UNIVERSITÀ DEGLI STUDI DI MILANO

PHD COURSE IN TRANSLATIONAL MEDICINE

PhD Thesis

DAILY ACTIVITY LEVELS AND SLEEP QUALITY IN BREAST CANCER

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Table of contents

Abstract

Rest-activity circadian rhythm (RAR) analysis is a valuable tool to evaluate daily physical activity levels and sleep quality in breast cancer (BC) women, including BC survivors, a population less considered in the scientific literature. Indeed, the role of physical activity is recognised even in tertiary cancer prevention due to its action either on physical or psychological human spheres. In managing the quality of life in BC women, sleep assessment and its relationship with physical activity also raise attention. Several studies reported that an increase in physical activity practice might lead to better sleep quality. All these aspects have been less investigated in BRCA1/2 carrier women. BRCA1/2 are deleterious and high-invasive gene mutations, predisposing to a very aggressive breast and/or ovarian cancer also at a young age.

The present PhD thesis evaluates RAR, sleep, and their relationship in two populations: a cohort of 5-year BC survivors and a sample of BRCA1/2 women.

For the first study, 28 women (15 5-year BC survivors and 13 healthy controls) were 7-day long actigraph monitored and RAR analysis was performed with both parametric and non-parametric approaches. BC survivors showed a statistically lower MESOR (Midline Estimating Statistic of Rhythm), amplitude, L5 (nocturnal activity), and M10 (daily activity), while IV (Intradaily Variability) was higher than the control group. These results are the first experimental evidence that RAR alterations persist

after 5 years since the primary diagnosis. Furthermore, BC survivors are less active than healthy controls and need practical intervention to increase their activity levels.

For the second study, 27 women with BRCA1/2 mutations were 7-day long actigraph monitored, while 63 filled in the PSQI (Pittsburgh Sleep Quality Index) and the GSL-TPAQ (Godin Shepard Leisure-Time Physical Activity Questionnaire) questionnaires to assess sleep and physical activity, respectively. The 27 actigraph-monitored women were stratified, based on the development of cancer diagnosis, in affected and unaffected. RAR and actigraphic sleep analysis showed no statistically significant differences between the two groups, even though the affected women seemed to sleep worse than the unaffected. Based on the PSQI score, the women were stratified into good and bad sleepers: good sleepers were significantly more active than bad sleepers. Based on the GSL-TPAQ score, women were stratified into active and inactive: active women showed a better body composition and significantly lower insulin level and better sleep than inactive women. Finally, the regression analyses disclosed the positive effect of physical activity on sleep. More specifically, the prevalence ratio of being a good sleeper significantly increased with the increase in amount, intensity, and frequency of physical activity. This cross-sectional analysis of 63 women sheds light on a possible association between physical activity and sleep in BRCA1/2 mutation carriers. Considering the large attention that the BRCA1/2 carriers' quality of life is receiving, a physical activity intervention could potentially improve the sleep quality in these women, also reflecting in an enhanced quality of life.

Prologue

The present thesis deals with the principal research topics, which have been deepened and consolidated during my PhD course. The main research lines have focused on analysing activity levels, rest-activity circadian rhythm, sleep, and physical activity in women with breast cancer. Firstly, I assessed these parameters, predominantly activity levels and rest-activity circadian rhythm, in women diagnosed and survived sporadic breast cancer. Later, I focused on women carrying high-penetrance gene mutations (*BRCA1/2 genes*) predisposing to breast and ovarian cancer.

The thesis is divided into two sections: the former is the introduction, which is based on the literature review of the topics I focused on during the three years of my PhD; the latter is the section of the doctoral studies, which lies on four chapters exposing the collected data and conclusions on both sporadic and hereditary/genetic breast cancer and a chapter exposing all other research items I dealt with during my PhD course.

Particularly, the first chapter reports the latest findings in the relationship between physical activity and cancer risk factors, analysing it during all cancer stages (i.e., before, during, and after diagnosis or treatment). The second chapter illustrates the influences of physical activity on sleep, considering how this relationship could enhance health status. The third chapter explains breast cancer pathology in several aspects, starting from the incidence and typology, passing from the diagnosis, and ending with genetic/hereditary aspects of the pathology. In this section, *BRCA1/2 genes* are

treated with a specific focus on how the mutations diagnosis and the consequent treatments could be detrimental to women's quality of life. The fourth chapter is a methodological one explaining the chronobiology, the circadian rhythms, their parameters with a particular focus on *Single Cosinor Method*, and the actigraphy, a methodology used to record and evaluate rest-activity circadian rhythm and sleep. The fourth chapter, the last of the introductory chapters, brings together all the topics treated in the previous chapters; thus, physical activity and sleep in the specific context of breast cancer and, when data and studies are available, of *BRCA1/2* carriers. Furthermore, rest-activity circadian rhythm is contextualized in breast cancer pathology.

The second section (devoted to explaining the doctoral studies) starts with chapter six, illustrating the rest-activity circadian rhythm assessed in five-year survival breast cancer women compared to a healthy sample. The evaluation has been carried out through the cosine method analysis and, more specifically, with the parametric parameters of the cosine method. The subsequent chapter, the seventh, continues the rest-activity circadian rhythm analysis with non-parametric parameters of the cosine method. Finally, chapter eight is dedicated to *BRCA1/2* carrier women, with a rest-activity circadian rhythm and sleep analysis, involving data collected with the actigraph and analysed with the cosine methods (both parametric and non-parametric parameters) physical activity and sleep data collected through questionnaire administration. In these three chapters, the introduction session has been omitted because it is widely illustrated in the preceding chapters; thus, only the aim of the study, participants, methods, results and discussion are reported. The women involved in these studies were recruited and enrolled in collaboration with *IRCCS Istituto Nazionale dei Tumori di Milano* (Milan, Italy). After these three chapters of the primary doctoral studies, the ninth chapter advances the major conclusions of these studies.

The last chapter briefly summarizes all other articles I worked on during my PhD course. They are grouped based on the treated topic. The first reports the analysed data during my research stay at

the *Technische Universität – Dortmund* under the supervision of Prof. Dr. Dr. Philipp Zimmer; the first involved haematological cancer patients, while the second healthy subjects both evaluated in sleep, physical activity, and fatigue. Subsequently, three publications follow on a sample of shift nurses (recruited in collaboration with *IRCCS Istituto Ortopedico Galeazzi*, Milano, Italy) with their sleep analysis, rest-activity circadian rhythm, and muscular fatigue. The penultimate includes a group of publications involving the chronotype assessment in relation to other aspects (sleep, physical and academic performance). Finally, three works involving subjects with *Metabolic Syndrome* and *Binge Eating Disorders* (recruited in collaboration with *IRCCS Istituto Nazionale dei Tumori di Milano*, Milano, Italy and *IRCCS Ospedale San Raffaele-Distaccamento Turro*, Milano, Italy) close this chapter.

First Part

Introduction

1. Physical activity and cancer

Physical activity is described by Caspersen and colleagues (1985) as:

"…*any bodily movement produced by skeletal muscles that results in energy expenditure. Physical activity can be categorised in a variety of ways. (…) The simplest categorisation identifies the physical activity that occurs while sleeping, at work, and at leisure. (…) Leisure-time physical activity can be further subdivided into categories such as sports, conditioning exercises, household tasks (for example, yardwork, cleaning, and home repair), and other activities."*[1]

Physical activity practised during the day is an issue of great concern in health-related literature. Indeed, the decreased level of physical activity and the resulting reduced energy expenditure may be the principal causes of several chronic pathologies of the new millennium, including some types of cancers. Prolonged sitting time while working or at home and enhanced use of means of transport are the principal attitudes that characterise the contemporary age's sedentary lifestyle. Furthermore, prolonged sitting time increases mortality rates, while physical activity frequency reduces the mortality risk.[2]

Sedentary or less active habits enormously decrease energy expenditure, which is not counterbalanced by a diminished energy intake. When the caloric intake is not sufficiently disposed of, the food surplus is stored as adipose tissue, leading to obesity.[3,4]

If countermeasures are not adopted, obesity is considered the new and more significant epidemic pathology and problem for the present and future generations.[5] Obesity is responsible for promoting many cancer types, because many cancer onset pathways are obesity-dependent. Fortunately, obesity is a reversible situation sensitive to physical activity practice.[3]

The following chapters introduce the principal cancer-related risk factors, the majority linked to obesity. Furthermore, it will explain the action of physical activity against the described risk factors and the different outcomes depending on the cancer stage.

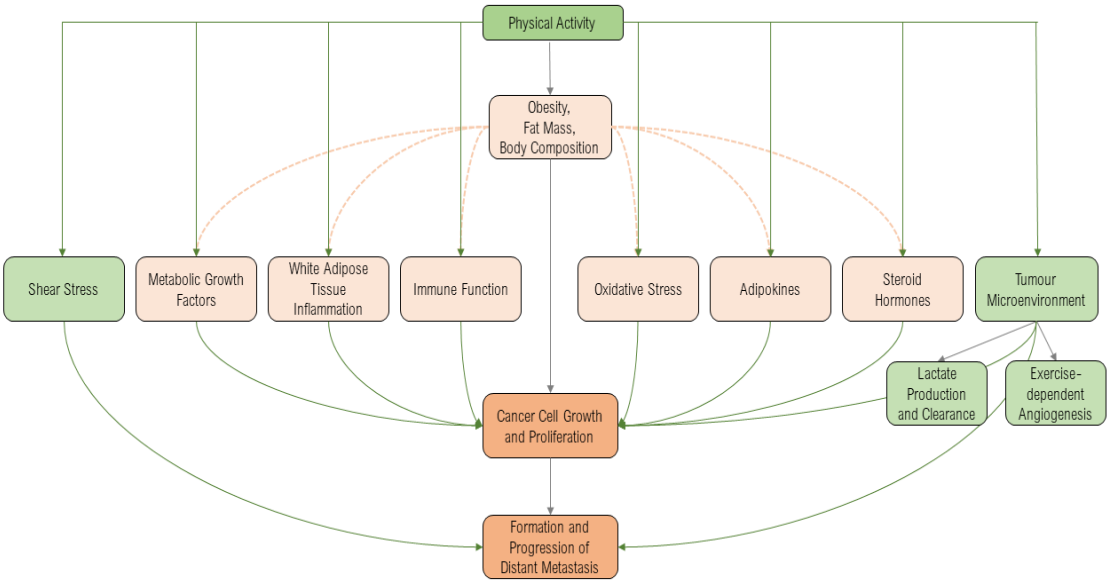

Figure 1 *Hypothesised mechanisms underlying the association between physical activity and cancer onset development. The effects of physical activity may be mediated, in part, by body composition.*

1.1 Cancer-related risk factors

Risk factors predisposing to cancer onset are described as *non-modifiable* risk factors (sex, age, ethnicity, familiarity, gene mutations, etc.) and *modifiable* risk factors (physical activity, diet, smoking habits, sleep, alcohol assumption, etc.). The latter ones are also named *lifestyle or environmental factors.* They are described as a potent contributing factor to the onset of the most common cancers. Indeed, modifiable lifestyle factors account for 35-50% of the most commonly diagnosed cancer and cancer-related deaths.[6] In this view, lifestyle modification could prevent between 20% and 40% of all cancer cases, and the percentages are even more prominent when considering specific cancer types and risk factors (such as lung cancer and smoking habits or liver cancer and alcohol consumption).[6]

Cancer cases attributable to modifiable lifestyle factors have increased during recent years.[6] More specifically, cancer causes derived from energy balance compete with smoking habits as the leading preventable cause of cancer.[7] A higher than needed chronic energy intake associated with a lower energy expenditure results in obesity. In addition to predisposing to several cardiovascular diseases, obesity is recognised for its cancerogenic aspect and one of the major risk factors for cancer.[3,8] Obesity is usually assessed through the *Body Mass Index* (*BM*I) calculation. The review by Renehan and colleagues (2008) reports a positive relationship between BMI and risk ratio for oesophageal, thyroid, colon, renal, liver gallbladder, rectum (only men), pancreas, prostate, endometrium, breast and ovarian cancer, as well as for malignant melanoma (only men), multiple myeloma, leukaemia, and non-Hodgkin lymphoma.[9]

Cancers whose primary risk factor is obesity are named *obesity-dependent*, *obesity-associated* or *obesity-related* cancers.[9] However, not all cancers are obesity-related. Indeed, fatness and greater body weight or BMI are still not recognised as leading causes of the onset of all cancer types,

or cancer could also develop in a non-obesity or overweight condition.[9] The obese-associated cancers are usually allocated near an adipose deposit, such as breast and visceral cancers.[10]

Even though several studies prove the connection between chronic obesity status and cancer development, mechanisms involving obesity in cancer onset are still not fully understood. It is supposed that obesity, particularly the more represented fat tissue, can create a favourable environment for the tumour and promote dysregulation in numerous regulatory networks that are able to predispose to cancer.[7,11] The main dysregulations in pathways and regulatory networks comprise metabolism, inflammation, oxidative stress, adipokine and sex hormones balances (Figure 1). For these mechanisms, the greater adipose tissue representation acts as a mediator that is able to modify the host tissue or tumour microenvironment, creating favourable conditions for the cancer onset and growth.[7] This is possible because the adipose tissue is not a passive and inactive mass, as initially supposed; on the contrary, it participates actively in a network of endocrine, paracrine and autocrine signals.[12]

Another hypothesis supports the idea that cancer outset may be promoted and facilitated by obesity because of its nutrients' support. Cancer stems from a neoplastic transformation due to a mutagenic mutation during the division and replication of the cells. After the development, mutated cells could survive and grow only if they are nourished and continuously supported in their growth process through growth factors stimulation.[13] The obesity condition, activating the growth factors signalling pathways, guarantee nutrient and growth stimulation support to the cancer cells.[3]

1.1.1 The role of metabolic growth factors

The chronic overrepresentation of the fat tissue in body composition facilitates tumorigenesis through insulin and growth factors stimulation. Pancreatic beta cells secrete insulin in response to enhanced blood glucose levels. Insulin links its specific receptors (*Insulin Receptors - IR*) on the cell's surface and induces a factorial cascade with different outcomes depending on the stimulated tissue.[3]

In several cancers, IRs are overexpressed, and this may explain cancer's sensitivity to hyperinsulinemia.[14] Furthermore, IR could express two splice variants: IR-A and IR-B, and cancer type could differentiate their characteristic based on one of these two IR variants' overexpression.[15–17]

