of MS, 39.1% (N=52) had three altered parameters, and 14.3% (N=19) five altered parameters. Females differed from males in the sex-sensitive variables such as height, weight, waist circumference and HDL, as well as in fasting

glucose level. In particular, they were shorter and leaner, but with similar BMI, and showed lower levels of fasting glucose and higher levels of HDL than males. The X^2 test did not show differences of sex in the percentage of number of altered MS parameters (p=0.09). The highest percentage of females (47.3%) had three altered parameters, while the highest percentage of males (55.9%) had four altered parameters.

8.1.2 Sleep characteristics

Actigraphic data for all 133 participants were analyzed to assess the sleep quality. Table 4 shows the sleep parameters for the total sample and female and male groups.

SLEEP PARAMETERS	**TOTAL SAMPLE** *(N=133)*	**FEMALES** *(N=74)*	**MALES** *(N=59)*
Actual sleep time *(hh:mm)*	06:04 ± 00:04	06:10 ± 00:06	05:57 ± 00:07
Actual wake time *(min)*	60.6 ± 1.9	61.4 ± 2.4	59.6 ± 3.1
Sleep efficiency *(%)*	80.3 ± 0.6	80.2 ± 0.8	80.5 ± 1.1
Sleep latency *(min)*	22.1 ± 1.6	21.9 ± 2.2	22.3 ± 2.4
Immobile time *(%)*	83.6 ± 0.5	84.3 ± 0.7	82.7 ± 0.7
Mean activity score *(a.c.)*	21.1 ± 1.2	21.4 ± 1.2	20.6 ± 2.3
Fragmentation index	34.8 ± 1.0	33.5 ± 1.3	36.3 ± 1.6

Table 4. *Sleep parameters in the total sample and in the two sexes. Data are reported as mean ± se.*

At baseline the average amount of hours spent sleeping was 6 hours. Only 29.3% of the population presented a sleep efficiency higher than 85%, and only 15.8% a sleep duration higher than 7 hours; while the 72.9% showed a sleep latency lower than 30 minutes (cut-off value recommended by the National Sleep Foundation; Hirshkowitz et al. 2015, Ohayon et al. 2017). The data suggest that only a small part of the sample had a good sleep condition. No differences were found for all parameters stratifying the sample by sex. Participants with a median sleep efficiency value greater than 85% were more active, and their adherence score to word cancer research food recommendation was higher than participant with lower sleep efficiency values. No statistically significant correlations were found between MS and sleep parameters.

Then, the difference between the severity of MS and the sleep quality was assessed, adjusting the analysis for age. Table 5 shows sleep parameters by stratifying the sample based on MS severity. No

differences were found in the sleep parameters comparing people with 3, 4 or 5 altered MS parameters.

SLEEP PARAMETERS	**3 ALTERED PARAMETERS** *(N=52)*	**4 ALTERED PARAMETERS** *(N=62)*	**5 ALTERED PARAMETERS** *(N=19)*
Actual sleep time *(hh:mm)*	06:08 ± 00:07	06:02 ± 00:07	06:00 ± 00:12
Actual wake time *(min)*	61.1 ± 2.7	58.3 ± 2.7	66.8 ± 5.6
Sleep efficiency *(%)*	80.1 ± 1.1	80.7 ± 0.9	79.6 ± 1.4
Sleep latency *(min)*	21.2 ± 2.9	22.9 ± 2.3	21.8 ± 3.4
Immobile time *(%)*	84.1 ± 0.8	83.6 ± 0.9	82.2 ± 1.3
Mean activity score *(a.c.)*	23.0 ± 2.7	19.0 ± 1.1	22.3 ± 2.3
Fragmentation index	34.0 ± 1.6	34.2 ± 1.5	38.9 ± 2.4

Table 5. *Sleep parameters in the sample stratified by the severity of MS. Data are expressed as mean ± se.*

Subgroup analyses did not reveal statistically significant differences between males and females within each group with 3, 4 and 5 altered MS parameters; even within the male and female groups, no differences were found between subjects with 3, 4 and 5 altered parameters.

8.1.3 Rest-activity circadian rhythm characteristics

Among the 133 subjects, 12 males and 24 females removed the actigraph for several hours during the day, therefore the data could not be used for the RAR analysis. Actigraphic data of the remaining 97 participants, 50 female (62 ± 6.2 years) and 47 male (64 ± 6.0 years), were analyzed using both parametric and non-parametric analysis. The mean period of missing data did not differ between male and female groups.

Parametric analysis

By the single cosinor analysis, a statistically significant RAR was found for all subjects. The population-mean cosinor showed a statistically significant RAR for both male and female groups. Statistically significant differences were found for MESOR and Amplitude between males and females using the Hotelling T^2 test. Specifically, female group showed higher MESOR and

Amplitude values than male group. No statistically significant differences were found in Acrophase between the male and female groups (Table 6).

PARAMETRIC PARAMETERS	**FEMALES** *(N=50)*	**MALES** *(N=47)*
MESOR *(a.c.)*	243.3 ± 20.0*	197.6 ± 17.9*
Amplitude *(a.c.)*	184.5 ± 18.5°	144.2 ± 17.2°
Acrophase *(hh:mm)*	14:55 ± 00:41	14:38 ± 00:44

Table 6. *Parametric analysis of activity level: comparison of RAR parametric parameters between female and male groups. The values are reported as mean ± SD. *p=0.001, °p=0.002.*

Figure 4 shows the cosine functions fitting the activity level data derived by population-mean cosinor analysis for male and female groups.

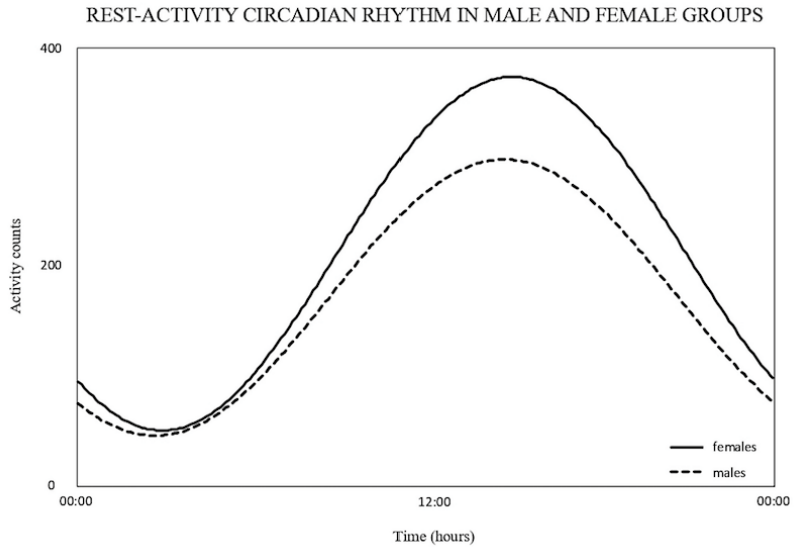

Figure 4. *Rest-activity circadian rhythm (expressed in activity counts): best-fitting 24-h cosine curves resulting from the population-mean cosinor analysis. Dashed black line denotes the male group, continued black line denotes the female group.*

Non-parametric analysis

Table 7 reports the results of the non-parametric statistical analysis for females and males. The two groups differed in IV and M10, with females having lower values than males. No statistically significant differences were found for IS and L5 between the two groups. $L5_{st}$ and $M10_{st}$ confirmed that L5 represents the night activity ($L5_{st}$ 01:07:26 ± 01:17:32 hh:mm in females; 00:50:22 ± 00:56:04 hh:mm in males) and M10 the daytime activity ($M10_{st}$ 07:33:44 ± 01:45:24 hh:mm in females; 07:05:42 ± 01:50:28 hh:mm in males).

NON-PARAMETRIC PARAMETERS	FEMALES *(N=50)*	MALES *(N=47)*
IS *(a.c.)*	0.64 ± 0.02	0.62 ± 0.02
IV *(a.c.)*	0.75 ± 0.03§	0.85 ± 0.03§
L5 *(a.c.)*	19.13 ± 1.54	19.63 ± 1.87
M10 *(a.c.)*	379.08 ± 16.43#	295.13 ± 12.88#

Table 7. *Non-parametric analysis of activity levels: comparison of RAR non-parametric parameters between male and female groups. The values are reported as mean ± SD. §p=0.026, #p=0.001.*

RAR parameters and sex

The multivariate correlation analysis in females (Table 8) showed an inverse correlation between MESOR and age (p=0.018; r=-0.33), Amplitude and BMI (p=0.03; Spearman p=-0.30), M10 and age (p=0.04; Spearman p=-0.30). No statistically significant relations were found between other parameters. Linear regression analysis for females showed an inverse relation between MESOR and age (p=0.02; F=5,968; R^2=0.11), Amplitude and BMI (p=0.02; F=5.558; R^2=0.10), M10 and age (p=0.03; F=4.754; R^2=0.09). No statistically significant relations were found between other parameters. Thus, in females, aging is associated with a decrease of daily activity and obesity, affecting the Amplitude of RAR.