The principal metabolic growth factors are two peptide hormones: the *Insulin-like Growth Factor-1* (*IGF-1*) and *the Insulin-like Growth Factor-2* (*IGF-2*), both released by the liver. The *Insulin-like Growth Factor Binding Proteins-1* (*IGFBP-1*) regulates the IGF-1 action, binding the IGF-1 and preventing the binding with the IGF-1 receptors. IGF-2 activity is mediated through insulin and IGF-1 receptors. The growth hormone and the heightened insulin level stimulate their secretion.[3] The IGF-1 upregulation has been detected in some cancer types (such as prostate, breast, endometrial, and non–small-cell lung cancers)[15,18–20], while increased expression of IGF-2 has been revealed mainly in colon cancer.[21]

Fatness is usually associated with altered circulating glucose, whose upregulation stimulates insulin secretion, leading to insulin resistance and hyperinsulinemia. Chronic higher circulating insulin levels enhances the bioavailability of the IGF-1 by intensifying its secretion and, at the same time, reducing the action of the IGFBP-1. Insulin and IGF-1 induce anabolic processes in nonendocrine tissue; thus, their upregulated secretion leads to greater nutrients uptake supporting the uncontrolled cell growth, survival, and proliferation, also involving mutated cells.[3]

In conclusion, the dysregulation, particularly the upregulation of the metabolic regulatory networks, concomitance to mutagen factors (such as oxidative stress products), and other epigenetic shifts in gene regulation predisposes the obese subjects to cancer development risk.[22,23] The cancer risk factors are even augmented in case of specific mutations, such as alterations in the *phosphatidylinositol 3-kinase* (*PI3K*). The PI3K is a pathway whose mutation makes cells more resistant to caloric restraint, aiming to improve obesity and the associated higher insulin and IGF-1 actions. Thus, diet interventions in PI3K alterations are less efficient than in subjects without alterations in this pathway.[24]

1.1.2 The role of white adipose tissue inflammation

Adipose tissue, more specifically the white adipose tissue, is the substrate mainly characterising the obesity phenotype and the gusset linking the inflammation of the tumour microenvironment, obesity, and cancer.[10,25] In some conditions, the tumour microenvironment closely resembles the environment of a healing wound that can stimulate the local inflammatory response and, as a consequence, alter the angiogenesis' process.[26,27] The newly formed pro-neoplastic microenvironment could favour malignant cells' reproduction and promote cancer genesis and growth.[19]

As a consequence of the adipose tissue's outgrowth and hypertrophy in fat depots, the blood supply results insufficient to support and nourish all the needings of adipocytes, causing their suffering and, most of the time, deaths. The immune system recognises this situation as similar to an injury situation and triggers all the local pro-inflammatory responses. As well as any other inflammatory response, several complementary factors stimulate the neutrophil chemotaxis, which, in turn, activates the platelet-derived growth factor cascade. The process leads to the transformation and activation of the *growth factor-b*, *monocyte chemoattractant protein-1* (*MCP-1*), *interleukin* (*IL*)*-1b,* and *tumour necrosis factor-a* (*TNF-a*). Furthermore, the pro-inflammatory process guides the circulating macrophages to the suffering adipose tissue.[10,28]

Macrophages, in particular, engage in the phagocytosis process against suffering or dead adipocytes. They encircle the fat cell, forming a typical inflamed adipose tissue structure termed *crown-like structure (CLS)*. The entrapped and under macrophages' digestion adipocytes release the *free fatty acids* (FFAs) that can activate the *toll-like receptor 4* (TLR) allocated on the macrophage plasma membrane. Its stimulation increases the expression of some pro-inflammatory genes, such as the *nuclear factor kappa B-dependent*, which includes TNF-a, IL-1b, and *cyclooxygenase-2* (*COX-2*). TNF-a and other pro-inflammatory cytokines further sustain the white adipose tissue inflammation because they can additionally stimulate the lipolysis and release of FFAs.[10] The inflammation of the white

adipose tissue is a kind of inflammation that persists for a long time and is usually defined as *chronic low-grade inflammation*.

The CLS presence helps detect the white adipose tissue inflammation, and studies have assessed the CLS presence in visceral, subcutaneous, and breast fat deposits on the majority of examined obese subjects.[29] However, the CLS presence is not the only signalling factor of adipose tissue inflammation; most of the time, it is associated with an elevated expression of *nuclear factor-kappa B,* responsible at the same time for inflammation and cancer progression.[30] The observed higher CTS and *nuclear factor kappa B* levels are supposed to be responsible for reduced cancer survival, shortened distant recurrence-free, and cancer-related disease in patients with cancer.[31] Additionally, unfavourable clinical outcomes have also been estimated in cancer patients with a normal BMI. Thus, in some cases, white adipose tissue inflammation could develop independently from obesity.[10,29,32]

1.1.3 The role of the immune system

In cancer patients, the immune system alterations also comprise the adaptative immune system branch (not only the innate one as described previously) with both myeloid and lymphoid compartments.[33] The role of obesity on immune function alterations is still not delineated; however, recent studies, predominantly on animal models, have discovered possible mechanisms.[34,35]

In general, the immune system can recognize and eliminate mutated cells because they are recognized by specific immune cells (e.g., *cytotoxic antigen-specific T cells*). The myeloid compartment responds with the production of *dendritic cells*, while the lymphoid system reacts with the activation of *natural killer* (*NK*)-, *γδ T*-, *T-helper*- (*CD4+ T* and *CD8+ T*), and *B*- cells. However, cancer can escape the immune system controls and continue its growth in some conditions, such as chronic low-level inflammation and immune-compromised or obese individuals.[33,34]

Obesity could decrease the adaptative immune system surveillance effectiveness and inhibit its cancer counteraction in different ways. Firstly, lipid cell accumulation impairs the action of the dendritic cells and, at the same time, weakens their number and response. Secondly, obese individuals are deficient in NK numbers and citoxocity, probably because NK in obese incorporate lipids droplets instead of perforin granules. Thirdly, lipid uptake could inhibit the *rapamycin complex 1* (*mTORC1*), a crucial pathway for the activation and function of NK cells. Finally, concordant results on both animals model and human tissues advance the hypothesis that, when stimulated, the NK cells of obese individuals cannot increase oxidative phosphorylation in order to augment their basal metabolism.[35]

In such a situation, cancer can dissimulate adaptive system function checkpoints and continue its expansion.[34] Liver cancer is the primary cancer type in which the role of obesity on the immune system has been recognized.[36]

1.1.4 The role of oxidative stress

Obesity status is usually characterised by a higher level of oxidative stress, a cellular state in which unstable oxidant molecules override the antioxidant defence mechanisms.[37,38] The unstable oxidant molecules are the *reactive oxygen species* (*ROS*) and the *reactive nitrogen species* (*RNS*), while the defence mechanisms are the *antioxidants* (*AOX*), which can scavenge both ROS and RNS. However, the over-threshold ROS production makes the AOX insufficient to contrast the ROS adverse effects. The ROS excess can alter and damage the mitochondrial and genomic DNA; particularly, ROS can invert GC pairs with TA pairs during DNA replication. The unfavourable change between the two DNA pairs results in damage and mutation in the DNA and altered signal pathways that, together, are the basis for cancer onset, promotion, and progression.[38]

Severe obesity is correlated with lipid peroxidation resulting from elevated oxidative stress.[28] Two main mechanisms could justify the higher oxidative stress and the consequent lipid peroxidation.

The first is that obese subjects require enhanced mechanical and metabolic loads to the myocardium, which, in turn, increases myocardial oxygen consumption and the mitochondrial respiration that naturally produces ROS. The second mechanism refers to the pressure of the great body mass, provoking progressive cell injuries and the consequent release of cytokine responsible for ROS production.[39]

The ROS oxidative action on the polyunsaturated fatty acids results in *lipid peroxyl radicals* and *lipid hydroperoxides*, two lipid peroxidation products. These products are led to degradation by different aldehydes typologies (among them *malondialdehyde* and *4-hydroxy-2-nonenal*), genotoxic products highly responsive to cellular macromolecules that can produce DNA-additive proteins. By interacting with genomic DNA, these proteins can mutate the DNA sequence and promote cancer initiation. [28] As previously mentioned, DNA mutation, induced by increased oxidative stress and its related consequences, is recognised as a promoter of cancerogenesis, cancer progression, and metastasis settlement.[40,41] Furthermore, inflammation conditions could also alter ROS production, disable AOX action and favour adverse ROS consequences on DNA.

1.1.5 The role of adipokines imbalance

Adipokines are a wing of cytokines secreted by the adipose tissue; among all adipokines, *leptin* and *adiponectin* are the most relevant in the cancer field. As the other cytokines, these two pleiotropic adipokines are involved in cellular and systemic signalling and inflammatory pathways; furthermore, they are responsible for satiety. In particular, leptin regulates appetite and energy balance and, in obese conditions, its level increases in contrast with adiponectin levels, which decrease with higher fat mass representation.[3,42]

An excess of leptin secretion is known to inhibit cell apoptosis, promote cell proliferation, and activate multiple signalling cascades and pathways, including the PI3K, *mitogen-activated protein kinase* (*MAPK*), and IL-6 production. Thus, it is supposed that enhanced circulating leptin could increase

cancer risk or proliferation. However, results are disparate depending on the types of cancer, sex, and age. Recent developments state that the leptin/adiponectin ratio better expresses cancer risk related to adipokines.[3,42]

On the other hand, adiponectin inhibits angiogenesis, induces cell apoptosis, and, in some conditions, impedes leptin and IL-6 proliferation. Thus, its action counterbalances the pro-cancerogenesis effects of leptin. Related to this, in several studies and reviews, the adiponectin impact has been described as a protective role in cancer.[3,42]

In this context, by unbalancing the ratio between leptin and adiponectin favouring the former, the obese status and enhanced fat mass could be considered predisposing to cancer onset.[3,42]

1.1.6 The role of steroid hormones

The principal and recognised steroid hormones linking obesity to cancer risk are *oestrogens* and *androgens*. In the context of obesity, pathways and interactions are better described and understood for oestrogens and typical female cancer, while androgens' action in prostate cancer still raises some doubts.[3,42]

Ovaries are the organs in which the aromatase process converts androgens into oestrogens. Therefore, when ovaries cease their function at the outset of the menopause period, fat tissue becomes the principal contributor to the aromatase process. From this point of view, an excess of fat tissue intensifies the aromatase process; in other words, the more considerable amount of fat tissue converts a significant volume of androgens into oestrogens. Furthermore, the excess of adipose tissue in concomitant hyperinsulinemia decreases the *Sex Hormone Binding Globulin* (*SHBG*) action, a globulin that can link estrogenic hormones, limiting their effects.

The higher level of blood circulating oestrogens hyperstimulates the cells exposing oestrogen-receptors in proliferating and reducing apoptosis. Oestrogens potentially induce mutagenesis

because they can be metabolised into reactive DNA metabolites. These are all favourable conditions for endometrial and hormone receptor-positive breast cancer development in postmenopausal conditions. That is why targeting oestrogen levels in plasma is helpful to modulate endometrial and breast cancer risk in obese postmenopausal women.[3,10,42,43]

Regarding men and typically male cancers, such as prostate cancer, it is less clear how obesity and cancer are interrelated. Indeed, obesity is associated with a lowered level of circulating androgens because the aromatase process converts them into oestrogens. However, obesity and aggressive prostate cancer are strongly associated. It is supposed that androgens can crosstalk with circulating cytokines (e.g., IL-6) and growth factors (e.g., IGF-1), activating the androgen receptors and stimulating some pathways leading to cancer initiation. Alternatively, androgens receptors could lose their specificity, IL-6 and/or IGF-1 could activate the androgen receptors, which promiscuously bind ligands driving cell survival and proliferation.[5,8,33,44]

1.2 Physical activity and cancer prevention

Physical activity (PA) is one of the tools adopted in cancer prevention; indeed, a large body of literature sustains the linear relationship between PA and reduced cancer risk. In other words, the more PA is practised, the greater cancer prevention benefits.[45] In 2018, the *Physical Activity Guidelines Advisory Committee* concludes that robust evidence links PA with a lower risk of colon, breast, kidney, endometrial, bladder oesophageal adenocarcinoma, and stomach-cardia cancers.[46]

International Health Agencies well know the benefits of PA concerning cancer prevention. In 2018, the *World Cancer Research Fund* (*WCRF*) and the *American Institute for Cancer Research* (*AICR*) updated their previous recommendations (*WCRF/AICR 2007*) for cancer prevention. From the initial 150 min/week of light-to-moderate PA or 75 min/week of moderate-to-vigorous PA (MVPA),

new guidelines recommend engaging in light-to-moderate PA for 150 to 300 min/week or in MVPA for 75 to 150 min/week. The optimal situation is to exceed the 300 min/week of PA. Additionally, new recommendations focus on sedentary time: sitting and lying down should be limited as possible (*WCRF/AICR 2018*).[45,47]

Kaluza and colleagues (2020) state that people following the WCRF/AICR 2018 recommendations, specifically higher PA, average weight, and limited alcohol consumption, reduce cancer incidence. The results are in line with the previous *European EPIC* study conclusions that assess how following PA and body weight recommendations reduce the total cancer incidence.[48,49]

Despite the increasing evidence of the PA's role in population-based cancer prevention, many debates are still open about the intensity, typology, and PA assessment method related to cancer-risk prevention.[50,51] Moore and colleagues (2016) and Matthews and colleagues (2020) state that leisure-time PA (7.5-15 MET-hours/week; 2.5-5.0 hours/week of moderate-intensity PA) can prevent 13 and 7 cancer types, respectively.[50,52]

Furthermore, some types of cancer, such as colon, breast, endometrial, oesophageal, and head and neck, show a linear dose-response curve, which means that the preventive PA's role increases when the PA practice is over the recommended threshold and the intensity shifts to moderate-vigorous. Other cancer types (e.g., kidney, gastric, and liver) show a curvilinear association with PA, which means that risk reduction is maximum with the recommended PA amounts. In other words, an increased PA amount in these three cancer types does not lead to higher cancer-risk prevention.[50] These differences are probably attributable to the different effects of PA on tissues and physiological processes, which show different impacts depending on the cancer's nature and characteristics.[48]

Notwithstanding the evidence of large-scale studies, conclusions of other studies are sometimes discordant on the PA's role and benefits in cancer prevention. For example, the review of Capece and colleagues (2020) concludes that PA seems to be more efficacious on mortality rather than

prevention in prostate cancer.[53] In another study on lung cancer, the authors state that PA does not prevent lung cancer in the general population when genetic factors for lung cancer are also considered.[54] In contrast, previous studies on current or former smokers show PA's positive improvements in contrasting smoking's adverse effects, particularly on women.[55,56] Contrasting results can also be found for pancreatic cancer. The evidence in this field is still limited, and the PA role, if it should exist, on pancreatic cancer is still debated.[57,58] The most recent findings sustain the hypothesis that a generally healthy lifestyle (including active habits) could prevent pancreatic cancer.[59]

Another important aspect is the period of life during which the PA is practised. In a recent meta-analysis by Hidayat (2020), the PA practised at a young age is associated with a lower colon and breast cancer risk. Moreover, the lifetime PA decreases cancer-related risk for colon, breast, and endometrial cancer.[60] Even though the number of studies is limited, even for pancreatic cancer, a conclusion has recently been advanced, delineating how cumulative PA throughout the lifespan and during young age could be a protective factor against pancreas malignancies.[61]

1.3 Physical activity in primary prevention

The beneficial action against cancer-related risk factors could be carried out in several ways, and several biological pieces of evidence support the role of PA in cancer prevention. Firstly, PA impact has been related to cancer-related risk factors by reducing or preventing body weight increase and improving body composition with increased fat-free mass. From this point of view, PA's beneficial influence on body weight mediates the PA benefits in cancer prevention. Several cancer-risk factors are obesity-related; thus, in improving body weight, PA could indirectly improve the adverse consequences of some obesity-related risk factors predisposing to cancer (Figure 1).[60,62]

Even though body weight management represents an important goal in cancer prevention, evidence suggests that weight loss alone is insufficient to decrease cancer-related risk factors. By promoting, improving, and withdrawing some systemic and physiological functions to normal, PA may effectively prevent cancerogenesis in specific cancer types.[45]

Exercise, and consequently PA, induces several physiological modifications through complex and highly coordinated responses. This is possible because the skeletal muscle tissue is recognised as an endocrine organ with paracrine and endocrine signalling characteristics, stimulating interorgan communication.[64] During contraction, skeletal muscle tissue produces the myokines (cytokines expressed, produced, and released by the skeletal muscle tissue) responsible for the "crosstalk" between the muscle and other organs, such as bone, heart, liver, brain, and adipose tissue.[65] Interorgan communication allows reprogramming multiple regulatory systems, including metabolism and immunity. In this context, exercise is a potent-whole body environmental challenge, and the chronic exercise-induced perturbations could promote physiological adaptations affecting the tumour microenvironment.[7,65]

1.3.1 The effect of physical activity on metabolism and metabolic growth factors

Through insulin-independent mechanisms, chronic and constant PA substantially intensify glucose uptake and clearance in skeletal muscle, leading to reduced basal blood glucose, insulin, and IGF1 levels. Thus, PA helps in regulating glucose homeostasis.[66] In this way, in the first step, PA reduces the volume of available growth factors and nutrients in the systemic milieu, while, in the second step, it improves and/or restores the subsequent whole-body metabolic homeostasis.[65,67]

This improved or restored homeostasis involves the potential tumour microenvironment or tumour cells, which, through PA's chronic action, are not supplied anymore with enough nutrients and growth factors stimulation. Studies supporting this concept are emerging, even though tests have

been carried out only on animal models. For example, after PA intervention, delay in cancerogenesis and simultaneous reductions in circulating insulin and IGF1 are documented on a chemically induced mammary carcinogenesis model. In another study, again on an animal model, the PA can also reduce the PI3K activation, a pathway resistant to the lonely food restriction.[48]

1.3.2 The effect of physical activity on inflammation and immune system

PA can modify the two branches of the immune system; however, the responses vary depending on the acute or chronic habit of practising PA. In general, chronic PA lowers the pro-inflammatory circulating cells rather than increasing them, as happens after an acute bout of exercise.[67] In this way, leisure-time PA and PA practised in daily routines, such as active transportation to work, are established to decrease inflammation levels (e.g., circulating fibrinogen and C-reactive protein).[68]

The role of PA on inflammation and innate immunity is traceable in the endocrine function of the skeletal muscle. The targeting of the skeletal muscle tissue as an endocrine organ started by observing the IL-6 as a myokine, thus as a skeletal muscle tissue secreted factor. The production and release of IL-6 during the contraction are somewhat unique, both for its transcription process and function. During muscle contraction, IL-6 release increases up to 100-fold and can inhibit the TNF's production. TNF is one of the factors responsible for low-grade inflammation, predisposing to cancer development. To sum up, through its action on TNF, IL-6 secreted by the skeletal muscle during the contraction is targeted with an anti-inflammatory function rather than a pro-inflammatory function as it is usually described.[69]

Additionally, chronic PA favours the decrement in pro-inflammatory monocyte and cytokine amounts and modulates NK cells activity. In animal models, macrophage and neutrophil accumulation are reduced in rats exercising regularly, indicating that PA could avoid the concentration of these cells in fat tissue or a possible tumour microenvironment.[70] Finally, several studies prove the responsive

traits of NK cells to chronic PA. It seems that exercise and PA enhance the cytotoxin function of the NK cells, probably mediated by the releasing of the IL-6.[67,71]

Focusing on adaptative immunity, chronic PA seems to enhance the circulating lymphocytes, such as T cells. Additionally, in regularly active subjects, PA seems to prevent T cells from commitment to apoptosis.[49] Relating to cancer, 12 weeks of moderate- to high-intensity exercise reduces circulating concentrations and proportion of some growth factors, cytokines, and TNF in conjunction with a trend towards an increased number of activated $CD4^+$.[72] Particularly for $CD4^+$, its increment is seen with a high-intensity PA rather than a moderate-intensity PA. [49,73]

1.3.3 The effect of physical activity on oxidative stress

The ROS' downregulation may have antitumoral effects by promoting cell apoptosis. In this view, oxidative stress level modulation could help decrease cancer risks. PA is considered a helpful tool in regulating oxidative stress and, consequently, preventing cancer onset and development. Indeed, the PA mechanism in the context of oxidative stress may be summarised in two ways.[74]

Firstly, PA is usually associated with either an augmented AOX activity or an enhanced DNA error repair.[75] Taken together, these two actions have important implications in both cancer prevention and the long-term quality of life of cancer survivors.[76] However, especially in postmenopausal women, the role of PA on oxidative stress is more efficient when combined with a correct diet.[74]

Secondly, simultaneously with the antioxidant effect, PA shows a prooxidant activity. Generally, the tumour microenvironment and its balance between ROS and AOX production are sensitive to exogenous ROS. In this situation, the exogenous ROS induced by PA can disrupt the balance in the tumour microenvironment, and PA shows co-adjuvant characteristics during cancer therapies.[77,78] This effect is most visible in some genetic polymorphisms, which induce different and more potent responses.[74,79]

1.3.4 The effect of physical activity on adipokines imbalances

PA reduces the body mass, particularly the fat mass and, consequently, induces changes in circulating adipokines, a product of the fat mass. More specifically, between leptin and adiponectin, adipokines' unbalances are primarily rebalanced by PA to reduce the body fat mass. Chronic long-term (>12 weeks) exercise training protocols are usually accompanied by a reduction in the serum leptin level and increased adiponectin.[80]

Concerning acute exercise, studies state that the decrease of the leptin level needs a long-term (>60 minutes) with a high caloric expenditure (>800 kcal). This is probably due because exercises respecting these conditions can stimulate the FFA release and mobilisation, making the action of leptin unnecessary and, as a result, decreasing its circulating level.[80] This process is an evident link between obesity, PA, and cancer-related risk factors.[81]

1.3.5 The effect of physical activity on steroid hormones

PA may exert its anti-cancer effect on female steroid hormones directly and indirectly.[81] The first consists of a proven PA action on oestradiol hormone levels in both pre- and postmenopausal women. In other words, vigorous exercise can directly decrease the level of circulating oestradiol. The second expects the mediation of the PA action on the adipose tissue. Adipose tissue is one of the principal sites of sex hormones production in premenopausal and, especially, in postmenopausal women.[81] In a study conducted on 1092 women, the increased BMI corresponds to a decreased concentration of SHBG.[82] Hence, the reduced adipose tissue due to PA intervention weakens the aromatase process and, consequently, on the one hand, diminishes the circulating oestradiol, while on the other hand augments the circulating SHBG.[81–83]

Concerning the male steroid hormones (androgens), the most considered and studied is testosterone. Indeed, high testosterone bioavailability is linked to prostate cancer in men. The

testosterone bioavailability depends either on its concentration or on the SHBG circulating levels. Aerobic PA can reduce the first and increase the second; therefore, molecular evidence suggests the link between PA and reduced prostate cancer risk.[84] The study by Teixeira and colleagues (2012) shows that anaerobic PA can also modulate prostate cancer risk inducing specific cell apoptosis and balancing the equilibrium between androgen and oestrogen hormones in men.[85] Indeed, the lowered oestrogen level seems to play a critical role in preventing prostate cancer.[85] However, the association between PA and testosterone could be transient.[86]

1.3.6 The effect of physical activity on other cancer-related risk factors

Until now, PA's role in avoiding cancer development has been illustrated concerning the obesity-related risk factors. However, PA could exert several benefits in preventing carcinoma independently of the obesity condition. These other mechanisms are represented by the production and clearance of lactate and exercise-dependent angiogenesis.[52]

Regarding lactate, it is known that malignant cells and the tumour microenvironment alter the extracellular milieu's metabolic composition to create a favourable environment for cancer development. The alterations involve the utilisation of extracellular glucose and glutamine with a consequent higher lactate accumulation.[87] The increased lactate levels in the tumour microenvironment are responsible for the immunosuppression of immune cells, angiogenesis, and cancer cell invasion. All these consequences reflect a pro-tumorigenic effect of lactate accumulation.[13,67]

Even though tested only on a mouse model, seven weeks of PA reduced the cancer growth and the lactate accumulation in the tumour microenvironment. Therefore, it could be speculated that exercise may act with two mechanisms to reduce lactate concentrations. The first is that exercise and muscle contraction require and utilise nutrient availability by shifting from glycolysis to alternative processes (such as oxidative phosphorylation), producing energy with lower levels of lactate

accumulation. The second mechanism involves the enhanced post-long-term exercise vascularisation that may improve the lactate clearance in the tumour microenvironment.[13,67]

With regard to angiogenesis, chronic PA is a potent stimulator of exercise-dependent angiogenesis.[52] Trained muscles increase their capillarization to provide a larger surface area for oxygen flux and diffusion.[88] One of the principal mechanisms inducing angiogenesis is activating the *Hypoxia-Inducible Factor 1* (*HIF1*), which, in turn, induces the expression of the *Vascular Endothelial Growth Factor* (*VEGF*).[89] Explanations of the mechanisms by which exercise causes a hypoxic environment (among them a sufficient decrease in the partial pressure of oxygen in myocytes to activate HIF1) are controversial.[52]

However, the factor cascade inducing exercise-dependent angiogenesis is considered an effective and helpful exercise consequence to reduce the tumour microenvironment hypoxia condition.[52] Indeed, cancers show "abnormal" blood vessels, characterised by impaired oxygen delivery and low oxygen tension, leading to hypoxia. Hypoxia condition in solid cancer activates HIF1.[90] Unlike physiological angiogenesis, HIF1-induced angiogenesis paradoxically causes pathological angiogenesis, characterised by either impaired perfusion or oxygenation of the tumour microenvironment, thus creating an enhanced hypoxia condition.[91]

The translation of the regulatory effects of exercise on physiological angiogenesis in skeletal muscle to solid cancers has received attention. [52] The potential and supposed mechanisms in improving cancer hypoxia condition are four:

i. exercise may promote vessel and vascular maturity;[92]

ii. the exercise-induced shear stress may stimulate angiogenesis;[93]

iii. exercise increases the bioavailability of nitric oxide, which mediates vascular maturity and lymphatic vessel function;[94]

iv. cardiac output and blood flow are redistributed during exercise, and an animal model shows an increased blood cancer perfusion during exercise.[95]

1.4 Physical activity during cancer treatment

PA practice is extended and recommended after the cancer diagnosis, after surgical intervention, and during chemotherapy or other medical treatments (adjuvant treatments) for the newly diagnosed cancer pathology.[96,97] Indeed, cancer symptoms and treatment usually have a detrimental effect on body functioning and health-related quality of life, compromising daily life. During adjuvant treatments, the most reported clinical symptoms are constipation, diarrhoea and vomiting (responsible for weight loss following chemotherapy), pain, sudden menopause, decreased sexual function, and osteoporosis; additionally, cognitive and psychosocial impairments and symptoms as depression and anxiety may occur.[98]

Impaired physical functioning compromises most of the physical performance in cancer patients, limiting their social and daily life. In this view, PA, improving the required muscle activity for daily life tasks, is suggested as an intervention instrument and a potent countermeasure to reduce cancer treatment's side effects.[99,100]

1.4.1 The role of physical activity on chemotherapy outcomes, side-effects, cancer-related surgery, and quality of life

Studies show the feasibility and efficacy of PA in decreasing fatigue, anxiety, depression, nausea, sleep disturbance, and exhaustion and improving physical and physiological functioning, treatments- or cancer-related symptoms, quality of life, and emotional well-being.[96,101–103] In prostate cancer patients, active individuals (500-100 MET-min/week) report a higher quality of life than the

inactive counterpart. However, only the most active subjects (>1000 MET-min/week) also report emotional well-being benefits.[104] Furthermore, succeeding in engaging in PA protocol during adjuvant treatment is considered helpful by cancer patients also for their return to work.[96]

Although all these benefits are essential for cancer patients, also healthy or non-cancer-affected people experience them. What is gaining attention in the cancer field is the potential relationship between PA and treatment outcomes. Developing evidence is emerging on the PA's positive impact on treatment efficacy. Better chemotherapy completion rates are promising in patients undergoing PA intervention.[105] Studies are still minimal, but they show how the exercise-dependent angiogenesis could improve vascularisation in the tumour microenvironment and, consequently, reduce the hypoxia condition. In rat models, during exercise, the blood flow in the tumour microenvironment increases by 200% and the hypoxia reduces by 57%. A lowered hypoxia and a more oxygenated tissue improve both the radiotherapy efficacy and the delivery of chemotherapy drugs to the tumour microenvironment.[93] If this is the case, PA could be considered an adjunct therapy in cancer.[106]