FEMALES									
RAR PARAMETES	**Age**	**Weight**	**BMI**	**Wc**	**Sbp**	**Dbp**	**FaGlu**	**HDL**	**Trg**
IS	.164	.003	-.156	-.014	-.135	-.055	.063	.001	.269
IV	.060	-.070	.117	.059	-.027	.040	-.055	.022	-.052
L5	-.203	.146	.198	.157	.144	.162	-.046	-.182	.068
M10	-.295*	-.166	-.248	-.170	-.034	-.041	.121	-.048	.075
MESOR	-.333*	-.195	-.263	-.158	-.077	.004	-.025	.014	.023
Amplitude	-.126	-.212	-.303*	-.198	-.127	-.161	.131	-.064	.048
Acrophase	-.235	-.057	-.063	-.149	-.125	-.225	.117	.113	-.028

Table 8. *Coefficients of correlation between RAR and metabolic syndrome parameters in females. BMI=body mass index; Wc=waist circumference; Sbp=systolic blood pressure; Dbp=diastolic blood pressure; FaGlu=fasting glucose; HDL=high density lipoprotein; Trg=triglycerides. *p≤0.05.*

By contrast, the multivariate correlation analysis in males (Table 9) showed a direct relation between MESOR and HDL (p=0.02; r=0.34), Amplitude and HDL (p=0.04; r=0.30), L5 and fasting glucose (p=0.05; Spearman p=0.29). No significant correlations were found between the other parameters. For male participants, linear regression showed a positive relation between MESOR and HDL (p=0.02; F=5.733; R^2=0.11); Amplitude and HDL (p=0.04; F=4.469; R^2=0.09). No statistically significant relations were found between the other parameters. These findings indicate that men with higher daily activity levels had higher levels of HDL.

MALES									
RAR PARAMETES	**Age**	**Weight**	**BMI**	**Wc**	**Sbp**	**Dbp**	**FaGLu**	**HDL**	**Trg**
IS	.193	.189	.260	.182	.076	-.009	.065	.114	-.028
IV	-.035	-.167	-.165	-.189	.248	.102	.226	-.008	.012
L5	.230	.117	.190	.095	-.033	-.042	.288*	.118	-.132
M10	.162	-.017	-.028	-.008	-.063	-.045	-.038	.087	.142
MESOR	-.034	-.022	.043	-.027	.085	.059	.022	.336*	-.006
Amplitude	-.151	.107	.197	.111	.087	.001	-.043	.301*	.065
Acrophase	.007	-.080	-.078	-.049	.057	.204	.009	-.025	.232

Table 9. *Coefficients of correlation between RAR and metabolic syndrome parameters in males. BMI=body mass index; Wc=waist circumference; Sbp=systolic blood pressure; Dbp=diastolic blood pressure; FaGlu=fasting glucose; HDL=high density lipoprotein; Trg=triglycerides.* $^{*}p \leq 0.05$.

In table 10 are shown the RAR parameters, stratifying the sample according to the severity of MS in the two sexes. We did not find statistically significant differences in RAR parameters comparing people with 3, 4 or 5 altered MS parameters, both in females and males. In people with 3 altered parameters of MS we found a difference between sexes for M10, MESOR and Amplitude. Same differences were found in people with 4 altered MS parameters, MESOR, and Amplitude. In both cases, females showed higher value of these variables than males. By contrast, no differences were found in people with 5 altered parameters of MS.

RAR PARAMETERS	GROUPS	3 ALTERED PARAMETERS	4 ALTERED PARAMETERS	5 ALTERED PARAMETERS
IS *(a.c.)*	Females	0.63 ± 0.10	0.64 ± 0.13	0.65 ± 0.13
	Males	0.62 ± 0.11	0.60 ± 0.11	0.67 ± 0.15
IV *(a.c.)*	Females	0.77 ± 0.22	0.70 ± 0.20	0.83 ± 0.14
	Males	0.85 ± 0.22	0.81 ± 0.24	0.97 ± 0.17
L5 *(a.c.)*	Females	19.68 ± 11.49	15.77 ± 6.58	28.10 ± 15.80
	Males	17.24 ± 8.68	20.09 ± 13.48	25.97 ± 20.56
M10 *(a.c.)*	Females	358.26 ± 106.54$^{@}$	399.11 ± 119.81^{+}	395.60 ± 146.46
	Males	281.95 ± 109.13$^{@}$	313.52 ± 70.33^{+}	274.67 ± 63.40
MESOR *(a.c.)*	Females	234.36 ± 25.15*	251.46 ± 36.61^{ç}	251.47 ± 95.06
	Males	191.20 ± 26.97*	205.79 ± 30.86^{ç}	190.47 ± 52.38
Amplitude *(a.c.)*	Females	178.68 ± 24.95°	191.16 ± 32.09$^{\#}$	185.92 ± 88.27
	Males	141.77 ± 24.79°	155.31 ± 26.53$^{\#}$	114.59 ± 70.92
Acrophase *(hh:mm)*	Females	14:59 ± 01:08	14:56 ± 01:04	14:40 ± 02:41
	Males	14:44 ± 01:03	14:28 ± 01:05	15:04 ± 05:35

Table 10. *RAR parameters of the sample stratified by the severity of metabolic syndrome. Data are expressed as mean ± SD. 3 altered parameters: females N=24 and males N=20; 4 altered parameters: females N=20 and males N=21; 5 altered parameters females N=6 and males N=6.* $^{@}p=0.024$, $^{+}p=0.008$, $^{*}p=0.036$, $°p=0.032$, $^{ç}p=0.031$, $^{\#}p=0.044$.

8.2 Post-intervention conditions

At follow-up, 105 healthy subjects with MS out of 133 completed the study. After one year of intervention with metformin/placebo treatment and cooking/general awareness courses, 45.7% (N=48, 21 females and 27 males) out of 105 participants improved their MS condition. In 37.5% of them (N=18, 7 females and 11 males) it was no longer possible to diagnose the MS, as they had less than three out of five altered parameters.

We found an improvement of MS condition in people treated both with placebo (placebo group: N=52) and metformin (metformin group: N=53). However, metformin group showed higher percentage of subjects that improved their syndrome condition compared to the placebo group, with double the number of people without MS at the end of the study. Indeed, 49.1% (N=26, 12 females and 14 males) of metformin group improved their syndrome condition, and 46.2% of them (N=12, 6 females and 6 males) were no longer diagnosed the MS. For placebo group, 42.3% of subjects (N=22,

9 females and 13 males) improved their syndrome condition, 27.3% of them (N=6, 1 female and 5 males) were no longer diagnosed the MS (Table 11).

GROUPS	ALTERED PARAMETERS	BASELINE	FOLLOW-UP	% OF SUBJECTS IMPROVED
Placebo (N=52) (N=F 25, M 27)	0	0	0	42.3% 27.3% NO METABOLIC SYNDROME
	1	0	0	
	2	0	11.5%	
	3	34.6%	48.1%	
	4	55.8%	25.0%	
	5	9.6%	15.4%	
Metformin (N=53) (N=F 27, M 26)	0	0	0	49.1% 46.2% NO METABOLIC SYNDROME
	1	0	7.5%	
	2	0	15.1%	
	3	43.4%	41.5%	
	4	41.5%	32.1%	
	5	15.1%	3.8%	

Table 11. *Percentage of change in severity of metabolic syndrome from baseline to follow-up after one year of intervention with metformin or placebo treatment and cooking/general awareness courses.*

8.2.1 Metabolic syndrome parameters

The comparisons of MS parameters at baseline and after one year of intervention showed a reduction in waist circumference for both groups. However, delta analyses showed a higher decrease in metformin group compared to placebo group. At follow-up waist circumference has lower values in people treated with metformin compared to placebo group. Furthermore, only metformin group showed statistically significant lower values for diastolic blood pressure and fasting glucose at follow-up compared to baseline. The results before and after intervention, and delta analyses are shown in table 12.

MS PARAMETERS	GROUPS	BASELINE	FOLLOW-UP	Δ	Δ *p*
Waist Circumference *(cm)*	Placebo	103.2 ± 2.0*	100.5 ± 2.0*	-2.7 ± 0.7	**0.026**
	Metformin	99.3 ± 1.4#	95.1 ± 1.4#	-4.2 ± 0.7	
Systolic Blood Pressure *(mmHg)*	Placebo	143.9 ± 2.1	144.6 ± 1.7	0.7 ± 1.8	0.406
	Metformin	147.0 ± 2.3	144.4 ± 2.2	-2.6 ± 2.1	
Diastolic Blood Pressure *(mmHg)*	Placebo	89.4 ± 1.3	88.8 ± 1.0	-0.5 ± 1.3	0.090
	Metformin	89.3 ±1.3°	85.8 ± 1.2°	-3.5 ± 1.3	
Triglycerides *(mg/dL)*	Placebo	127.6 ± 7.5	122.8 ± 7.9	-4.8 ± 5.7	0.827
	Metformin	109.8 ± 5.6	108.0 ± 7.0	-1.83 ± 5.3	
Fasting glucose *(mg/dL)*	Placebo	103.4 ± 1.5	102.3 ± 1.6	-1.10 ± 5.7	**<0.001**
	Metformin	101.9 ± 1.6+	97.6 ± 1.3+	-4.32 ±1.1	
HDL *(mg/dL)*	Placebo	52.2 ± 1.9	52.5 ±1.9	0.31 ± 1.0	0.652
	Metformin	59.2 ± 2.1	59.8 ± 2.3	0.70 ± 1.2	

Table 12. *Metabolic syndrome parameters in metformin and placebo groups: comparison before and after intervention and delta analysis. Placebo group: N=52; metformin group: N=53. The data are reported as mean ± se.* *, #, +*p<0.001; °p=0.013.*

Table 13 shows the MS parameters data referring to the male group, at baseline and follow-up. At baseline males treated with placebo differed by males treated with metformin in waist circumference (p<0.008). No differences were found for the other MS parameters. Before and after analysis showed an improvement in the waist circumference in both placebo and metformin group, with higher effect in males treated with metformin, although not statistically significant (Δp=0.07). Moreover, it also shown an improvement in HDL parameters in the two groups. As expected, metformin-treated males showed a statistically significant decrease of fasting glucose level.