Another critical regulation promoted by PA is on a chemotherapy-induced inflammation state. Chemotherapy excessively activates pro-inflammatory cytokines inducing dysregulation in the inflammatory function with a no longer balanced amount of pro- and anti-inflammatory cytokines. Hence, cancer patients undergoing chemotherapy experience a chronic pro-inflammatory status. In healthy subjects, PA is known for its effects on the inflammatory response, and, usually, IL-6 is the pro-inflammatory cytokine most represented and considered when PA is called into question. During the contraction, the muscle releases anti-inflammatory IL-6, which induces the anti-inflammatory IL-10 to reduce and inhibit either the pro-inflammatory IL-6 or the IL-1β concentrations.[107]

Klecker and colleagues (2019) demonstrate that a combined walking and resistance PA protocol can improve cancer patients' inflammatory profile undergoing adjuvant treatments. More specifically, the pro-inflammatory cytokines decrease after six weeks, while the anti-inflammatory increases

from pre- to post-intervention. Furthermore, the exercise group exhibits a positive correlation in changes between IL-6 and IL-10 and between the cytokines and receptors, suggesting an improved inflammatory profile. Due to the interleukins and inflammatory state's contribution to fatigue, pain, neuropathy, and cognitive impairments, the inflammatory profile's ameliorations could also apport benefits to these diseases.[108]

In addition to favourable outcomes in well-being, daily life, and adjuvant therapies, PA is also associated with better outcomes after cancer-surgery treatments.[109] In this context, You and colleagues (2020) report that higher PA levels (>12 METs) help in reducing the postoperative complications and mortality rates and improving long-term outcomes in patients with colorectal cancer stages from I to III.[110] In the same way, in lung cancer patients, PA and respiratory exercises help decrease the post-operative pulmonary complications (when practised before surgical treatments) and faster pulmonary capacity recovery (when practised after the surgical treatments).[111] Still, even though studies on osteosarcoma are still sparse, the first studies advance the idea that PA intervention protocol before surgical amputation may improve muscle strength, cardiovascular capacity, and joint mobility. Moreover, these benefits may pass on in the post-surgical and rehabilitation phases.[112]

1.4.2 Barriers to the physical activity practice and psychological aspects

Although the benefits of exercise are well documented, the majority of cancer patients reduce their PA levels and show low engagement in the PA programs after the diagnosis, immediately before, and during cancer treatment. Indeed, newly diagnosed cancer patients (independently of the cancer type) decrease their PA level and frequency after the diagnosis, and this represents an issue because these levels are likely to decrease with the advancing of therapies and treatments.[113–116] Lowered PA levels correlate with lower-level of cardiorespiratory fitness and body muscle functions, which are probably destined to decrease even more during adjuvant therapy.[117]

In order to facilitate PA participation, the exercise group is an essential factor because of its social context. Intervention groups favour contact with other subjects in the same situation, support from peers, empathic understanding, and increased motivation. In the study by Ax and colleagues (2020), the participants report that they meet others in the same situation, and the consequent social support takes the focus away from cancer-related thoughts and enhances the engagement in PA and the quality of life.[96] Mental well-being, empowerment, feeling healthy, less tired, and more independent are the most reported feelings in cancer patients undergoing PA protocols.[118,119]

The causes which prevent cancer patients from PA are several and are usually described as:

- physical barriers (physical symptoms, cardiovascular, musculoskeletal and pulmonary diseases, increased weight gain, and fatigue);
- psychological barriers (depression, scarce motivation, fears, lack of knowledge, and awareness about PA and exercise program);
- environmental/social-related barriers (employment situation, access to facilities, bad weather, and lack of time).[114,120,121]

However, the most reported motivation for not participating in PA are the more experienced therapy side effects, such as tiredness, lack of energy, and physical deconditioning.[114,121] Indeed, most cancer patients suffer from cancer-related fatigue (C-RF), characterised by excess weakness and tiredness different from the drowsiness experienced by healthy people.[122] The particular characteristics of C-RF are predominantly determined by sides effects, comorbidities, and complications related to adjuvant treatments.[123]

PA is recommended as a non-pharmacological intervention for C-RF treatment.[124] The study by Nilsson and colleagues (2020) sustains the hypothesis of a dose-response relationship between PA and C-RF during adjuvant treatment. More specifically, they state that higher MVPA positively impacts C-RF both at baseline and after 24 months of follow-up.[125] In patients with colorectal cancer,

medium intensity PA can effectively reduce fatigue and improve life quality, while excessively intense or prolonged PA leads to opposite effects.[123] Furthermore, subjects more active at the baseline report less fatigue at the follow-up and tend to be more active during adjuvant treatments.[114,125]

1.5 Physical activity in tertiary cancer prevention

In concomitance with the rising cancer incidence, the cancer survival rate (five years since diagnosis) is also escalating, making attention to cancer survivors' health an increasing necessity.[126] Upon completion of adjuvant cancer treatments, cancer patients and survivors remain at risk of physical deconditioning and sarcopenia (or loss of muscle mass), besides psychosocial sequelae such as depression, anxiety, and fatigue. Hence, although these patients may be cured of their cancer, the ability to achieve physical, psychological, and emotional well-being may be a greater challenge.[127]

1.5.1 The role of physical activity on secondary cancer, metastasis, and quality of life

Regarding physical and physiological aspects, adjuvant treatments result in many bodily changes, such as reducing mitochondrial function, the impairment in the oxidative phosphorylation in skeletal muscles, and the high possibility of toxic effects. The consequences, such as sarcopenia and reduced lung capacity or cardiac reserve, can be attenuated by PA, principally by aerobic exercise. Indeed, most of the mechanisms, able in contrasting factors predisposing to cancer onset, are the same that could avoid recurrences and metastasis. Through physical (e.g., blood flow and shear stress), endocrine (e.g., stress hormones and myokines), immune (e.g., mobilisation/infiltration of innate cytotoxic immune cells), and metabolic (e.g., lactate) mechanisms, PA and exercise have the potential to affect cancer growth kinetics and metabolism.[127]

In confirmation of what was affirmed above, Wang and colleagues' (2020) meta-analysis states that active cancer survivors show improvements in insulin levels and resistance, C-reactive protein, and glucose levels compared to inactive survivor controls' levels. Differences in IGF-1 levels are less accentuated and probably depending on the baseline's insulin level, with higher levels predisposing to more accentuated IGF-1 decrement.[128]

Additionally, PA-related blood circulation modifications could also prevent the metastatic process. Firstly, one of the physical mechanisms induced by PA, i.e., the fluid shear stress in the vasculature, may contrast or reduce the metastatic initiation and progression. The fluid shear stress, similar to that of vascular blood flood, directly affects cell viability, alters intracellular characteristics, retards growth rates, and attenuates the metastatic potential of circulating tumour cells.[62] Secondly, exercise-related angiogenesis is supposed to impact metastatic seeding and/or colonisation through a decreased hypoxia condition. The hypoxia condition in the primary tumour site is the prerequisite for stimulating the release of the pro-tumorigenic factors. These factors promote the establishment of secondary or metastatic cancer. However, the improved vascularisation through exercise-related angiogenesis decreases the hypoxia condition, the pro-tumorigenic factors, the communications between the first and second cancer sites, and consequently, lowers the risk of metastasis.[67]

In summary, there is ample evidence that unhealthy lifestyles, including sedentary and low PA levels, increase either the risk of developing a second primary cancer or recurrence, as well as metastasis and other cancer survivors' comorbidities.[129] On the contrary, an active lifestyle is associated with a 26% to 69% reduction in cancer-related mortality.[130]

As previously seen for the prescription of being physically active during adjuvant treatments, PA is recognised as an effective instrument in reducing C-RF in cancer survivors. Even though most studies focus on breast cancer survivors, recent studies on mixed cancer types find improvements in fatigue, quality of life, depression, anxiety, and health management in subjects practising a regular PA

protocol for at least twelve weeks. These results seem to be independent of the intensity and kind of PA.[131–133] Hence, even a light intensity PA improves the quality of life and fatigue in colorectal cancers.[134] In this view, a recent innovative study, reallocating sedentary time in PA and sleep time with an isotemporal substitution approach, designed the light PA as the most suitable solution for kidney cancer survivors.[135] These results are significant for older or impaired subjects who cannot afford MVPA level.[134] Furthermore, to maintain PA benefits, the importance of ongoing regular and constant habits of PA is underlined throughout the survivorship, and these habits should not be decreased or interrupted.[136]

1.5.2 The physical activity status in cancer survivors

It might be supposed that the cancer survivors, having experienced a malignancy in some cases evitable with a healthy lifestyle, should be aware of the importance of healthful habits. Surprisingly, analysis on lifestyle and, in particular, on PA in these subjects are in contrast.[137] The recent study by Cortés-Ibáñez and colleagues (2020) describes a Dutch cancer survivor population as physically active but, in contrast, has a higher BMI and more sedentary behaviour compared to the general population without a cancer history.[138] The opposite findings concerning PA are present in the study by Knowlton and colleagues (2020), in which less than half of the study participants adhere to the PA guidelines.[115] However, the inactivity tendency or insufficient PA practice of cancer survivors are usually more frequent.[139–141]

The possible factors explaining the differences in the conclusions are sex, age, type of cancer, and time since diagnosis.[138] Regarding the last point, in a study on young cancer survivors, Bøhn and colleagues (2021) state that only one of four participants meet the combination of being physically active, normal weight, and non-smokers; moreover, the majority of the sample is not active enough. The mean time from the diagnosis to the present study is ca. 15 years, and the authors advance the

hypothesis that the rate of meeting guidelines is lower in long-term survivors than in short-term survivors.[142]

Notwithstanding the scarce adherence to PA guidelines in cancer survivors, interviewers demonstrate the subjects' willingness to start cancer-specific PA training. Usually, younger people, women, and better-educated individuals are more prone to being physically active or starting a new activity. Moreover, subjects more active before the diagnosis tend to adhere to the PA guidelines after the end of adjuvant treatments. This is probably due to the consciousness of the PA benefits.[143] However, the particular physical and psychological situation of the cancer survivors population needs peculiar attention, well-prepared instructors, and specific PA training counselling and programs.[144,145] In this context, also the oncologist plays a key role in counselling starting or continuing PA. However, oncologists are still reluctant in prescribing exercise to cancer patients or survivors, and actions in making them understand the importance of being physically active should be considered.[127]

2. Physical activity and sleep

A general definition of *sleep* describes it as:

"*A normal active state of all living creatures in which the mind and body are less responsive. Sleep is a naturally recurring state of mind that is characterised by altered consciousness, the inhibition of almost all voluntary muscles, generally inhibited sensory activity, and a marked reduction in our interactions with our surroundings*."[146]

Sleep is an intrinsic hallmark of human beings, and it is probably assigned an evolution meaning. Sleep shows circadian rhythm characteristics; its patterns change during the lifespan and are defined based on behavioural and physiological changes.[147,148]

During sleep, numerous processes occur, such as memory consolidation, clearance of brain metabolites, and restoration of nervous, immune, skeletal, and muscular systems, making sleep essential for optimum health. However, complete and exhaustive explanations of sleep functions still remain a scientific enigma.[147–149]

The contemporary society is more predisposed to experience disordered sleep and a decrement in the overall sleep quality. It is supposed that disordered or impaired sleep predisposes to developing a long list of chronic diseases, including cardiovascular diseases, type 2 diabetes, and

cancer. PA and exercise are advocated as effective interventions for treating disordered sleeping in subjects suffering from these pathologies. An exhaustive explanation of the relationship between PA and sleep has not been formulated yet; however, many hypotheses refer to sleep functions.[150]

Many authors explore several sleep functions, ranging from immune, caloric, and neuronal roles.[150] Probably, some of these functions represent the bidirectional link between sleep and PA.[148] In this view, numerous studies have explored the relationship between sleep deprivation, restriction, or extension on physical performance outcomes (usually in athletes).[151,152] However, in the following paragraphs, it is explored how increased PA could improve self-sleep perception, quality, and health in the general population.

2.1 Sleep architecture and parameters

Sleep shows circadian timing characteristics in promoting sleep onset and arousal, as well as rhythmicity features during the sleep period. During the day and the wake time, sleep pressure is accumulated, and, in normal conditions, the pressure raises its peak during the evening. Circadian and hormonal stimuli promote sleep onset, and the sleep state is prolonged for a sufficient amount of time. After that period, other circadian, hormonal, and, in modern society, also external stimuli promote sleep end and awakening.

Sleep is split into two function- and control-independent states:

i. *non-rapid eye movement* (*NREM*) sleep, subsequently subdivided into four stages (NREM stages 1 to 4), and whose slow-wave sleep (*SWS*) stages (3 and 4) dominate the first third of sleep;

ii. *rapid eye movement* (*REM*) sleep, which dominates the last third of sleep.

The NREM and REM phases cyclically alternate themselves for 4/6 cycles, and each cycle lasts on an average from 90 to 110 minutes. In adult humans, NREM sleep accounts for 75-80% of total sleep time, while REM sleep accounts for 20-25%. Thus, there is a cyclic progression in the normal adult from wakefulness to sleep onset to NREM and then to REM sleep.[147]

The two phases differentiate for the types and shapes of waves recollected during the electroencephalogram (EEG) and physiological changes and features. A progressively decreased responsiveness to external stimulation and muscle tone characterises the NREM sleep. Furthermore, this phase is accompanied by slow eye movements followed by EEG slow-wave activity associated with spindles and K complexes. Further reduction of responsiveness to stimulation and absent muscle tone depicts REM sleep. During this phase, it is possible to observe rapid eye movements in all directions, low-voltage fast EEG activities mixed with distinctive saw-tooth waves. In addition to phasic rapid eye movements, there are also phasic swings in blood pressure and heart rate, irregular respiration, and phasic tongue movements, leading to few periods of apnoea.[147]

Studies involving the utilisation of EEG can investigate and assess if a particular modulator (e.g., PA, stress, use of drugs, etc.) is able to interfere and modify the different sleep phases. However, numerous studies, most of which include large sample size, use other instruments to assess sleep, such as questionnaires and actigraphy. In this way, sleep phases cannot be identified; however, sleep can be described through sleep duration, latency, efficiency, awakening after sleep onset, sleep disturbances, and self-perceived sleep quality.