MALES					
MS PARAMETERS	**GROUPS**	**BASELINE**	**FOLLOW-UP**	**Δ**	**Δ *p***
Waist Circumference *(cm)*	Placebo	113.3 ± 2.6*	110.1 ± 2.7*	-3.2 ± 1.5	0.070
	Metformin	105.8 ± 2.6+	99.3 ± 2.2+	-6.5 ± 1.8	
Systolic Blood Pressure *(mmHg)*	Placebo	150.4 ± 3.5	148.6 ± 2.7	-1.8 ± 2.8	0.214
	Metformin	153.5 ± 3.7§	145.4 ± 4.1§	-8.1 ± 3.7	
Diastolic Blood Pressure *(mmHg)*	Placebo	92.4 ± 2.3	90.7 ± 1.6	-1.7 ± 2.6	0.183
	Metformin	89.6 ± 1.8	86.2 ± 2.1	-3.4 ± 2.6	
Triglycerides *(mg/dL)*	Placebo	125.7 ± 13.5	115.9 ± 11.2	-9.8 ± 10.2	0.887
	Metformin	111.9 ± 10.3	104.4 ± 12.7	-7.5 ± 11.2	
Fasting glucose *(mg/dL)*	Placebo	107.4 ± 2.4	105.4 ± 2.4	-2.0 ± 1.5	0.101
	Metformin	108.0 ± 1.7#	102.0 ± 2.9#	-6.0 ± 2.4	
HDL *(mg/dL)*	Placebo	48.7 ± 2.6°	51.4 ± 3.3°	2.7 ± 1.5	0.992
	Metformin	53.6 ± 3.4ç	56.3 ± 3.4ç	2.7 ± 2.5	

Table 13. *Metabolic syndrome parameters in metformin and placebo groups: comparison before and after intervention and delta analysis in males. Placebo group: N=20; metformin group: N=16. The data are reported as mean ± se.* $^{*}p=0.040$, $^{+}p=0.002$, $^{§}p=0.043$, $^{\#}p=0.025$, $^{\circ}p<0.001$, $^{ç}p<0.001$.

The data referring the female sample are summarized in the table 14. At baseline females treated with placebo differed by females treated with metformin in HDL level (p<0.023). No differences were found for the other MS parameters. A statistically significant decrease in waist circumference was found only in placebo group. Only metformin-treated females had a statistically significant reduction of diastolic blood pressure. Conversely, the placebo group showed an increase of diastolic blood pressure. No statistically significant differences were found for triglycerides both in placebo and metformin groups, although a statistically significant opposite trend was observed. Females treated with metformin decreased the levels of triglycerides, while females treated with placebo increased it. An opposite trend was found regarding the HDL variable. In fact, females treated with placebo decreased HDL levels, while those treated with metformin increased it.

FEMALES					
MS PARAMETERS	**GROUPS**	**BASELINE**	**FOLLOW-UP**	**Δ**	**Δ *p***
Waist Circumference *(cm)*	Placebo	98.3 ± 3.1*	95.6 ± 3.2*	-2.7 ± 1.1	0.842
	Metformin	93.3 ± 2.3	91.1 ± 2.9	-2.2 ± 1.6	
Systolic Blood Pressure *(mmHg)*	Placebo	137.8 ± 3.7	142.9 ± 3.5	5.1 ± 4.5	0.408
	Metformin	143.6 ± 2.9	139.4 ± 3.4	-4.2 ± 3.7	
Diastolic Blood Pressure *(mmHg)*	Placebo	85.5 ± 2.3	86.8 ± 1.7	1.3 ± 2.3	**0.033**
	Metformin	86.2 ± 2.5+	81.5 ± 2.8+	-4.7 ± 2.1	
Triglycerides *(mg/dL)*	Placebo	141.8 ± 17.9	148.7 ± 22.4	6.9 ± 12.3	**0.049**
	Metformin	105.2 ± 12.5	88.1 ± 9.5	-17.1 ± 10.2	
Fasting glucose *(mg/dL)*	Placebo	99.9 ± 2.2	100.3 ± 2.6	0.4 ± 2.8	0.186
	Metformin	97.9 ± 3.6	95.4 ± 1.6	-2.5 ± 2.6	
HDL *(mg/dL)*	Placebo	52.4 ± 3.1°	50.9 ± 3.1°	-1.5 ± 2.2	0.473
	Metformin	66.9 ± 4.9§	68.9 ± 6.1§	2.0 ± 2.2	

Table 14. *Metabolic syndrome parameters in metformin and placebo intervention group: comparison before and after intervention and delta analysis in females. Placebo group: N=12; metformin group: N=13. The data are reported as mean ± se.* $^{*}p=0.027$, $^{+}p=0.042$, $^{\circ}p<0.001$, $^{\S}p=0.014$.

8.2.2 Sleep characteristics

Table 15 summarizes the results referring to sleep parameters, before and after treatment with placebo and metformin. At baseline, the placebo group (N=52) and metformin group (N=53) did not differ in their sleep conditions. The comparisons of sleep parameters before and after the treatment showed that metformin is able to improve the sleep quantity and quality in healthy people with MS. A statistically significant increase in actual sleep time was found in metformin group at follow-up compared to baseline. By contrast, placebo group showed a statistically significant increase of sleep latency at follow-up compared to baseline. Delta comparisons showed statistically significant differences in actual sleep time and sleep efficiency between the two groups. Metformin-treated subjects showed an improvement of actual sleep time and sleep efficiency, while people treated with placebo displayed no change in actual sleep time and a worsening in sleep efficiency. Although not significant, metformin group also showed a good trend in sleep efficiency, sleep latency, immobile time, mean activity score and fragmentation index parameters; while opposite trends were found in people treated with placebo in all the same parameters, with the exception for the mean activity score.

SLEEP PARAMETERS	GROUPS	BASELINE	FOLLOW-UP	Δ	Δ *p*
Actual sleep time *(hh:mm)*	Placebo	05:56 ± 00:07	05:58 ± 00:08	00:02 ± 00:07	**0.011**
	Metformin	06:08 ± 00:08*	06:30 ± 00:07*	00:22 ± 00:08	
Actual wake time *(min)*	Placebo	64.9 ± 3.5	65.7 ± 3.6	0.8 ± 3.8	0.571
	Metformin	58.3 ± 2.7	59.8 ± 3.0	1.5 ± 3.1	
Sleep efficiency *(%)*	Placebo	79.5 ± 0.9	78.5 ± 1.0	-1.01 ± 1.1	**0.042**
	Metformin	80.8 ± 1.1	81.9 ± 7.6	1.1 ± 1.1	
Sleep latency *(min)*	Placebo	21.4 ± 2.4°	27.0 ± 2.2°	5.6 ± 3.1	0.108
	Metformin	23.1 ± 2.9	22.3 ± 2.2	-0.8 ± 3.7	
Immobile time *(%)*	Placebo	83.4 ± 0.9	83.2 ± 1.0	-0.2 ± 1.0	0.344
	Metformin	83.6 ± 0.9	84.7 ± 0.7	1.1 ± 0.8	
Mean activity score *(a.c.)*	Placebo	22.4 ± 1.5	21.8 ± 1.7	-0.6 ± 1.9	0.165
	Metformin	20.5 ± 2.5	18.4 ± 1.1	-2.1 ± 2.5	
Fragmentation index	Placebo	35.7 ± 1.7	36.2 ± 2.0	0.5 ± 1.8	0.506
	Metformin	34.2 ± 1.5	33.4 ± 1.6	-0.7 ± 1.4	

Table 15. *Sleep parameters in placebo and metformin groups: comparison before and after intervention, and delta analysis. Placebo group: N=52; metformin group: N=53. The data are reported as mean ± se.* *$p=0.012$, °$p=0.047$.*

In addition, a subgroup-analysis was performed to investigate whether the effects of metformin were different in the two sexes (Table 16). Males treated with metformin (N=26) showed a statistically significant increase of the actual sleep time at follow-up compared to baseline. By contrast, males treated with placebo (N=27) showed a statistically significant increase of sleep latency at follow-up compared to baseline. Delta comparisons showed statistically significant differences between the two groups. Males treated with metformin showed an improvement of actual sleep time, sleep efficiency and sleep latency, while males treated with placebo displayed no change in the actual sleep time and a worsening in the other two parameters.

MALES					
SLEEP PARAMETERS	**GROUPS**	**BASELINE**	**FOLLOW-UP**	**Δ**	**Δ *p***
Actual sleep time *(hh:mm)*	Placebo	05:47 ± 00:10	05:49 ± 00:11	00:02 ± 00:05	**0.003**
	Metformin	06:05 ± 00:11*	06:35 ± 00:09*	00:30 ± 00:12	
Actual wake time *(min)*	Placebo	65.9 ± 5.5	66.9 ± 4.8	1.0 ± 4.9	0.516
	Metformin	56.7 ± 3.7	61.5 ± 4.3	4.8 ± 4.2	
Sleep efficiency *(%)*	Placebo	79.4 ± 1.3	77.6 ± 1.4	-1.8 ± 1.4	**0.007**
	Metformin	81.0 ± 1.9	83.0 ± 1.1	2.0 ± 2.0	
Sleep latency *(min)*	Placebo	19.0 ± 2.4°	27.8 ± 2.5°	8.7 ± 3.6	**0.007**
	Metformin	24.8 ± 4.7	16.8 ± 2.1	-8.0 ± 5.3	
Immobile time *(%)*	Placebo	82.4 ± 1.1	81.7 ± 1.3	-0.7 ± 1.1	0.261
	Metformin	82.6 ± 1.5	83.9 ± 1.3	1.3 ± 1.5	
Mean activity score *(a.c.)*	Placebo	21.7 ± 2.1	20.2 ± 1.8	-1.5 ± 2.2	0.194
	Metformin	21.3 ± 4.7	18.0 ± 1.7	-3.3 ± 4.8	
Fragmentation index	Placebo	37.7 ± 2.3	39.2 ± 2.9	1.5 ± 2.2	0.554
	Metformin	35.1 ± 2.4	35.9 ± 2.7	-0.8 ± 2.4	

Table 16. *Sleep parameters in placebo and metformin groups: comparison before and after intervention, and delta analysis. Placebo group: N=27, metformin group: N=26. The data are reported as mean ± se.* *$p=0.021$, °$p=0.002$.*

On the other hand, the same analysis performed on the female sample did not show differences between the two groups (Table 17).