The *Pittsburgh Sleep Quality Index* (PSQI) is one of the most used questionnaires to evaluate sleep subjectively.[153] The self-perception of sleep and its related improvement motivate people to abandon sleep drugs and believe in non-pharmaceutical treatments for sleep improvements rather than requesting medical assistance.[154] Moreover, a better self-perceived sleep quality enhances daytime functioning, life satisfaction, and mental health symptoms.[155]

Nonetheless, sleep can also be assessed with an objective methodology, such as polysomnography or actigraphy. The study by Jurado-Fasoli and colleagues (2020) reports that PA, particularly a high-interval training program, improves both subjective and objective (assessed with the actigraph) sleep evaluation in a group of middle-aged sedentary subjects.[156]

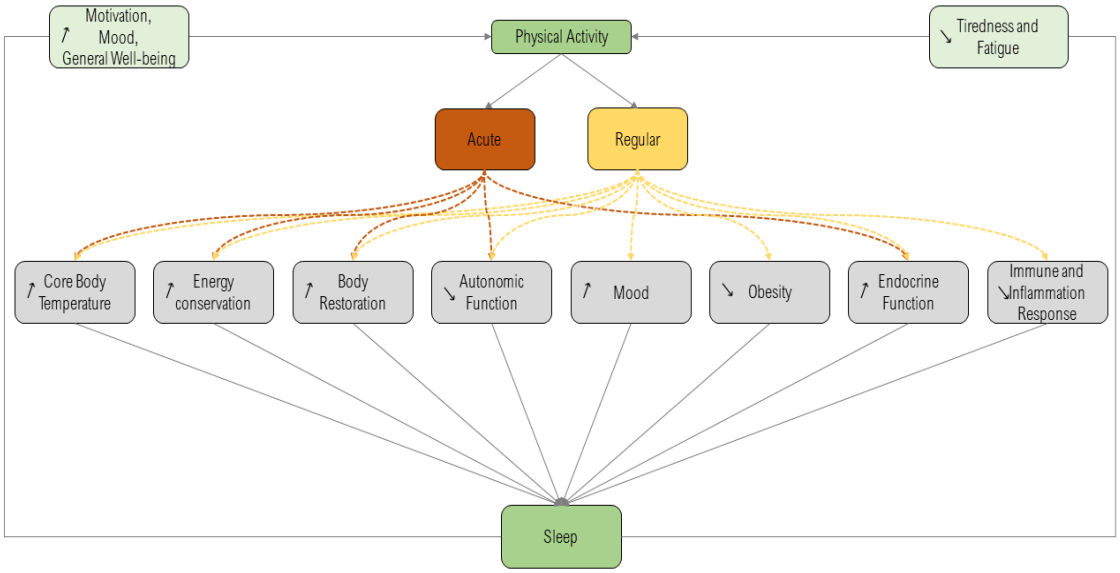

Figure 2 Possible effects of acute or regular physical activity on sleep and, vice versa, of sleep on physical activity practice (bi-directional relationship).

2.2 The relationship between physical activity and sleep

Sleep disturbances and problems, resulting in a lowered sleep quality, are common problems in modern society, with higher percentages of subjects reporting disturbed sleep. Sleep problems are known to affect either daily life or subjects' health negatively. Indeed, subjects suffering from impaired sleep usually show a lowered functional level for daily activities and an increased rate of specific and chronic pathologies, such as cardiovascular disease, diabetes, and obesity.[157–160] Furthermore, subjects reporting better sleep quality or those having a healthy sleep, and thus being satisfied with the

way they sleep, usually show an increased self-perception of health.[161] Hence, improving sleep problems could lead to the decline or resolution of some pathologies.

The treatment of sleep problems includes both pharmaceutical and non-pharmaceutical approaches. According to the *American National Sleep Foundation* (2019), non-pharmacological treatment options are the preferred first choice of treatment for sleep problems because of avoiding some side effects provoked by pharmaceutical treatment. Otherwise, pharmaceutical options should be prescribed in combination with a non-pharmaceutical treatment.[162]

Non-pharmaceutical interventions usually include diet and sleep hygiene counselling, cognitive behavioural therapy, relaxation exercises, and PA.[163] PA and exercise are described as two effective and powerful tools to improve sleep quality in several ways and decrease the risk of suffering from disturbed sleep (Figure 2).[164] On the contrary, as Morgan (2003) states, physical inactivity or low PA levels are a significant and independent risk for inadequate sleep and late-life insomnia.[165]

More recently, Vanderlinden and colleagues' review (2020) demonstrates that PA significantly improves sleep quality, sleep latency, sleep duration, sleep efficiency, and daytime functioning. Moreover, in subjects over 40 years, PA decreases sleep medication use when assessed with the PSQI.[163]

Concerning sleep stages, it is possible to summarise that the effects of PA on subsequent night sleep result in a more extended NREM sleep period (meaning a more restorative and deeper sleep) and shorter REM sleep.[166]

The role of PA as a means to improve sleep is recognised throughout the lifespan. Regular PA lasting at least twelve weeks is described, on the one hand, to decrease NREM stage 1 (very light sleep) and, on the other hand, to increase REM sleep latency, sleep continuity, and sleep efficiency in adolescents.[167] Another study states that increased PA promotes improvement in sleep quality rather than sleep duration.[168] Furthermore, PA and regular sleep schedules are the two main factors associated with adolescents' more favourable metabolic profile.[169]

Focusing on young adults, also in this population, individuals who engage in high PA levels are more likely to express a better sleep quality.[170] The positive influences are even more evident when body composition is taken into account. 15 weeks of PA show evident improvements in sleep quality in those classified as overweight/obese, probably due to a decrease in the body fat percentage that is likely to improve sleep quality.[150]

Active young women report a better overall sleep quality during old age with longer sleep duration and less awakening during the night than sedentary women. In addition to the possible physiological linkages between PA and sleep, one possible explanation from the authors is that those who practice more PA are more exposed to sunlight, which is in one of the principal circadian rhythm synchronizers. Especially in the elderly, who are physiologically subjected to a circadian rhythm desynchronisation, sunlight exposition could help them maintain a more regular circadian rhythm and, consequently, reflect improvements in sleep parameters.[171,172]

Although these are just a few studies involving PA protocols and sleep evaluation, it is possible to understand how the relation between PA and sleep appears strong. However, the linking mechanisms and the exact outcomes are still unclear. Probably, numerous variables (e.g., PA regularity, type of PA, time of day of PA, subject's fitness level, eventual obesity situation, sedentary behaviours, etc.) interfere differently with sleep parameters and outcomes.[154] Thus, in the following paragraph, different exercise attributes relevant to sleep are described.

2.2.1 Effects of acute and regular exercise on sleep

The term "*acute exercise*" indicates a single bout of exercise. "*Regular or chronic exercise*" denotes training sessions repeated several times during a specific time. In sleep reviews, regular exercise is not well defined, probably depending on the different study protocols.[157]

The review by Chennaoui and colleagues (2015) highlights that acute exercise has minor effects on sleep architecture by increasing SWS, REM sleep latency and decreasing REM sleep amount.[173] Besides, Kredlow and colleagues' (2015) review demonstrates that acute exercise has minor beneficial effects on sleep efficiency and NREM stage 1 and substantial effects on sleep disturbances.[155] Particularly, the decease of NREM stage 1, which is considered the "light-sleep," and the increase of SWS, the "deep-sleep," duration suggest that PA could be able to shift the sleep cycles towards a more restorative sleep.[155]

Sleep duration is the more consistent moderator on the acute effects of exercise on sleep.[173] In general, exercise should last more than one hour to achieve effective improvements in sleep quality. The type of exercise seems not to affect the favourable effects of acute exercise on sleep.[154]

Focusing on chronic exercise, in their study lasting twelve months, King and colleagues (2008) demonstrated how a regular and moderate-intensity endurance exercise could improve either objective or subjective sleep parameters. More specifically, participants improved the percentage time of NREM sleep stage 2, probably associated with a more positive sleep perception. Furthermore, sleep disturbances and latency decrease. All these improvements result in an improved rested-feeling in the morning.[174]

The review by Chennaoui and colleagues (2015) confirms these findings. Regular exercise is associated with increased SWS, total sleep time, decreased REM sleep, sleep latency, and awakening after sleep onset. Moreover, in adolescents, regular PA improves overall sleep efficiency. However, PA interventions lasting at least 16 weeks seem only to improve subjective sleep parameters, while for objective sleep improvements, longer interventions are needed.[173]

Kredlow and colleagues (2015) state that both acute and regular exercise positively affects overall sleep. Acute exercise is reported to have a small effect on total sleep time, sleep latency, SWS,

and decreased REM sleep. Moderate-to-largely strong positive effects are reported for regular exercise, improving all the PSQI subscales, including total sleep time, sleep efficiency, and latency.[155]

Even though both acute and regular exercise promotes sleep (Figure 2), it could be concluded that the highest proportion of beneficial effects of exercise on sleep are obtained with three training sessions per week.[154,155,163] Indeed, regular exercise substantially changes somatic functions that are supposed to be related to better sleep. These somatic changes include long-term improvements in body weight and composition, basal heart rate, cardiac and immune function, and glycaemic control. In addition to somatic changes, regular exercise improves mood, anxiety, and stress states, which are known to influence sleep.[166] Moreover, regular exercise and healthy PA habits may at first help people abandon detrimental behaviours for sleep, such as the use of alcohol and caffeine. Secondly, they increase total sleep time, SWS, deep sleep and reduce REM sleep or awakening time after sleep onset.[154]

2.2.2 Effects of time of day of physical activity on sleep

The effects of PA on sleep and its parameters also depend on the time of day the exercise is performed. Existing researches suggest there is no comprehensive-time of day during which it is best to exercise for sleep.[175] However, some general assumptions could be inferred by the current research conclusions.

It is usually suggested that exercise earlier in the day may improve the quality of nocturnal sleep. Sleep improves when the parasympathetic nervous system enhances its activity, and this could be stimulated when the sympathetic activity diminishes after a greater activation due to exercise.[176] From this point of view, morning exercise is shown to significantly diminish sleep latency, maximise and prolong deep sleep duration, while awakenings after sleep onset decrease after evening exercise in a sample of young adults.[163,177]

Brand and colleagues (2014) obtained different results; in their study, exercise an hour and a half before bedtime is associated with increased deep sleep. More specifically, decreased light sleep and increased deep sleep are shown by those who report a higher self-perceived exertion, meaning that self-perceived exercise exertion and objectively assessed sleep are positively associated.[178]

The differences in these studies could probably be ascribed to the exercise intensity. Traditional sleep hygiene principles impute an increased heart rate, core body temperature, and adrenaline levels to intensive exercise three hours before sleep. Altogether, these body conditions negatively impact sleep by lowering sleep efficiency and increasing time awake after sleep onset. A different situation is depicted when moderate-intensity exercise is practised between 4 and 8 p.m. or even later. In this case, exercising before bed may not produce any adverse effects, and the individuals training late in the evening fall asleep quickly, experience an adequate amount of deep sleep, more SWS, less stage 1 (light) sleep, increased latency for REM sleep and wake up feeling well-rested. Thus, only vigorous exercising before bedtime could be inherently harmful.[179]

The different sleep outcomes related to the time of day of the training can be explained by another factor: the chronotype of the subject. Subjects with an evening chronotype are less sensitive to sleep problems when exercise happens in the evening because their internal circadian rhythm is aligned with more activation during the evening hours.[175]

2.2.3 Effects of physical activity intensity on sleep

The intensity of exercise on improving sleep is probably dependent on the subject's age and physical fitness level. In middle-aged and elderly, low-to-moderate-intensity exercises reported better sleep outcomes such as PSQI overall score, subjective sleep quantity, and fewer difficulties falling back to sleep than the vigorous exercise program.[163]

In adults, moderate-to-vigorous exercise can increase sleep quality by reducing the time to fall asleep and the time spent awake in bed during the night. Additionally, exercise extends its benefits during the day by alleviating daytime sleepiness and reducing the need for sleep medications.[179]

Strenuous and prolonged exercise is known to have detrimental effects on sleep, probably due to increased cytokine levels. Indeed, after very high-intensity exercise, the skeletal muscle releases inflammatory cytokines, including IL-1 and TNF, which, in turn, contribute to the delayed onset of muscle soreness. Altogether, these consequences are able to disturb and disrupt sleep.[154]

2.2.4 Effects of the type of physical activity on sleep

The most suggested exercise type is aerobic exercise that can improve sleep and battle insomnia when performed daily and at least 150 minutes per week.[163] Aerobic exercise increases total sleep time, SWS (particularly stage 4), and delays REM latency.[154]

Additionally, strength training is suggested in order to reduce sleep latency and awakenings after sleep onset, while yoga or relaxation exercises are primarily advised when stress prevents people from falling asleep.[163] Indeed, Siddarth and colleagues (2015) report significantly better mood, mental health, and sleep after mind-body exercise intervention compared to individuals participating in aerobic exercise.[180] In this context, incorporating a blend of aerobic and resistance movements at a moderate intensity emphasises relaxation and the release of tension. Summing up, it seems that a mixture of different types of exercise is most successful in improving different sleep parameters simultaneously.[163]

Finally, Wennman and colleagues' (2014) study on the Finnish population concludes that leisure-time PA is more effective in increasing sleep quality. On the contrary, occupational PA is associated with worse sleep.[181]

2.3 Possible mechanisms linking physical activity and sleep

The potent sleep-promoting effect of PA on sleep is evident from the numerous studies proving it and the several PA variables tested to verify the connection between these two spheres of human lives. Quite a few hypotheses and explanations have been advanced to explain these influences. However, definitive evidence and conclusions about some influencing processes are still inconclusive.

2.3.1 The thermogenic hypothesis

Sleep onset is promoted by the body cooling down, thus dropping the core body temperature. The heat dissipation occurs through vasodilation and increased sweating.