FEMALES					
SLEEP PARAMETERS	**GROUPS**	**BASELINE**	**FOLLOW-UP**	**Δ**	**Δ *p***
Actual sleep time *(hh:mm)*	Placebo	06:05 ± 00:09	06:07 ± 00:11	00:02 ± 00:11	0.364
	Metformin	06:10 ± 00:10	06:24 ± 00:10	00:14 ± 00:12	
Actual wake time *(min)*	Placebo	63.8 ± 4.4	64.4 ± 5.5	0.6 ± 6.0	0.686
	Metformin	59.8 ± 4.0	58.1 ± 4.3	-1.7 ± 4.5	
Sleep efficiency *(%)*	Placebo	79.7 ± 1.3	79.5 ± 1.5	-0.2 ± 1.8	0.923
	Metformin	80.7 ± 1.1	80.9 ± 1.0	0.2 ± 1.2	
Sleep latency *(min)*	Placebo	23.9 ± 4.3	26.1 ± 3.9	2.2 ± 5.0	0.499
	Metformin	21.5 ± 3.6	27.5 ± 3.6	6.0 ± 5.0	
Immobile time *(%)*	Placebo	84.4 ± 1.3	84.9 ± 1.4	0.5 ± 1.8	0.887
	Metformin	84.5 ± 0.8	85.5 ± 0.8	1.0 ± 0.7	
Mean activity score *(a.c.)*	Placebo	23.1 ± 2.1	23.5 ± 3.0	0.4 ± 3.3	0.408
	Metformin	19.7 ± 1.8	18.8 ± 1.6	-0.9 ± 1.8	
Fragmentation index	Placebo	33.5 ± 2.5	32.9 ± 2.5	-0.6 ± 3.1	0.722
	Metformin	33.2 ± 1.7	31.0 ± 1.9	-2.2 ± 1.5	

Table 17. *Sleep parameters in placebo and metformin groups: comparison before and after intervention, and delta analysis. Placebo group: N=25, metformin group: N=27. The data are reported as mean ± se.*

Another subgroup-analysis was performed to investigate whether the effects of metformin on sleep parameters were different between people with and without MS at follow-up (Tables 18 and 19). No statistically significant differences were found in placebo group (Table 18) between people with and without MS, except for sleep efficiency in people without MS. People with MS at follow-up also showed a decreasing trend for the same parameter. By contrast, in metformin group (Table 19) an improvement trend was found for most sleep parameters. Indeed, delta analysis showed a general improvement of actual sleep time, sleep efficiency, sleep latency, immobile time, mean activity score and fragmentation index, with a greater effect in people without MS at follow-up. Only for the fragmentation index we found a statistically significant delta *p* value, showing a greater improvement in people without MS. Moreover, they showed a statistically significant *p* value in the before-after analysis in immobile time and fragmentation index parameters.

PLACEBO GROUP					
SLEEP PARAMETERS	**MS AT FOLLOW-UP**	**BASELINE**	**FOLLOW-UP**	**Δ**	**Δ *p***
Actual sleep time *(hh:mm)*	NO	05:53 ± 00:31	05:40 ± 00:23	-00:13 ± 00:19	0.459
	YES	05:56 ± 00:07	06:00 ± 00:08	00:04 ± 00:08	
Actual wake time *(min)*	NO	56.6 ± 10.0	56.6 ± 8.9	-0.1 ± 7.0	0.499
	YES	65.9 ± 3.8	66.9 ± 3.9	0.9 ± 4.2	
Sleep efficiency *(%)*	NO	81.9 ± 3.0*	79.2 ± 2.6*	-2.7 ± 1.1	0.894
	YES	79.2 ± 1.0	78.4 1.1	-0.8 ± 1.3	
Sleep latency *(min)*	NO	13.8 ± 5.6	24.8 ± 7.1	11.1 ± 9.0	0.784
	YES	22.4 ± 2.6	27.2 ± 2.4	4.9 ± 3.3	
Immobile time *(%)*	NO	85.1 ± 2.7	82.0 ± 3.7	-3.1 ± 1.7	0.640
	YES	83.2 ± 0.9	83.4 ± 1.0	0.23 ± 1.2	
Mean activity score *(a.c.)*	NO	19.1 ± 4.6	19.4 ± 4.4	0.3 ± 3.0	0.898
	YES	22.8 ± 1.5	22.1 ± 1.8	-0.7 ± 2.2	
Fragmentation index	NO	31.8 ± 5.1	38.2 ± 7.8	6.4 ± 4.3	0.363
	YES	36.2 ± 1.8	35.9 ± 2.0	-0.3 ± 2.0	

Table 18. *Sleep parameters in the placebo group: comparison before and after intervention, and delta analysis. The sample was divided in two groups: subjects without (N=6) and with (N=46) MS at follow-up. The data are reported as mean ± se.* *$p=0.052$.

METFORMIN GROUP					
SLEEP PARAMETERS	**MS AT FOLLOW-UP**	**BASELINE**	**FOLLOW-UP**	**Δ**	**Δ *p***
Actual sleep time *(hh:mm)*	NO	05:59 ± 00:11	06:33 ± 00:19	00:34 ± 00:19	0.609
	YES	06:11 ± 00:09	06:29 ± 00:07	00:19 ± 00:10	
Actual wake time *(min)*	NO	60.3 ± 4.8	61.5 ± 6.0	1.2 ± 5.1	0.890
	YES	57.7 ± 3.2	59.3 ± 3.5	1.6 ± 3.7	
Sleep efficiency *(%)*	NO	80.4 ± 1.9	82.0 ± 1.9	1.6 ± 1.3	0.828
	YES	80.9 ± 1.3	81.9 ± 0.8	1.0 ± 1.4	
Sleep latency *(min)*	NO	23.8 ± 3.6	20.8 ± 5.4	-3.0 ± 6.1	0.765
	YES	22.9 ± 3.7	22.7 ± 2.4	-0.2 ± 4.5	
Immobile time *(%)*	NO	83.0 ± 1.5*	85.4 ± 1.0*	2.3 ± 1.0	0.408
	YES	83.7 ± 1.0	84.5 ± 0.9	0.7 ± 1.0	
Mean activity score *(a.c.)*	NO	19.1 ± 3.4	18.2 ± 2.5	-0.9 ± 1.8	0.933
	YES	20.9 ± 3.1	18.5 ± 1.3	-2.4 ± 3.2	
Fragmentation index	NO	36.4 ± 3.2°	31.1 ± 1.8°	-5.4 ± 1.8	**0.018**
	YES	33.5 ± 1.6	34.1 ± 2.1	0.7 ± 1.7	

Table 19. *Sleep parameters in the metformin group: comparison before and after intervention, and delta analysis. The sample was divided in two groups: subjects without (N=12) and with (N=41) MS at follow-up. The data are reported as mean ± se. *p=0.039, °p=0.012.*

Due to the small size of the sample, it was no possible to perform a subgroup-analysis by sex, both in placebo and metformin groups, and within the groups of people who had or had not the MS at follow-up. However, an investigative analysis showed that metformin-treated males who had no MS at follow-up appeared to increase the actual sleep time and sleep efficiency, and to reduce the actual wake time, sleep latency and fragmentation index.

8.2.3 Rest-activity circadian rhythm characteristics

At baseline, in our cohort of 133 subjects, 12 males and 24 females removed the actigraph for several hours during the day, thus making the data unusable for the RAR analysis. Therefore, actigraphic data from 97 participants were analyzed at baseline using both parametric and non-parametric analysis. At follow-up, of 97 healthy subjects with MS, 61 participants (25 females and 36 males) had a 7-day monitoring with sufficient data for an actigraphic analysis of the RAR. For the other 36 subjects (25 females and 11 males), who had removed the actigraph for several hours during the day, the amount of the data was not enough to evaluate the RAR.

At baseline, the comparison of parametric and non-parametric RAR parameters in the female sample did not show statistically significant differences between placebo and metformin groups (Table 20). Same results were also found for the male sample (Table 21), except for L5 parameters (p=0.036), as placebo-treated males showed higher level of L5 than those treated with metformin. The before and after intervention comparisons of parametric and non-parametric RAR parameters in the two female groups, placebo and metformin, showed no statistically significant differences. By contrast, the same comparisons in male subjects showed a statistically significant decrease in IS values from baseline to follow-up for both groups, placebo and metformin, and a statistically significant increase in L5 values from baseline to follow-up only in metformin group. Moreover, the table 21 also shows a postponement of Acrophase in males treated with placebo, while an advance of the Acrophase in those treated with metformin. Delta comparisons in females did not show differences between placebo and metformin groups (Table 20). Instead, in males we found a statistically significant opposite trend for Acrophase between males treated with placebo and those treated with metformin (Table 21).