PA can mimic the body cooling in preparation for rest. Indeed, body temperature increases during exercise, and afterwards, it drops through dissipation mechanisms, including peripheral vasodilation. The similarities between the changes in body core temperature during/after PA and before falling asleep could help to signal to the brain that it is time to fall asleep. Driver and Taylor (2000) affirm that exercising at a constant workload with an intensity of 70% of VO_{2max} may raise the core body temperature by approximately 2°C after 15–20 minutes. The consequent drop of the core body temperature reproduces the trigger point to favour sleep onset.[154] Furthermore, regular PA induces a more regular decline in body temperature, reflecting in more regular sleep.[173] The core body temperature elevation before bed timing is associated with a higher percentage of SWS because the mechanisms that promote SWS are the same that reduce the core body temperature.[166]

One of the trigger hormones for body temperature regulation is melatonin. Clearly, melatonin has either a hypothermic or a hypnotic effect with sleep propensity characteristics. It is supposed, and some studies even prove this, that endogenous melatonin concentration is affected by PA.[182] The study by Atkinson and colleagues (2005) supports the hypothesis of the link between PA and melatonin

secretion.[183] However, melatonin is so sensitive to many factors, such as light, time of day, sex, age, etc., that it is difficult to establish a close and stable connection between PA and endogenous melatonin secretion.[182]

2.3.2 The energy conservation hypothesis

Metabolic requirements and expenditure are significantly reduced during the sleep period compared to that of awake. Thus, sleep is an instrument to preserve energy use among all other functions, promote energy conservation, and, generally, have a role in energy regulation.

PA is the leading stimulus in depleting energy and, consequently, impacting sleep. Hence, after PA training, a larger energy amount is depleted, and more sleep or a night of more restorative sleep is needed to restore energy balance and preserve the energy extent still present in the human being.[154]

2.3.3 The compensatory hypothesis

The fundamentals for the body or tissue restoration hypothesis are similar to the energy conservation ones. Either the energy conservation or body restoration hypothesis advance the idea of a high need for sleep after PA.[154]

Evidence of this hypothesis is related to deep sleep. Restful sleep usually results after a night with increased SWS and activity and a delayed and reduced REM time. Thus, if sleep is assigned a restorative function, the principal mechanism that is able to stimulate this function is PA and the related energy expenditure.[154]

PA during the waking period involves a catabolic activity for the metabolic processes. During sleep, the principal activity is the anabolic one, stimulated or promoted by PA during the previous day. From this point of view, PA is a stress factor able to facilitate sleep.[154]

2.3.4 The cardiac and autonomic system effect

Studies on PA's influences on *heart rate* (*HR*) or *heart rate variability* (*HRV*) during sleep are still in their infancy, and firm conclusions are still lacking. However, studying how the HR modifies during sleep after PA would make it possible to understand the autonomic function changes and adaptations when HR is basically stationary.[184]

In normal conditions, sleep, particularly during the SWS periods, is characterized by a decline in sympathetic and an increase in parasympathetic activity. This situation provokes changes in cardiac and autonomic regulation, making HR slow down. Sleep problems or insomnia cause sympathetic overactivity, thus preventing the HR from slowing down and HRV from increasing during sleep. The cardiac adjustments during the night are promoted by the vagal stimulation, which is supposed to be the linking mechanism between PA and declined HR during sleep.[173]

This hypothesis is based on Sandercock and colleagues' study (2005) conclusions. They find that regular exercise enhances vagal modulation inducing bradycardia during the night and higher HRV. The vagal modulation reflects parasympathetic activity, which, in turn, could improve mood and sleep.[184]

2.3.5 Anxiety, stress and depression

A stressful or anxious, and depressed period can intrude on the ability to fall asleep; in other words, it prolongs the sleep latency.[185] Furthermore, Dzierzewski and colleagues (2014) suggest that

mood regulation and arousal are proponents that may heavily impact sleep.[186] It appears that mind-body disciplines may assist in alleviating symptoms of anxiety, arousal, or bad mood. Furthermore, the regular practice of PA raises the endorphin secretion during the exercise bouts.[187] Endorphins are hormones able to improve mood and sleep quality.[185]

With regard to depression, one of the main symptoms is disturbed sleep. PA is even more and more advisable and used as an alternative treatment of depression. It is plausible that regular PA helps in improving depression symptoms by improving sleep quality.[173] One possible mechanism explaining this relation is the *Brain Derived Neurotrophic Factor* (*BDNF*) hypothesis. PA is well known to increase BDNF concentration, a mediator factor also involved in depression. In depressed subjects practising PA regularly, BDNF concentrations are regularized, and depression symptoms attenuated.[166]

From a different point of view, the relation PA-BDNF-Sleep can also be described as Sleep-BDNF-PA. In other words, the loss of sleep quality leads to higher stress levels, stimulating the cortisol secretion and, simultaneously, suppressing the BDNF's production. Diminished BDNF production exposes to a great depression vulnerability; however, PA is considered one of the tools able to improve BDNF balance, stress, and sleep.[188]

2.3.6 Obesity and fat mass

Some studies evidence how sleep improves after PA intervention, probably the results of a decreased body mass and, sometimes, the fat mass.[150] Obese subjects are more likely to develop sleep disorders, and shortened sleep duration is strongly associated with obesity in young adults and adults.[189] By improving body composition through a PA intervention, it is possible to reduce sleep problems and enhance sleep quality.[150]

In this view, Farnsworth and colleagues' (2015) study shows that obesity is associated with an elevated risk of sleep problems and poor sleep quality independently of the PA or sedentary level.

The significance of this finding is that by improving body composition, sleep quality also improves. Consequently, PA, by improving obesity and composition, may improve sleep indirectly.[190]

In fact, in a cohort of type 2 diabetes patients under PA intervention, body weight and total abdominal fat reduction are accompanied by a drop in sleep disturbances.[191] In this condition, PA affects sleep indirectly by reducing excessive body weight, making the individuals less likely to experience obstructive sleep apnoea symptoms. Indeed, obstructive sleep apnoea is one of the major symptoms disturbing sleep, and roughly 60% of obstructive sleep apnoea cases have been ascribed to obesity.[179]

Another biological explanation for the parallel changes in body weight and sleep may be the abnormal responses in ghrelin, leptin, and orexin after sleep restriction. On the one hand, obesity risk increases with a chronic sleep duration <6 hours. On the other hand, the lack of equilibrium between these metabolic hormones is responsible for overeating and increasing body mass. PA, influencing sleep quality and duration, could also be responsible for the re-equilibrium of these hormones.[182,192]

2.3.7 The endocrine functions

The main hormonal secretions responding to exercise and influencing sleep are those of the *Growth Hormone* (*GH*) and melatonin.[173]

Studies on GH concentrations after PA and during sleep are sometimes inconclusive and in contrast.[173] The principal findings show that GH concentrations are relatively unchanged by resistance exercise bout during the subsequent night; what is substantially altered is the shape of the GH peaks and the secretory events, thus the dynamic of the secretion. Exercise bout results in a less orderly GH release characterized by a greater frequency of the secretory bursts with a smaller quantity of GH secreted per burst. The physiological significance of the altered secretion profile and its eventual significance on health are inconclusive.[193]

Concerning melatonin, PA has an effect also on this hormone: however, its modifications are linked to several indirect effects, such as light exposure, time of day, sex, age, etc., and it is still not possible to clearly define the effect of PA on melatonin in improving or modifying sleep architecture.[173]

2.3.8 The immune-inflammation response

Some cytokines regulating the inflammatory process are also intricated in the sleep-wake cycle regulation, and, principally, the IL-6 and the and TNF-α are sensitive to sleep homeostasis.[194] Sleep loss or total sleep duration restriction are two factors elevating pro-inflammatory cytokines expression during the night. Indeed, the elevation of circulating IL-1β, IL-6, and TNF-α are already visible after few days of sleep restriction. The association between reduced sleep and inflammation is visible in some pathologies sensitive to enhanced inflammation, such as cancer and depression.[195,196] Additionally, also sleep disturbances are strongly correlated with an enhanced inflammation status. Indeed, the presence of sleep disturbances is reported in association with and increased IL-6 and C-reactive proteins in the meta-analysis by Irwin and colleagues (2016).[197]

In such a situation of chronic sleep restriction, PA could act directly and indirectly to reduce the pro-inflammatory circulating factors. Firstly, PA can directly decrease IL-6, TNF-α, and other circulating pro-inflammatory cytokines; secondly, PA can reduce them by improving sleep duration.[198]

2.4 The bi-directional relationship between physical activity and sleep

Most of the studies are focused on describing sleep improvements in those subjects who experience a more considerable amount of PA. However, it is well known that PA and sleep do not act uni-directional, but they can interfere with each other in a bi-directional way. Therefore, sleep quality

and amount can predispose the subjects to feel more or less inclined to practice PA during the subsequent day.[149]

The role sleep plays in PA levels has not been studied thoroughly yet; however, most studies conclude that individuals experiencing poor sleep and suffering from sleep pathologies or disorders are usually less active than those with healthy sleep. In particular, people with certain sleep disorders are not likely to exercise during the day.[149,179] For example, the study by Schmid and colleagues (2009) advances the hypothesis that, if the short-term sleep loss reduces both the PA and its activity the following day, a chronic sleep duration curtailment could reduce PA in the long run, thereby increasing the risk for obesity, diabetes, and adverse cardiovascular events.[199]

Factors that can prevent the subjects from PA after a night of non-restful sleep are several: motivation, mood, general well-being, tiredness, fatigue, etc.[154]

Despite these results, the research has not conclusively proven that healthier sleep leads to increased PA levels. Evidence suggests that a good night's sleep can help the subject feel well-rested and more motivated to exercise the following day, but healthy sleep alone may not be sufficient to enhance PA habits.[179]

3. Rest-activity circadian rhythm and sleep assessment: actigraphy

In nature, all living organisms, starting from the singular cell and ending with the human organism, show several functions that recur periodically and with different frequencies. The discipline dedicated to the study of the rhythmicity of functions is chronobiology, which is defined as:

"…the science that quantifies and objectively investigates mechanisms of biological time structure, including rhythmic manifestations of life. Among various subspecialties, chronobiology includes chronophysiology, which describes temporal manifestations of physiological processes. It evaluates cyclical nervous, endocrinal, metabolic, and other interactions within the organism that underlie biological temporal characteristics and interactions with the environment." [200]

The primary impulse of the periodic phenomena is located in the *Central Nervous System* (*CNS*), which includes several structures proposed at various times as the primary circadian rhythm generator: the pineal gland, the hypophysis or part of it, the hypothalamus, and the suprachiasmatic nucleus. The last one is the structure most frequently proclaimed by physiologists as the biological

clock. On the top of the optic chasm, its location allows it to process and integrate the signals from the eyes, the released hormones and other neuronal signals, functioning as a trigger point for behavioural and physiological events.[200]

However, the suprachiasmatic nucleus does not work independently; indeed, all the CNS's structures are integrated and work in a network. The first demonstration of this dynamic dates back to 1977, when Halberg and colleagues (1979) report a modification in the circadian rhythm of body temperature in rats but not its disappearance after laser ablation of the bilaterally symmetrical suprachiasmatic nuclei.[200,201]

After much evidence from other experiments, it is possible to conclude that the CNS transmits rhythmic information to other brain regions and, secondly, to peripheral tissues through a multiplicity of outputs (e.g., endocrine signals, body temperature rhythms, and indirect cues provoked by oscillating behaviour). Hence, the main circadian-the suprachiasmatic nucleus-and secondary oscillators are located in the brain, while in the periphery, circadian clocks are functional in most body cells.[202]

3.1 The Rest-activity circadian rhythm

The biologic rhythmicity is, among several other indices, defined through frequencies that describe rhythms as:

- circadian: 20 hours $\leq \tau \leq$ 28 hours, thus rhythms with a frequency of one cycle in 24 ± 4 hours;[203]
- ultradian: $\tau <$ 20 hours, thus rhythms with a frequency greater than one cycle in 20 hours and higher than the circadian rhythm;[200]
- infradian: $\tau >$ 28 hours, thus rhythms with a frequency lower than circadian.[200]

Most human functions, including the sleep-wake cycle, show a rhythmical periodicity around or slightly longer than 24 hours. The 24-hour sleep-wake cycle includes the control of the rest-activity rhythm, which, lasting around 24 hours, is usually called Rest-Activity Circadian Rhythm (RAR).[200]

The circadian sleep-wake cycle, and generally the circadian system, is a complex functional system strongly linked to all organism's functions and promoted by the interaction of the homeostatic process. Indeed, sleep, or the rest period during the 24 hours, is promoted by accumulating sleep-pressure during the day or wakefulness. The sleep-pressure is dissipated during the sleep period and, at the end of which, a wake-promoting signal is built up to promote the awakening. Thus, the sleep-wake cycle and RAR are favoured by sleep-pressure and wake-promoting signals that should respect regular timing. Indeed, the peak of sleep-pressure should happen immediately before the sleep start, while its nadir shortly before wake time.[204,205]

Compliance with regular timing and signals oscillators is essential in order to maintain the organism's health. The desynchronization in these two oscillators, sleep and awake, leads to the desynchronization of RAR, predisposing to unhealthy status up to chronic disease.[205] This desynchronization could be favoured by artificial or external conditions, such as working on shift-work or during the night, or natural and physiological processes, such as ageing.[172,206]

In shift-workers or night shift-workers, the oscillation between the sleep-pressure and wake-promoting signal is not more aligned with the day-night alternation, one of the prominent synchronizers of all circadian rhythms.[206] In the elderly, probably the deterioration of the suprachiasmatic nucleus weakens the circadian rhythm. This condition leads to a higher possibility to develop chronic diseases, such as type 2 diabetes, obesity, and cardiovascular diseases.[172,207]

Finally, in some chronic pathologies, such as cancer, neurodegenerative or metabolic diseases, an altered or desynchronized sleep-wake cycle and RAR has been highlighted.[208–212]

3.2 Parameters of the rhythm

Every rhythmic biological function, including RAR, could be described and analysed by examining its rhythm, described as:

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

which is mathematically described by the following form:

$$f(t) = M + A \cos (\omega t + Ø)$$

where ω = angular frequency and t = time. This is the form of an approximating function, which represents a sinusoidal shape used in the *Rhythmometric Cosine Method*.[213]

The parameters of the sinusoidal shape and the characteristics of a rhythm, considering the best fitting cosine, are shown in Figure 3 and are usually described as follows:

- *Acrophase*: the measure of timing. It is the temporal distance from the point of the curve to the crest time or the peak of the mathematical function that appropriately approximates the rhythm; it is also defined as the phase angle of the crest in relation to the specified reference time point.[214] It could be reported with different measuring units, such as angular measures, degrees, radians, time units, or physiological episodic units;[215]
- *MESOR* (*Midline Estimating Statistic of Rhythm*): is the average value of the rhythm, in other words, the value in between the highest and lowest points of the function, which approximates the rhythm. It is described with the original physiological units;[216]