FEMALES					
RAR PARAMETERS	**GROUPS**	**BASELINE**	**FOLLOW-UP**	**Δ**	**Δ p**
IS *(a.c.)*	Placebo	0.64 ± 0.10	0.64 ± 0.07	0.00 ± 0.11	0.571
	Metformin	0.67 ± 0.12	0.63 ± 0.16	-0.04 ± 0.12	
IV *(a.c.)*	Placebo	0.77 ± 0.19	0.75 ± 0.17	-0.02 ± 0.19	0.868
	Metformin	0.67 ± 0.18	0.72 ± 0.16	0.05 ± 0.16	
L5 *(a.c.)*	Placebo	19.53 ± 13.85	13.83 ± 6.03	-5.70 ± 14.90	0.182
	Metformin	15.59 ± 7.14	18.36 ± 8.50	2.77 ± 9.13	
M10 *(a.c.)*	Placebo	408.12 ± 142.54	369.63 ± 104.51	-38.49 ± 95.91	0.671
	Metformin	420.3 ± 130.98	383.06 ± 150.41	-37.24 ± 159.36	
MESOR *(a.c.)*	Placebo	250.57 ± 90.46	223.39 ± 58.43	-27.18 ± 68.31	0.530
	Metformin	255.31 ± 78.23	239.56 ± 91.58	-15.75 ± 82.36	
Amplitude *(a.c.)*	Placebo	212.03 ± 74.43	196.19 ± 52.46	-15.84 ± 57.02	0.657
	Metformin	197.63 ± 59.44	196.15 ± 72.26	-1.48 ± 65.45	
Acrophase *(hh:mm)*	Placebo	15:37 ± 01:05	15:13 ± 00:43	-00:24 ± 00:57	0.689
	Metformin	14:43 ± 01:05	14:40 ± 01:01	-00:03 ± 00:55	

Table 20. *RAR parameters in the female group: comparison before and after intervention, and delta analysis. Placebo group: N=12, metformin group N=13. Data are expressed as mean ± SD.*

MALES					
RAR PARAMETERS	**GROUPS**	**BASELINE**	**FOLLOW-UP**	**Δ**	**Δ p**
IS *(a.c.)*	Placebo	0.61 ± 0.11*	0.52 ± 0.14*	-0.09 ± 0.17	0.936
	Metformin	0.60 ± 0.12°	0.51 ± 0.15°	-0.09 ± 0.13	
IV *(a.c.)*	Placebo	0.81 ± 0.21	0.83 ± 0.21	0.02 ± 0.27	0.330
	Metformin	0.92 ± 0.26	0.88 ± 0.25	-0.04 ± 0.36	
L5 *(a.c.)*	Placebo	25.17 ± 16.10	29.71 ± 17.34	4.54 ± 19.85	0.131
	Metformin	15.42 ± 8.48#	20.87 ± 10.62#	5.45 ± 7.18	
M10 *(a.c.)*	Placebo	299.29 ± 96.59	302.16 ± 86.27	2.87 ± 129.11	0.247
	Metformin	262.77 ± 77.04	257.16 ± 92.30	-5.61 ± 109.11	
MESOR *(a.c.)*	Placebo	197.72 ± 62.54	198.90 ± 55.13	1.18 ± 59.85	0.161
	Metformin	183.72 ± 48.07	161.34 ± 63.41	-22.38 ± 46.64	
Amplitude *(a.c.)*	Placebo	143.75 ± 44.98	153.72 ± 40.64	9.97 ± 50.58	0.285
	Metformin	149.04 ± 50.11	138.68 ± 49.77	-10.36 ± 47.17	
Acrophase *(hh:mm)*	Placebo	14:32 ± 14:58§	14:58 ± 00:41§	00:26 ± 00:47	**0.003**
	Metformin	14:58 ± 01:32ç	14:32 ± 01:04ç	-00:26 ± 00:44	

Table 21. *RAR parameters in the male group: comparison before and after intervention, and delta analysis. Placebo group: N = 20, metformin group N=16. Data are expressed as mean ± SD. *p=0.037, °p=0.021 #p=0.008, §p=0.023, çp=0.033.*

9. DISCUSSION

Metabolic syndrome is a cluster of metabolic risk factors, that increases the risk of several pathologies (Hanson et al. 2002, Alberti et al. 2009, Motillo et al. 2010). Environmental factors, as well as genetic and metabolic factors, can play an important role in the development of MS (Ferrannini et al. 1991, Park et al. 2003, Janja 2007, Mirmiran et al. 2008, Buckland et al. 2008, Munoz Contreras et al. 2013, Xu et al. 2015, Kielbasa et al. 2016). Metformin is a drug used to reduce liver glucose production, decrease glucose absorption, and increase insulin sensitivity of target cells. It is indicated as an adjunct to diet, physical exercise, and lifestyle changes to improve glycemic control in type 2 diabetes mellitus. Therefore, its main effects are on the fasting plasma glucose level, but it also acts on other related variables of MS, such as hypertension. Indeed, insulin plays an important role on the blood pressure regulation, acting as a vasodilator by means of the liberation of nitric oxide from the vascular endothelium and facilitates tubular reabsorption of sodium through the endothelial action of the Angiotensin Converting Enzyme system (Chaudhary et al. 2011). Also, Vitale et al. (2005) demonstrated that the use of metformin not only improves the insulin resistance, but also the endothelial function. Several studies showed that people with MS are exposed to cardiovascular disease and type 2 diabetes mellitus (Hanson et al. 2002, Motillo et al. 2010). It has been hypothesized that insulin resistance, associated with obesity, was the main cause of MS (Munoz Contreras et al. 2013). Vascular diseases and diabetes risk related to MS have also been associated with genetic susceptibility (Shu et al. 2010). Therefore, the use of metformin could play an important role not only to prevent the insulin resistance, but also the hypertension. Based on these assumptions, my PhD project aimed to verify whether metformin, in addition to a healthy eating style, could represent a new strategy in the management of MS. Furthermore, many studies documented that people with MS suffer from sleep disturbances, such as sleep breathing disorders and sleep duration (Leineweber et al. 2003, Nilsson et al. 2004, Gruber et al. 2006, Gangswich et al. 2007, Hall et al. 2008, Nock et al. 2009, O'Connor et al. 2009, Troxel et al. 2010, Redline 2019). Recently, metformin was introduced as adjuvant agent for the management of sleep disorders, promoting the activation of AMPK and affecting insulin and IGF-1/Akt pathways, as well as testosterone and IGF-1 blood concentration. This determines a stimulation of fatty acid oxidation, promotion of glucose transport and glycolysis, and inhibition of gluconeogenesis; all processes affected by sleep disorders (Tasali et al. 2008, Spiegel et al. 2009, Tasali et al. 2009, Broussard et al. 2012, Mesarwi et al. 2013, Saner et al. 2018). Although it is shown that people with MS suffer from sleep disorders and that the use of metformin may improve the quality and quantity of sleep, so far no studies have investigated the effect of metformin treatment on sleep disorders in patients with MS.

After one year of intervention, with metformin/placebo treatment and cooking/general awareness courses, we showed an improvement of MS condition in almost 50% of the participants, in 37.5% of whom was no longer possible to diagnose the MS, since there were less than three altered parameters out of five. People treated with placebo showed an improvement only in waist circumference after one year of treatment. This could be related to the effect of the cooking or general awareness courses followed by all study participants and having received advice to follow on a more active lifestyle, or simply that participation in the trial may have prompted people to adopt a healthier lifestyle. By contrast, we found that the 49.1% of people treated with metformin improved their MS condition and, of these, 46.2% no longer had the MS. Particularly, they showed a reduction in waist circumference, diastolic blood pressure and fasting glucose. A good trend was also found for the other MS parameters. The results of this research project seem to support the idea that metformin may represent a new strategy in subjects with MS, together with adherence to a healthy eating stile.

Regarding the assumption that metformin may be able to improve the night sleep in subjects with MS, our results showed that people treated with metformin improved the sleep quantity and quality, with a more remarkable effect on actual sleep time. Moreover, delta comparisons showed statistically significant differences in actual sleep time and sleep efficiency between placebo and metformin groups. In particular, people treated with metformin showed an improvement in actual sleep time and sleep efficiency, while people treated with placebo displayed no change in the actual sleep time and a worsening in the sleep efficiency. Although not significant, metformin group also showed a good improvement trend in sleep efficiency, sleep latency, immobile time, mean activity score and fragmentation index parameters, while opposite trends were found in people treated with placebo in all the same parameters except for the mean activity score. In addition, we investigated whether the effect of metformin differed between people who had MS or not at follow-up. After one year of treatment with metformin, we found that people without MS at follow-up showed more time spent in sleep immobility and less fragmented sleep. Moreover, in both groups we found a good trend of improvement for most of the sleep parameters, such as actual sleep time, sleep efficiency, sleep latency, immobile time, mean activity score and fragmentation index, with greater effect in people without MS at follow-up. By contrast, we did not find substantially difference in placebo group between people with and without MS, except for sleep efficiency that decreased in people without MS. In my opinion, these findings are relevant considering that people with MS may also suffer from sleep disorders, and therefore get a double benefit from the treatment with metformin on metabolic condition and sleep.

Furthermore, sex also seems to play a role in the development of MS, with males that are more at risk of developing the syndrome (Buckland et al. 2008, Roomi et al. 2019, Han et al. 2020). Some authors have reported that males had higher values of waist circumference, triglycerides, blood pressure,

fasting plasma glucose and lower levels of HDL compared to females (Miccoli et al. 2004, Mirmiran et al. 2008). In agreement with these authors, the results of this present study show the influence of sex on each of the parameters involved in MS. At baseline, we found lower values of waist circumference and fasting glucose, and higher levels of HDL in females compared to males. After one year of intervention with metformin/placebo treatment an overall improvement in waist circumference and HDL parameters is observed in metformin-treated males. In females we showed a reduction of waist circumference in both groups. Moreover, females treated with metformin showed an improvement in blood pressure, triglycerides and HDL, while the placebo group showed an opposite situation for HDL and triglycerides. The overall improvement on the same parameters observed in males and females treated with both placebo and metformin could be explained by the positive effect of the cooking classes or by the general awareness courses followed by the participants, as well as by the advice to follow a more active lifestyle. Even just participating in the trial itself may have led to adopt a healthier lifestyle. However, the better trend and the statistically significant effect respectively showed by the males and females treated with metformin lead us to think of a greater effect if both treatments are used. This could be important if we consider that males are more at risk of developing MS.

Sex-related differences were also investigated by considering the effects of metformin on sleep parameters. Males treated with metformin showed a statistically significant increase of the actual sleep time at follow-up compared to baseline. Moreover, they showed an improvement of actual sleep time, sleep efficiency and sleep latency; these effects are not displayed in placebo-treated males, which showed no change in actual sleep time and a worsening in the other two parameters. Although it was not possible to demonstrate differences related to the effect of the treatment in female groups, the results obtained for the male groups seem to suggest the importance of considering the use of metformin in order not only to improve metabolic parameters, but also counteract the presence of any sleep disorders.

Regarding the RAR, the intervention with metformin/placebo did not show effects. This result was rather predictable, as a change in daily activity levels is needed to modify the RAR, for example by encouraging strategies aimed at increasing daily activity and decreasing sedentary behaviors. With this in mind, promoting structured physical activity session would have been important, as it was planned in my original plan. In fact, my PhD project also aimed to administer a physical activity protocol. Unfortunately, due to the COVID-19 pandemic and related restrictions, it was not possible to implement this protocol. Implementing a structured program of physical activity in people with MS could be a complementary and reinforcing strategy, not only in order to improve RAR, but also to help improve sleep, and modulate major health risk factors. Based on similar effects, the improvement obtained with the use of metformin could have been larger. In fact, physical exercise

induces the activation of AMPK and the transcription of the g receptor (PGC-1α) activated by the peroxisome proliferator, leading to an increase in mitochondrial content and function by improving insulin sensitivity, and interacts closely with clock genes improving sleep (Saner et al. 2018).

In conclusion, the results of this PhD research project support the hypothesis that metformin may be a new valid approach, together with a healthy eating style, for the secondary prevention not only of the metabolic syndrome but also of any related sleep disorders, particularly in males who are indicated as being more at risk of developing MS. Future studies that plan to associate the use of metformin with a structured program of physical activity in these subjects could be considered, in order to improve the same parameters on which metformin affects. Moreover, a chronobiological approach that investigate the optimal timing of metformin intake could be useful to improve its effects on sleep and SM condition.

This research project has led to the following publication:

Sex Differences in Rest-Activity Circadian Rhythm in Patients With Metabolic Syndrome

Mulè A., Bruno E., Pasanisi P., Galasso L., Castelli L., Caumo A., Esposito F., Roveda E., Montaruli A. (2021) *Frontiers in Physiology*, 34(4):443-444. Doi: 10.3389/fphys.2021.641461.

10. ARTICLES PUBLISHED

Effects of Shift Work in a Sample of Italian Nurses: Analysis of Rest-Activity Circadian Rhythm

Galasso L. & **Mulè A.**, Castelli L., Cè E., Condemi V., Banfi G., Roveda E., Montaruli A., Esposito F. (2021) *Environmental Research and Public Health,* 18(16),8378.
Doi: https://doi.org/10.3390/ijerph18168378.

Abstract

Background: The shift worker is exposed to an increased risk of manifesting a desynchronization of the rhythms that influence physiological and psychological functions. This is due to a temporary misalignment between working hours and physiological-behavioral functioning, with consequent impairment of health. In fact, shift work has been shown to increase the risk of developing insomnia, worsening sleep quality, reduced ability to work during waking hours and increased cardiovascular risk.

Aim: The aim of the study was to evaluate the effects of shift work on the rest-activity circadian rhythm (RAR) and health status of Italian orthopaedic nurses.

Materials and methods: We recruited 59 nurses: 44 worked the night shift and 15 worked the day shift. All participants carried out continuous 5 days actigraphic monitoring to assess RAR.

Results: The RAR analysis showed that, during the working period, the night shift nurses showed a significantly lower amplitude than the day shift nurses. The acrophase also differed between the two groups. When we stratified the two groups by median body mass index in normal weight (meadian: <25 kg/m^2) and overweight (median: ≥ 25 kg/m^2), during the working period, we noted a significantly lower amplitude for both the normal weight and the overweight nurses who worked the night shift.

Conclusion: The present results underscore the importance of further investigations evaluating the relationship between daily activity levels and shift work. Future studies are needed to determine whether physical activity trainings, scheduled in the nurses' leisure time or conducted at the workplace, could improve RAR and prevent alterations in circadian rhythms in shift workers.

Sex Differences in Rest-Activity Circadian Rhythm in Patients With Metabolic Syndrome

Mulè A., Bruno E., Pasanisi P., Galasso L., Castelli L., Caumo A., Esposito F., Roveda E., Montaruli A. (2021) *Frontiers in Physiology*, 34(4):443-444. Doi: 10.3389/fphys.2021.641461.

Abstract

Background: Rest-activity circadian rhythm (RAR) is considered a marker of the circadian timing system. Several studies investigated the relationship between irregular circadian rhythms and cardiovascular risk factors such as hypertension, obesity, and dyslipidemia; factors related to the metabolic syndrome (MS). MS is a clustering of metabolic risk factors that increases the risk to develop cardiovascular and metabolic diseases.

Aim: This cross-sectional study aimed to explore the RAR characteristics by actigraphy in subjects with MS, particularly focused on sex differences, using parametric and non-parametric analyses of RAR.

Materials and methods: The present study reports a cross-sectional analysis of baseline data of people with MS from the "Me.Me.Me." (Metabolic syndrome, Metformine and Mediterranean diet) study (ERC-AdG-2012 n.322752), a randomized controlled trial of Mediterranean diet and Metformin for the prevention of age-related non-communicable chronic diseases, developed by Fondazione IRCCS Istituto Nazionale dei Tumori di Milano (Milan, Italy). Anthropometric and MS condition of subjects was evaluated. Participants were instructed to wear an actigraph to investigate their RAR.

Results: We found that female participants had higher values than male participants of the Midline Estimating Statistic of Rhythm (MESOR), Amplitude and the most active 10-h period (M10). Furthermore, females showed higher daily activity level than males. Female participants also had lower Intradaily Variability than male participants, which indicates a more stable and less fragmented RAR.

Conclusion: These preliminary data provide the first experimental evidence of a difference in RAR parameters between male and female people with MS. The evaluation of RAR based on sex could prove to be a useful tool to monitor the daily activity level of individuals and possibly improving it by setting personalized physical activity trainings based on each person's daily activity profile.

Biological Rhythm and Chronotype: New Perspectives in Health

Montaruli A., Castelli L., **Mulè A.**, Scurati R., Esposito F., Galasso L., Roveda E. (2021) *Biomolecules,* 11:487. Doi: https://doi.org/ 10.3390/biom11040487.

Abstract

Background: The circadian rhythms play an important role in the regulation of biological functions, including sleep-wake preference, body temperature, hormonal secretion, food intake, and cognitive and physical performance. Alterations in circadian rhythms can lead to chronic disease and impaired sleep. The circadian rhythmicity in human beings is represented by a complex phenotype. Indeed, over a 24-h period, a person's preferred time to be more active or to sleep can be expressed in the morningness-eveningness concept. Interindividual differences in chronotypes need to be considered to reduce the negative effects of circadian disruptions on health.

Aim: In the present review, we examine the bidirectional influences of the rest-activity circadian rhythm and sleep in chronic pathologies and disorders. Moreover, we analyze the concept and the main characteristics of the three chronotypes (Morning-type, Neither-type and Evening-type).

Materials and methods: Studies on people of any age, gender, and medical condition were included as well as articles concerning animal studies. The article titles and abstracts were screened for relevance. The full text of all potentially relevant studies was reviewed to determine whether it met the inclusion criteria. Articles describing changes in chronotype across the lifespan, social jet lag, effects of disrupted rest-activity circadian rhythm (RAR) and sleep on health status, and interrelations between chronotype and melatonin were included.

Results: We evaluated 2103 articles. The number of final studies included in the review is 217. All 217 studies included in the review contained original data and were published in English. The data reported in this review highlight how alterations in RAR and sleep are related to physiological processes, such as aging, but also to pathological processes. Moreover, it has also been shown that circadian rhythmic expression can differ from person to person, and these individual differences in the chronotypes are able to influence both physiological and psychological functioning.

Conclusion: We could consider a bidirectional relationship between circadian rhythms and diseases. Therefore, the alteration of the circadian rhythm could lead to the development of diseases and physiological aging; on the other hand, the presence of pathology is able to increase the anomalies of the circadian rhythm. Moreover, it is necessary to take into account that regular habits and social commitments tailored to the chronotype should be considered important factors that should not be underestimated in order to avoid desynchronization in the rest-activity circadian rhythm and in the sleep.