- *Amplitude*: the measure of half of the rhythmic extent change in a cycle; it represents the difference between the maximum of the cosine curve and MESOR. It is measured with the original physiologic units.[217,218]

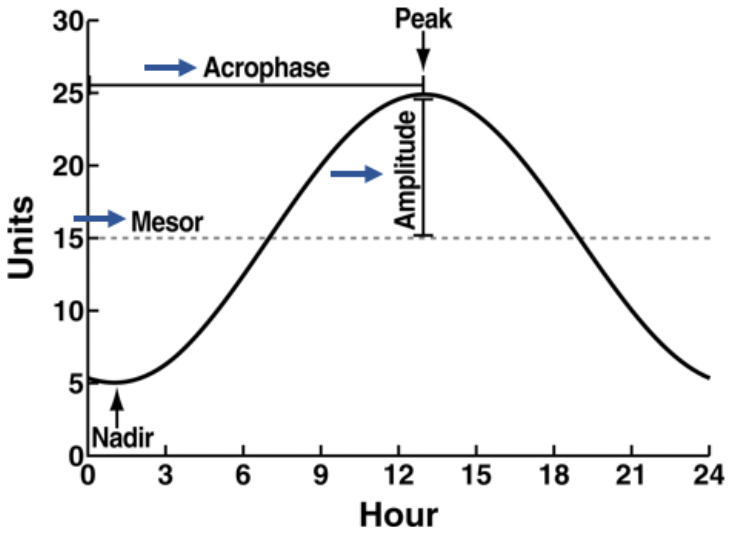

Figure 3 *Example of a biological rhythm and its parametric parameters: Acrophase (Ø), Amplitude (A) and MESOR (M).*

Acrophase, MESOR, and amplitude are the parametric parameters of the single cosinor model. This model depends on the assumption that the activity counts proceed on the time course as the results of the superposition of two components:

i. the trend, which is the smooth deterministic component;

ii. the natural time-to-time variability around the, which is the random component.

These two components can be portrayed as a signal to which a certain amount of noise is superimposed. However, through a visual inspection of the raw data, it is possible to affirm that the activity counts collected during an epoch of 60 seconds do not respect a well-behaved natural trend. More specifically, particularly during the daytime, rapid and wide-ranging activity counts are the norm rather than the exception. Due to their nature of identifying rhythm with a circadian periodicity, the parametric model is unsuitable to follow the high-frequency changes exhibited by the activity counts,

which are treated by the analysis as residuals resulting in a loss of data variability. An alternative or complementary approach is represented by the non-parametric approach (Figure 4), which depends on six other parameters:

- *Mean* (*M*): the mean value of the activity levels;
- *Variance* (*VAR*): the variance of the activity levels;
- *Interdaily Stability* (*IS*): the measure of rhythm stability (ranging between 0 = lack of synchronization and 1 = perfect synchronization); it provides information about the RAR synchronization and stability in concomitance with environmental stimuli;
- *Intradaily Variability* (*IV*): the measure of synchronization of the RAR to the 24 h light-dark cycle) (ranging between 0 = less fragmented rhythm and 2 = more fragmented rhythm); it indicates the fragmentation of the RAR, deriving from its changes of RAR, such as from a diurnal nap or a nocturnal awakening;
- *Nocturnal activity* (*L5*): the average activity during the least active five hours span of the 24 hours;
- *Daily activity* (*M10*): the average activity during the most active 10 hours span of the 24 h).[219–221]

Unlike the parametric approach, this methodology condenses the RAR time series by a set of numerical indices that do not require the investigator to postulate a specific functional form of RAR.

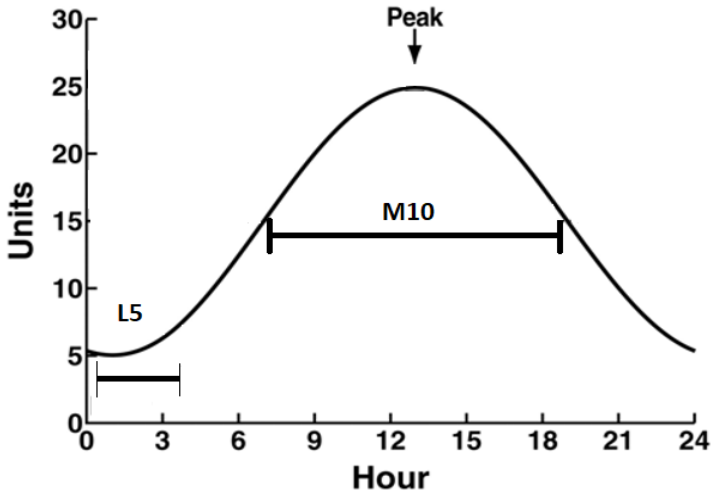

Figure 4 *Example of a biological rhythm and non-parametric parameters: Nocturnal activity (L5) and Daily activity (M10).*

3.3 Components of the rhythms: endogenous and exogenous factors

As stated above, the rhythmicity and frequency structure of all living organisms stem from the teamwork of neural, hormonal, and cellular systems. However, other factors could influence and modify the intrinsic and periodic characteristics of biological functions, such as the environmental factors, also called *synchronizers*.[200]

Based on their influence on the variable, the synchronizers are usually addressed as primary or secondary. For most living beings, including humans, the most influencing synchronizer is the light-dark alternation, reflecting rest-activity (RAR) cycles.

The action of synchronizers could be understood by observing Figure 5, in which the full line represents the circadian rhythm of core temperature in subjects living normally, while the dashed line shows the effects of a 24-hour "*constant routine*". The constant routine consists of remaining awake, sedentary, and relaxed for at least 24 hours without removing any individual's lifestyle or environment. In constant routine conditions, the rhythm of core temperature does not disappear; it only changes compared to the full line.

On the one hand, a possible explanation of the maintained rhythm is attributed to a "body clock" that conserves the endogenous component of the core temperature rhythm.[222] On the other hand, the difference between the two rhythms (i.e. the two lines) is due to the exogenous component of core temperature rhythm, which is dominated by the rest-activity cycle.[223] These conclusions are general and could be extended to all rhythms because all rhythms show a mixture of endogenous and exogenous components.[224]

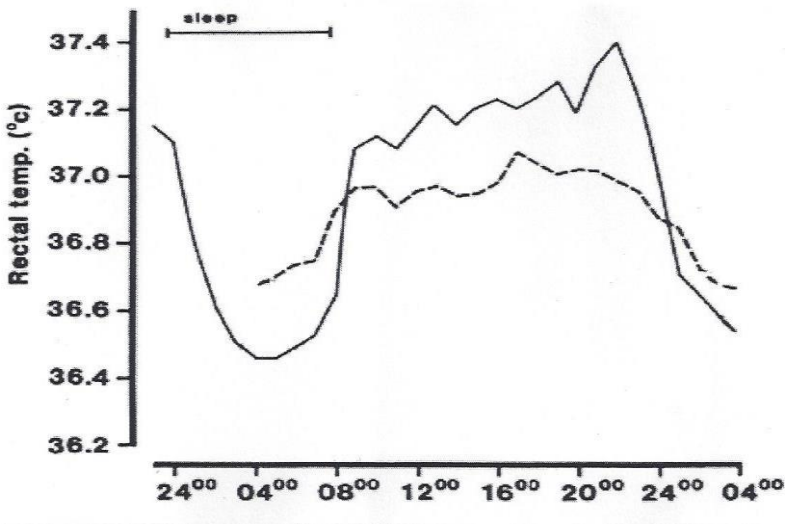

Figure 5 The daily rhythm of core temperature in a group of eight young men. Full line: under normal condition (sleep from 12 a.m. to 07 a.m. indicated by the bar). Dashed line: undergoing a 24-hour constant routine starting at 04 a.m.

3.4 An objective assessment of rest-activity circadian rhythm and sleep behaviour: the actigraphy

The RAR, daily physical activity, and sleep-wake patterns could be monitored using actigraphy, an objective assessment method that, compared with traditional polysomnography, appears more practical and convenient for extensive use. Actigraphy is simple to use and can record highly reliable and reproducible data for days, weeks or even longer, providing critical information in assessing a

person's sleep-wake patterns.[225–229] The activity levels are recorded over the 24 hours, making possible the analysis of RAR.

Referring to sleep, actigraphy has been validated against polysomnography, the gold standard in sleep studies.[230,231] Furthermore, it has been shown to provide specific information on the diagnosis of sleep and circadian rhythms disorders and sleep variability in insomnia patients.[225] Actigraphy enables the operators to collect both qualitative and quantitative sleep data.

3.4.1 The actigraph

Recording RAR and sleep data for actigraphy assessment are possible through a small device (similar to a wristwatch) called actigraph. It contains a piezoelectric, bi- or tri-axial accelerometer that produces a voltage when the device is in motion. Then, after the collection period, the data analysis is possible by connecting the actigraph to a specific analysis software through a computer or PC. Actigraphs are becoming more and more sophisticated and precise. For example, considering that voluntary human movement rarely exceeds 3–4 Hz, most single-axis acceleration devices today use band-pass filters that eliminate very slow (<0.25 Hz) and swift movements (>2–3 Hz).[225]

The digitalization of the analogue signal recorded by the actigraph involves different methods; the three most used are the *time above threshold, zero-crossing* and the *digital integration*. Time above threshold is based on setting a certain threshold (commonly 0.1–0.2 g) and then measuring the amount of time per epoch produced by the signal in response to motion above the fixed threshold. The other two methods do not need a threshold; the zero-crossing method calculates the number of times per epoch in which the activity signal level overcomes the zero value, while the digital integration implies selecting the accelerometer output signal at a high frequency, then calculating the area under the curve for each epoch.[232]

The actigraph is usually worn on the wrist of the non-dominant hand for at least one week; however, the best placement of the actigraph is still debated. Some studies compare different actigraphy placements and suggest that the actigraph detects more movements when placed on the wrist than the ankle or trunk placements. Furthermore, the placement on the dominant arm perceives more movements than the placement on the non-dominant one.[233] Concerning the length of the monitoring, three days are the recommended minimum length; however, for more robust data and also for a non-parametric approach, a more extended period is necessary.[226] Finally, the actigraphy monitoring is usually accompanied by a daily diary to recollect essential data about sleep habits.

3.4.2 Rest-activity circadian rhythm assessment

Actigraphy offers a non-invasively method to assess RAR. The actigraph recollects 60-second epochs (but also 30-second epochs) data, then transfers them to a personal computer, which, using specific software, obtains the activity and RAR data expressed in activity counts (a.c.).

In literature, different methods to analyse RAR data are explained. One of the most robust and used is the *single cosinor method*, of which mathematical function and rhythmometric parameters are listed and clarified in chapter 5.2.[200,234] The single cosinor method analyse RAR for each subject reporting with 95% confidence intervals. In order to obtain RAR and activity levels of the analysed sample and its eventual stratifications (population), data is then processed using the *population mean cosinor*.[234]

3.4.3 Sleep assessment

In addition to activity levels, actigraphy is also recognized for its role in evaluating sleep both in its quantitative and qualitative aspects. Furthermore, it is a valid instrument for identifying sleep

disturbances.[225,235] The activity data recollected by the actigraph shows a phase of about eight hours, in which the activity episodes are rare or absent. This data represents the sleeping periods and is used to evaluate the characteristics of a person's sleep.[236]

Also in this case, the data analysis is based on a sleep detection algorithm provided by specific software. In addition to the actigraph monitoring, sleep analysis is accompanied by a sleep log or diary, in which the bed- and wake-up-times are reported. This makes the data analysis procedure more manageable and reliable; furthermore, it allows researchers to macroscopically and visually study actograms to verify the absence of anomalies.

The analysis identifies several sleep parameters (more than 30 with specific software), which could be named differently depending on the utilised actigraph device and software. Notwithstanding minimal differences in names, the most relevant and reported sleep parameters, which have also been validated versus polysomnography, are:

- *Bedtime* (*BT*): a parameter derived from the diary; it is declared as the time when the subject goes to bed in bed;
- *Sleep Start* (*Ss*): a parameter obtained automatically by the actigraphic software; it marks the sleep start because the recognised movements are below the detection threshold;
- *Sleep End* (*Se*): a parameter obtained automatically by the actigraphic software; it marks the end of the sleep because the recognised movements are above the detection threshold;
- *Get up Time* (*GuT*): a parameter derived from the diary; it is declared as the time when the subject wakes up;
- *Time in Bed* (*TiB*): a parameter obtained automatically by the actigraphic software; it is the total time spent in bed between BT and GUT;

- *Assumed Sleep* (*AS*): a parameter obtained automatically by the actigraphic software; it is the time elapsing between the beginning and the end of sleep and is effectively spent sleeping;
- *Actual Sleep Time* (*AST*): a parameter obtained automatically by the actigraphic software; it is the total time spent in sleep, given by the difference between AS and AWT; it could also be expressed as a percentage of AS;
- *Actual Wake Time* (*AWT*): a parameter obtained automatically by the actigraphic software; it is the total time spent in wakefulness; it could also be expressed as a percentage of AS;
- *Sleep Latency* (*SL*): a parameter obtained automatically by the actigraphic software; it is the lag time between the time the subject goes to bed and the sleep onset;
- *Sleep Efficiency* (*SE*): a parameter obtained automatically by the actigraphic software; it is the total sleep time expressed as a percentage of TB; it is the most used parameter to express the sleep quality.
- *Immobile Minutes* (*IM*): a parameter obtained automatically by the actigraphic software; it is the total number of minutes categorised as immobile during AS; it could also be expressed as a percentage of AS;
- *Moving Minutes* (*MM*): a parameter obtained automatically by the actigraphic software; it is the total number of minutes categorized as mobile during AS; it could also be expressed as a percentage of AS;
- *Fragmentation Index* (*FI*): a parameter obtained automatically by the actigraphic software; it is the sum of the "Mobile time" (% time spent moving) and the "Immobile bouts" (% of immobile<1-min periods).[237]

Finally, more recent software can detect and evaluate nap periods during the day, the analysis of which is simplified with their registration in daily diaries.