Differences in Daytime Activity Levels and Daytime Sleep Between Night and Day Duty: An Observational Study in Italian Orthopedic Nurses

Roveda E., Castelli L., Galasso L., **Mulè A.**, Cè E., Condemi V., Banfi G., Montaruli A., Esposito F. (2021) *Frontiers in Physiology*, 12:628231. Doi: 10.3389/fphys.2021.628231.

Abstract:

Background: Working nonstandard work schedules is often associated with increased sedentary behavior and risk of sleep disorders. Night shift work expose the subjects to be prone to accumulating sleep debt, which is recovered by sleeping during the day. The effect on daytime activity levels is unknown.

Aim: The study aimed to objectively assess whether daytime sleep could affect daytime activity levels of shift worker nurses, resulting in an accumulation of their activity debt differently between working and rest periods.

Materials and methods: We recruited 37 orthopedic nurses working on a rotating schedule, including either a night shift (NS) or only day/afternoon shift (DS). Actigraph monitoring lasted both on the working and the rest period.

Results: For the NS nurses, the working period recorded higher daytime activity levels than the rest period, while daytime sleep during the working and rest periods was similar. Instead, DS nurses showed higher daytime activity levels and shorter daytime sleep during the working period. NS nurses were less active compared to DS nurses during the working period, probably because NS tended to have a longer daytime sleep. During the rest period, daytime activity levels for both groups decreased. Sleep of NS nurses recorded the better sleep parameters during the rest period, while that of DS nurses did not show significant differences between the working and the rest periods. Moreover, during the working period, NS nurses slept worse than the DS nurses. Both groups tended to accumulate a debt in daytime activity levels during the rest period.

Conclusion: Sleeping during the daytime may be a good strategy for night-shift workers to counteract the sleep debt accumulated during night work, while it could be useful to recover from the daily workload in day nurses. However, this exposes to a reduction of daytime activity levels. Indeed, since the more the rest taken during the daytime, the lower the daytime activity levels. Reducing daytime activity could seriously compromise shift worker health, leading to chronic disease, cardiovascular disease, and metabolic problems.

Reduced Neuromuscular Performance in Night Shift Orthopedic Nurses: New Insights From a Combined Electromyographic and Force Signals Approach

Cè E., Doria C., Roveda E., Montaruli A., Galasso L., Castelli L., **Mulè A.**, Longo S., Coratella G., D'Aloia P., Banfi G., Esposito F. (2020) *Frontiers in Physiology*, 11:693. Doi: 10.3389/fphys.2020.00693.

Abstract

Background: The working hours of hospital nurses rotate between day and night shifts. Moreover, night-shift workers routinely work against their internal biological clock. These factors expose subjects to suffer from sleepiness caused by desynchronization of their internal circadian clock and disturbance in their sleep-wake pattern. The effect of sleep-wake rhythm disruption on neuromuscular control and muscle fatigue is less studied. A combined approach based on the simultaneous analysis of surface electromyographic and force signals can objectively detect possible deficits in neuromuscular control and muscle fatigue in a sample of nurses.

Aim: The aim of the study was to investigate possible deficits in force generation capacity and in neuromuscular control possibly associated with poor sleep quality in the nurses who working night shifts (NS) compared to nurses who only work day shifts (DS).

Materials and methods: The study sample was composed by 71 nurses divided by their shift work schedule: night shift for 5 days (NS5), night shift for 10 days (NS10), and only day/swing shift (DS). Before and after the shift-work cycle, maximum voluntary contraction force (MVCF) and muscle activation, neuromuscular control, and muscle fatigability were measured in the finger flexor muscles. Physical activity levels of the subjects were assessed by using the Italian version of the International Physical Activity Questionnaire (IPAQ). Sleep and daily activity levels were also assessed.

Results: Daily activity levels and sleep during the shift-work cycle were recorded with a wrist actigraph. After the shift-work cycles, MVCF and muscle activation decreased, as well as neuromuscular control, while muscle fatigability increased in the NS5 and the NS10 group. Sleep quality was lower in the NS5 and the NS10 group, while the activity level for the three groups was similar.

Conclusion: The results of the present study showed a clear reduction in neuromuscular control and an increase in muscle fatigue in the night-shift nurses. These findings may inform of work schedule planning or recommendations for devising new recovery strategies to counteract neuromuscular alterations in night-shift nurses.

Rest-activity rhythm in breast cancer survivors: an update based on non-parametric indices

Galasso L., Montaruli A., **Mulè A.**, Castelli L., Bruno E., Pasanisi P., Caumo A., Esposito F., Roveda E. (2020) *Chronobiology International,* 37(6):946-951. Doi: 10.1080/07420528.2020.1756839.

Abstract

Background: In our previous study we evaluated by actigraphy the rest-activity circadian rhythm (RAR) in breast cancer (BC) survivors at 5 years from primary diagnosis, as well as in a control group with similar age and body mass index (BMI). RAR, analyzed by cosinor method, resulted significantly different in BC survivors compared to healthy subjects. In particular, we found in BC group lower Midline Estimating Statistic of Rhythm (MESOR) and Amplitude compared to Control group.

Aim: The aim of the present letter is to improve the results of the previous study with a new non-parametric analysis of the RAR.

Results: Using non-parametric RAR analysis we have detected Interdaily Stability (IS), Intradaily Variability (IV), nocturnal activity (L5), and daily activity (M10) on the same sample of previous study: 15 BC survivors at 5 years from the primary diagnosis (mean age = 56.7 ± 6.6 years; mean BMI = 24.5 ± 3.8 Kg/m^2) and 13 healthy controls (mean age = 54.4 ± 7.2 yeras; mean BMI = 25.2 ± 2.8 Kg/m2).

Results: We showed in BC group a statistically significant higher IV than in Control group, while L5 and M10 were significantly lower compared to Control group. No statistically significant differences were found in the other parametrs.

Conclusion: The data suggest that the analysis of RAR in all its components, parametric and non-parametric, is important to detect alterations in the rhythm and can be useful for developing new strategies for health protection, such as structured physical activity trainings, to improve circadian daily activity level in order to raise the quality of life in BC survivors.

Binge Eating Disorder: What Is the Role of Physical Activity Associated with Dietary and Psychological Treatment?

Galasso L., Montaruli A., Jankowski K.S., Bruno E., Castelli L., **Mulè A.**, Chiorazzo M., Ricceri A., Erzegovesi S., Caumo A., Roveda E., Esposito F. (2020) *Nutrients*, 12:3622.
Doi: 10.3390/nu12123622.

Abstract

Background: In literature, few studies were performed on binge eating disorder (BED) and physical activity. Binge eating patients present lower physical activity levels, which could be associated with lower exercise capacity. Individualized physical activity programs can ensure broad beneficial results relating to eating disorders, depression, and body mass index (BMI) in bulimia.

Aim: Our study aimed to investigate the effects of specific training as an addition to conventional treatment of eating disorder symptoms, anthropometric characteristics, and physical performance. Nineteen women with BED were recruited in the study and included in a dietary and cognitive-behavioral therapy program. After medical examination, 10 women carried out Combined Aerobic and Anaerobic Exercise Training in addition to conventional treatment (CAAET group), whereas the remaining 9 followed the conventional treatment alone (Control group) for six months.

Results: Both groups showed a significant decrease in binge episodes, weight, and body mass index, and an increase in exercise capacity. However, the CAAET group greater improved in aerobic performance than that observed in the Control group.

Conclusion: Our results suggest that both interventions similarly improved BED symptoms. The addition of physical activity could be important in the long-term maintenance of both weight loss and reduction in binge episodes in BED patients.

Effect of chronotype on motor skills specific to soccer in adolescent players

Roveda E., **Mulè A.**, Galasso L., Castelli L., Scurati R., Michielon G., Esposito F., Caumo A., Montaruli A. (2020) *Chronobiology International*, 37(4):552-563.

Doi: 10.1080/07420528.2020.1729787.

Abstract

Background: Circadian rhythms influence psychological and physiological functions, daily behaviors, as well as physical performance. There are three chronotypes based on the preferences that people typically show for activity at certain times of the day: Morning, Neither, and Evening types (M-, N- and E-types). The chronotype changes with age: E-types tends to be more prevalent in youth and M-types in older age. The progressive shift toward eveningness during adolescence creates misalignment with morning society schedules and can lead to a deterioration in intellectual and physical performance. Soccer is one of the world's most popular sports practiced by adolescents and soccer workouts are usually held after school in the afternoon or evening. Performance in soccer is related to a host of factors, including physiological variables and motor skills that have a circadian variation.

Aim: The aim of this study was to determine the effect of chronotype on motor skills specific to soccer. In particular, we investigated whether agility, aerobic endurance, and explosive power differ among the three chronotypes in relation to the time of day. For this study 141 adolescent soccer players filled in the Morningness-Eveningness Questionnaire (MEQ) for the assessment of chronotype. A subsample of 75 subjects, subdivided in M-types (n=25), N-types (n=25) and E-types (n=25), performed three tests (Sargent Jump Test - SJT, Illinois Agility Test - IAT, and 6-Minutes Run Test - 6MRT) at a morning and an evening training session (9:00 am and 6:00 pm).

Results: Mixed ANOVA was used to test the interactions between chronotypes, physical performance, and time. On all tests, M-types performed better during the morning than the evening session, whereas better performance during the evening than the morning session was observed for the E-types. No differences in test performance were detected for the N-types.

Conclusion: These findings underline the importance of a correct chronobiological approach to sports training. Scheduling training sessions according to an athlete's circadian preferences could be a valid strategy to enhance performance.

Effect of chronotype on rating of perceived exertion in active young people

Mulè A., Galasso L., Castelli L., Condemi V., Bisconti V., Esposito F., Roveda E., Montaruli A. (2019) *Sport Sciences for Health*, 16:331-336. Doi: https://doi.org/10.1007/s11332-019-00610-9.

Abstract

Background: The differences existing between individuals are determined by different expression of the circadian rhythm. In this way, individuals display preferences to be active at certain time of the day and based on these differences, they can be classified into three different chronotypes: Morning-types (M-types), whom get up and go to bed early and usually have their best mental and physical performances in the first part of the day; Evening-types (E-types), whom get up and go to bed later, and have their peak performances in the evening; Neither-types (N-types), whom show intermediate characteristics. Although many authors have shown a relationship between many variables related to sports performance and chronotype, few studies have shown the influence of day time on performance and whether the predisposition to a certain chronotype may affect this aspect.

Aim: The study aimed to evaluate the effect of chronotype on aerobic performance, heart rate (HR) and Rating of Perceived Exertion (RPE).

Materials and methods: We administered the Morningness–Eveningness Questionnaire (MEQ) to determine the chronotype's students attending the School of Sport Sciences (University of Milan). To investigate the effect of chronotype on aerobic performance, HR and RPE, 22 participants (11 M-types, 11 E-types) performed the Cooper test at 9:00 a.m. and at 5:00 p.m. Before and after the Cooper test, the RPE was detected using the Borg Scale CR 0-10.

Results: The two-way ANOVA showed an interaction between chronotype, time span and RPE. Specifically, M-types perceived less effort in the morning compared to the afternoon sessions, both before and after exercise. E-types felt more fatigued in the morning than in the afternoon sessions, both before and after exercise. Moreover, in the morning sessions, E-types had a greater perception of the effort than M-types. By contrast, in the afternoon sessions, M-types showed higher RPE values than E-types. Cooper Test and HR results did not show statistically significant differences. On the other hand, no interaction was found between chronotype, day time and performance or HR.

Conclusion: M-types perceive higher effort in the afternoon session, while E-types show an opposite trend and are more fatigued in the morning session. These findings may be useful to the coach to plane tailored training programs.

The multidisciplinary therapy in binge eating disorder is able to influence the interdaily stability and sleep quality?

Galasso L., Montaruli A., **Mulè A.**, Castelli L., Bruno E., Caumo A., Esposito F., Roveda E. (2019) *Cronobiology international*, 36(10)1311-1315. Doi: 10.1080/07420528.2019.1650059.

Abstract

Background: In our previous study we showed that Rest-Activity circadian Rhythm (RAR) significantly differed in women with Binge Eating Disorder (BED) compared to the Control group. In particular, BED patients showed statistically significant reduction of the Estimating Statistic of Rhythm (MESOR) and Amplitude levels compared to the Control group. Moreover, in the same study, the results of the actigraphic monitoring showed no differences in sleep parameters between the two groups.

Aim: The aim of the present letter is to improve the results of the previous study with a new non-parametric analysis of the RAR.

Materials and methods: We increased the original sample obtaining a total of 28 volunteered women, 14 BED women, and 14 Control group. A 5 days actigraphic monitoring was recorded in all 28 participants to detect sleep and the non-parametric RAR parameters: interdaily stability (IS), intradaily variability (IV), night activity (L5) and daily activity (M10). During the study, BED's women group kept an individual cognitive and nutritional therapy lasting five weekly days, from Monday to Friday. It was administered in out-patient care from 8:00 a.m. to 5:00 p.m. The combination of both our previous and current study results support the conclusion that the sleep quality of the BED group is significantly better compared to Control group. Moreover, the non-parametric indexes showed how IS, significantly correlated to sleep efficiency, was higher in BED group compared to the Control group, indicating a better synchronization of RAR.

Conclusion: The maintenance of a regular lifestyle, such as imposed by the multidisciplinary therapy, is important to avoid alterations in the sleep-wake circadian rhythm, particularly in BED patients.

Rest-activity circadian rhythm in breast cancer survivors at 5 years after the primary diagnosis

Roveda E., Bruno E., Galasso L., **Mulè A.**, Castelli L., Villarini A., Caumo A., Esposito F., Montaruli A., Pasanisi P. (2019) *Cronobiology international*, 36(8):1156-1165.
Doi:10.1080/07420528.2019.1621330.

Abstract

Background: Rest-Activity circadian Rhythm (RAR) can be used as marker of the circadian timing system. Some authors investigated the alterations of RAR in breast cancer (BC) patients, at different stages of clinical pathway. However, no studies to date have analyzed RAR alterations in breast cancer survivors several years after the diagnosis.

Aim: The aim of the present study was to evaluate RAR, by actigraphy method, in a population of BC survivors at 5 years after the primary diagnosis, and to compare their RAR characteristics with healthy controls.

Materials and methods: We recruited 28 women: 15 BC survivors at 5 years from the primary diagnosis (BC group) and 13 healthy controls (Control group), matched for age and body mass index. All participants have been monitored for 7-day by actigraphy to evaluate RAR.

Results: A statistically significant circadian rhythm (T=24) was found in all 28 subjects. The group analysis revealed a significant RAR both in BC and Control group. No differences in acrophase were found in the two groups. In contrast, BC group showed lower Midline Estimating Statistic of Rhythm (MESOR) and amplitude than Control group.

Conclusion: These results provide the first experimental evidence of alterations in RAR parameters in BC survivors at 5 years after the primary diagnosis. Larger prospective studies are needed to assess the role of RAR in the quality of life and prognosis in BC survivors.

Physical activity, chronotype and sleep in a sample of Italian elderly population

Castelli L., Galasso L., **Mulè A.**, Bruno E., Shokohyar S., Esposito F., Montaruli A., Roveda E. (2019) *Sport Sciences for Health*, 16:55-64. Doi: https://doi.org/10.1007/s11332-019-00573-x.

Abstract

Background: Aging is a natural process that induces several physiological changes. A continuous decline in body structures and functions, owing of metabolism, accumulation of body fat and sarcopenia contribute to modify the body composition that may be aggravated by a sedentary lifestyle. The aging-related modifications also affect sleep habits and chronotype expression. Many health benefits of physical activity have been evaluated passing from sedentary to active lifestyle.

Aim: The present study aimed to evaluate the role of physical habits and chronotype on sleep patterns in a sample of Italian elderly population.

Materials and methods: 100 Italian elderly people (50 males, mean age ± SD 70.9 ± 6.3 years) were enrolled and completed the Morningness–Eveningness Questionnaire (MEQ), the International Physical Activity Questionnaire Short Form and the Pittsburgh Sleep Quality Index (PSQI). Moreover, we evaluated the lipid profile (total cholesterol, triglycerides, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) values, total cholesterol/HDL and LDL/HDL ratios).

Results: Active subjects (AS) showed better PSQI score compared to inactive subjects (IS). The AS made less use of sleep medications and reported less sleep disturbances and daytime dysfunctions compared to IS. PSQI final and component scores for Neither types (N-types) were lower compared to those of Morning types (M-types). Referring to the females, active N-type collected lower PSQI value compared to active M-types. Active M-types made less use of sleep medications and had less sleep dysfunction compared to inactive M-types, while active N-types reported less daytime dysfunction compared to inactive N-types. Focusing on males, active M-types reported less disturbances compared to inactive N-types. IS showed lower HDL level compared to AS.

Conclusion: M-types had worst sleeping habits compared to N-types. IS had worse global sleep quality, more sleep disturbances and made greater use of sleep medications than AS. An active lifestyle could be considered as useful tool to improve sleep and reduces sleep problems during aging.

Effect of chronotype on academic achievement in a sample of Italian University students

Montaruli A., Castelli L., Galasso L., **Mulè A.**, Bruno E., Esposito F., Caumo A., Roveda E. (2019) *Chronobiology International*, 36(11):1482-1495, Doi: 10.1080/07420528.2019.1652831.

Abstract

Background: Circadian rhythms expression differs across individuals, determining the identification of three chronotypes: Morning-type (M-type), Neither-type (N-types) and Evening-types (E-type). University class schedules can sometimes conflict with E-types circadian preferences, compromising their academic performance. Conversely, M-types students, being more aligned with their daily schedule, might be more advantaged in their mental performance. In literature, the attitudes and performance of N-types students are little considered. Moreover, no studies to date have investigated academic achievement in relation to chronotype in an Italian student population.

Aim: To fill this gap, this study examined the relationship between chronotype and academic performance in a population of Sport Science Faculty in Milan, differentiating achievement in theoretical and practical subjects by chronotype.

Materials and methods: 423 university students (290 males and 133 females) were enrolled in the study and categorized by chronotype according to Morningness-Eveningness Questionnaire (MEQ) scores. Student transcripts were reviewed to obtain exam grades on three practical and three theoretical subjects.

Results: The mean grades on the theoretical and practical exams were higher for the M-types than for either the E-types or the N-types students. The N-types students had lower mean grades for the theoretical subjects than either the M-types or the E-types students, while the E-types performed worse than either the M-types or the N-types students on the practical exams. The same trend was observed for the total sample and when subdivided by sex. In the total sample, significant differences in theoretical and practical exam grades were noted between chronotypes: M-types *vs* E-types and M-types *vs* N-types. We found differences theoretical subjects between the males M-types compared to males N-types, and in the practical subjects between M-types and E-types and between M-types and N-types. No significant differences were noted between the females.

Conclusion: The results of the present study indicate overall better academic achievement by the M-types students, whereas the N-types had lower exam grades for the theoretical subjects and the E-types performed worse on the practical exams. We speculate that the higher intelligence expressed by the E-types students might have helped them compensate the disadvantage on the theoretical but not on practical exams, in which the effect of misalignment between circadian preferences and university class schedule was more evident.

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