4. Breast cancer

Breast cancer (BC) is a very intricate disease, showing several biological and genetic features with high genomic instability.[238] BC represents the most frequently diagnosed cancer among women all over the world.[239] In Italy, BC is the most diagnosed cancer in women in all age classes (0-49, 50-69, 70+ years), and its incidence has been slightly increasing during the last ten years. Among Italian women, the probability of developing BC is 1 in 8 and BC risk is the highest among all other cancer types; indeed, other frequent cancers in females show a lower risk, such as colon cancer (1 in 19), lung cancer (1 in 35), pancreas cancer (1 in 63), ovarian cancer (1 in 73), and uterus cancer (1 in 163). It is foreseen that during 2020 the 30,3% (54.976 new cases) of all female cancer will be BC. Once again, this percentage is the highest when considering all other types of cancer, and it is three times more than colon cancer (second cancer diagnosed among women in Italy).[240]

Notwithstanding the BC mortality rate has been decreasing in recent years (- 6,4%), BC still represents the first oncological death cause among women worldwide and in Italy (16.1%).[239] However, after five years from the diagnosis, the survival rate and prevalence are estimated to be around 87-90% (one of the highest in Europe) and 43%, respectively.[240]

4.1 Breast cancer risk factors

The cause or causes of BC are not yet fully understood; however, several risk factors have been identified. These risk factors can be divided into two classes:

i. *definite* or *known* risks, which have been shown to increase the risk of developing BC;

ii. *potential* or *suspected* risks, which have been hypothesized for increasing the risk of developing BC;[241]

The definite risk factors include:

- sex: being a female increases the risk of BC;
- age: the BC risk varies with age; less than 5% of all BC cases are diagnosed before the age of 40, its incidence strongly increases until the age of 70 and declines at an older age, with 80% of the cases that occur in women older than 50 years old;
- race/ethnicity: white women have a higher risk of developing BC; however, the risk of dying from BC is higher among black women;
- living in Western countries: BC rate tends to be higher in Western Europe and North America, while it is lower in Africa and the East Asian regions (however, BC rates are increasing in some Eastern regions);
- exposure to hormones: unopposed and lasting circulation of oestrogen could pre-dispose breast tissue to develop cancer; therefore, early menarche, late menopause, nulliparity, or first pregnancy after the age of 30/35, and not breastfeeding could increase the risk for BC;
- oral contraceptive pills: users of contraceptive pills are a more, even though slight and transient, risk of developing BC; however, the majority of studies are conducted

with older-style pills containing a higher dose of oestrogens; probably, new-style pills, which contain lower oestrogen dose, have a lower impact on BC risk; however, the risk almost vanishes after ten years from cessation. Furthermore, contraceptive pills are used during a life period during which the BC risk is lower than other periods, reducing the contraceptive pills risk related to BC;

- hormone replacement therapy: the use of combined progesterone-oestrogen therapy in menopausal women to alleviate climacteric symptoms of menopause is associated with an increased BC risk; however, this risk decrease as time from menopause increases and after 2-5 years of the discontinuation of the hormone replacement therapy;
- obesity: the higher obesity-related fat mass level and BMI are indicated as risk factors for BC, particularly in post-menopause women; indeed, fat mass is the primary oestrogen source due to the aromatization pathway in menopausal conditions;
- physical activity: there is an inverse association between the PA practice and the BC risk; the evidence is more robust for postmenopausal than the premenopausal BC, and PA during both adolescence and early adulthood is associated with a lowered BC risk;
- alcohol intake: Independent from the type of alcohol consumed, higher alcohol intake leads to a higher BC risk;
- benign breast disease: BC cancer risk could be enhanced by benign breast diseases, such as intraduct papilloma and atypical lobular or ductal hyperplasia;
- ionizing radiation: radiation exposure is known to increase the risk of BC;
- family history or genes mutations: the risk of developing BC increases with a strong family history of BC; the cause is identified in faulty genes, which favour a genetic

predisposition to BC; of these genes, the most common are the *BRCA1* and the *BRCA2*.[241–243]

The potential risk factors include:

- height: length at birth and increasing height is associated with an increase in BC, probably because height is influenced by some factors also inducing cancer onset and development, such as childhood nutrition, genetic predisposition and IGF-1 level;
- diet: although no specific component or particular nutrient of the adult diet is consistently associated with BC risk, there is a considerable agreement that diet or nutritional status plays a role in BC risk;

The above-listed risk factors can also be divided into three main categories based on their nature:

i. demographic and sociological factors: age, rich and western countries, and wealthy social class;
ii. constitutional, physiological, and reproductive factors: familial history for BC, high birth weight, early-age menarche, nulliparity, late first pregnancy, low or no breast-feeding, age at menopause, obesity or overweight at menopause, and high testosterone and oestradiol levels associated with low SHBG level at menopause;
iii. environmental factors: Ionizing radiation, hormone therapies for menopause, contraceptive pills, diet, scarce PA, and alcohol consumption;[238,244]

All these risks factors are considered together, determining a cumulative risk of developing or dying from BC. The cumulative risk changes during the lifespan, and it is important to calculate and know this in short interval years (10, 20 and 30 years) at various ages.[243]

4.2 Breast cancer types

BC is considered a family of diseases rather than a single disease or malignancy. The type of BC is defined according to the tissue from which it arises, most of the time from the *terminal duct lobular unit*.[241]

Cancer or carcinoma cells confined within the terminal duct lobular unit and adjacent ducts but not invading the basement membrane are known as *carcinoma in situ.* There are two types of carcinoma in situ: *Ductal Carcinoma In Situ* (*DCIS*) and *Lobular Carcinoma In Situ* (*LCIS*).[241]

When cancer cells escape from the terminal duct lobular unit and move to the basement membrane or other breast component, the carcinoma is named *invasive* or *infiltrating.* The most common invasive BC type is the *Infiltrating Ductal Carcinoma of no particular type*, followed by the *Infiltrating Lobular*, *Inflammatory*, *Mucinous*, *Medullary*, *Tubular*, *Papillary, Cribriform, Phylloides, Apocrine, Secretory, Aquamos-Cell, Metaplastic, and low-grade Adenosquamous carcinoma.*[241]

4.3 Diagnostic pathway of breast cancer

The BC diagnosis consists of different steps and methods to define the pathology and shed light on the stage and biochemical characteristics of the tumour.

In order to help in making the diagnosis and identifying the risk factors, women are asked about some historical details, such as age at menarche and menopause; the number of pregnancies, age at first pregnancy, babies breastfeeding, use of oral contraceptive pill and hormone replacement therapy, family history of breast and ovarian cancer, and eventual symptom and its duration. Following, the examination proceeds with the palpation, the inspection, and the clinical investigation (e.g., mammography, ultrasound, magnetic resonance imaging, biopsy, etc.).[241]

The screenings and histological inspections help determine cancer staging and the most appropriate treatment. The reported features for cancer staging are size, grade (based on the cell differentiation grade), lymphatic invasion and lymph node status, histological type, vascular invasion, proliferative activity (*Ki67*), the age of the patient, hormone receptor status (based on the presence or absence of *Oestrogen hormones Receptors* ($ER^{+/-}$), and *Progesterone hormone Receptors* ($PR^{+/-}$)), and of one specific proto-oncogenes receptor (*Human Epidermal growth factor Receptor – 2* ($HER2^{+/-}$)).[241,244]

The most common system used in the cancer staging is the *TNM System*, developed in France between 1943 and 1954 and that evaluates:

- T (Tumour) - the extent of the primary tumour;
- N (Node) - the absence/presence/extent of regional lymph node metastasis;
- M (Metastasis) - the absence/presence of distant metastasis.[245]

4.4 Genetic factors in breast cancer

All types of cancers, including BC, are always genetic at the cellular level because cancer is the final outcome of genetic changes and damage (mutations). The explication of what is said above lies in one specific function of some genes. Indeed, some genes control cell division and DNA replication, avoiding DNA errors and damages during the replication. When a mutation occurs in these genes, altered cells could replicate oddly and without control. Over time, mutations in particular cells and their uncontrolled growth may turn into cancer cells and, finally, cancer pathology.[241]

Mutations may have causes or insults of a different nature, including ageing, smoking habits, alcohol consumption, environmental radiation exposure, chemical factors (exogenous insults), hormones imbalances, metabolic process, intracellular oxidative distress, oncogene activation

(endogenous insults) as well as being inherited.[238] Due to these various causes, mutations are divided into sporadic and inherited; consequently, cancers are usually labelled as sporadic (occurring by chance and not associated with genetic predisposition) and inherited.[241]

Mutations for sporadic cancers usually take a long time to develop cancer pathology, which is why older persons are usually at a higher risk of developing cancer. Indeed, older individuals are subjected to a greater amount of cell replication and, consequently, have an enhanced risk of generating mutated cells, which, in time, evolve into cancer. Sporadic BC shows little family history for BC and accounts for most (85-90%) BC cases.[241]

A mutation is defined as inherited when the mutated gene is already present in one of the 23 pairs of chromosomes coming from the mother's egg and the father's sperm. In this case, the mutation is already present in the genetic makeup, and offspring cells have a 50% probability of carrying the mutation. The inherited gene mutation predisposes the individual to a higher risk than the person without the inherited mutation of developing cancer. This explains why, in some families, there are several cases of the same type of tumour throughout the different generations.[241]

The previous point has represented the trigger idea to start analysing possible genes involved in the inherited BC during the '90ies. In 1994 and 1995, the BR(east) CA(ncer) genes 1 and 2 (*BRCA1* and *BRCA2 – BRCA1/2*) are the first high-penetrance sequenced genes whose germline mutation is recognized to predispose to breast and ovarian cancer.[246,247] Other mutated genes involved in the hereditary BC and are classified based on their penetrance level:

- high-penetrance genes: *TP53*- (*Li-Fraumeni Syndrome*), CDH*1*- (*Hereditary Diffuse Gastric Cancer Syndrome*), *PTEN*- (*Cowden Syndrome*), and *STK11*- (*Peutz–Jeghers Syndrome*) gene;

- moderate-penetrance genes: *CHEK2*, *ATM*, *PALB2*, *NF1*, and the genes involved in the *Fanconi anaemia* (*FA*)-*BRCA* pathway and interacting in vivo with *BRCA1* and/or *BRCA2* (*NBN*, *RAD51C*, *RAD51D*, *BRIP1*, *RAD50*, and *MRE11*).[238]

The inherited or hereditary BC accounts for approximately 5–10% of the total BC cases and follows a Mendelian inheritance pattern (autosomal dominant). Hereditary BC differentiates from familial BC, which accounts for an additional 15–20% BC cases. Familial BC refers to women with two or more first- or second-degree relatives with the disease without an identified gene.[238]

4.4.1 High-penetrance *BRCA1/2* genes: Hereditary Breast and Ovarian Cancer Syndrome

BRCA1 and *BRCA2*, located on the chromosomes 17q21 and 13q12-13, respectively, are the most important and studied genes related to hereditary breast and ovarian cancers. Like any other gene, *BRCA1/2* show several functional domains, each of which binds to specific patterns.[248] Concerning *BRCA1*, it contains an N-terminal RING domain that, in the central portion, has E3 ubiquitin ligase activity, a nuclear export sequence, three nuclear localization signals (which helps nuclear import of *BRCA1*), a coiled-coil domain that binds the N-terminal of the partner and localizers of *BRCA2* (PALB2) protein, and a C-terminal BRCT domain that binds proteins phosphorylated by the mutated Ataxia telangiectasia and Rad3-related kinases.[249]

BRCA2 contains an N-terminal domain that interacts with the C-terminus of PALB2, eight BRC repeats that bind the RAD51 protein, and a C-terminal DNA-binding domain (DBD) that binds single-strand DNA (ssDNA) and double-strand DNA (dsDNA)[250]. The DBD contains five components: a 190-amino-acid-helical domain, three oligonucleotide-binding (OB1, OB2, OB3) folds that are ssDNA-binding modules, and a tower domain that protrudes from OB2 and binds dsDNA. The helical domain,

OB1 and OB2 also associated with the deleted split-hand/split-foot syndrome (DSS1) protein, which has been linked with *BRCA2* protein stabilization.[251]

As previously stated, cancers develop after uncontrolled replication of cells with DNA damages, and *BRCA1/2* are actively involved in the DNA damage repair and maintaining genomic integrity because they are two tumour suppressor genes. *BRCA1/2* can prevent potential carcinogenic cells replication by repairing damages in the double-stranded DNA breaks, which may occur during the homologous recombination. Additionally, *BRCA1* is involved in the repair of stalled and collapsed replication forks, transcriptional regulation, checkpoint control, ubiquitination, chromatin remodelling, X-chromosome inactivation, oxidative stress response and hormonal regulation.[252]

Germline mutation (functional loss) or deletion of one or both genes leads to genetic instability, predisposing to a higher risk of developing a tumour.[243] In fact, even a haploinsufficient state among *BRCA1/2* is considered insufficient to avoid disease development. Regarding *BRCA1* (a similar explanation is also feasible for *BRCA2*), it is supposed that the inheritance of the mutation, together with the loss of the caretaker function, may lead to the accumulation of mutations in the breast tissue. The increased cell division, predominantly during puberty and pregnancy, increases in concomitance with the rate of DNA synthesis, enhancing the possibilities of DNA damage in the breast. The compromised repair capacity due to the mutation and the loss of hormonal control leave mutated cells free to replicate. In fact, the *BRCA1*-associated tumorigenesis role is amenable to both caretaker and hormonal regulatory function.[252]

Some evidence supports the hypothesis that *BRCA1* carriers are more predisposed to BC because of the haploinsufficiency state associated with heterozygosity. This condition may accelerate the mutation rate of other crucial genes, including the second copy of *BRCA1*; therefore, several studies intend to find actions and/or factors that can stimulate and increase the expression of the unmutated *BRCA1* copy.[252]

Evidence shows that estimating the quantitative BC risks associated with specific *BRCA* mutations in carriers depends on several factors. In addition to the presence of a mutation on *BRCA1/2*, other factors such as environment, lifestyle factors, and mutation locations influence the BC risk. More specifically, breast and ovarian cancer risks vary with the precise location of the mutation in *BRCA1* or *BRCA2*.[238] In women with mutated *BRCA1*, mutations in the RING and BRCT domains are at higher risk of developing BC compared to mutations located in other gene regions. Differently, BC risk is increased when the mutation occurs at the 5' and 3' regions (approximately the PALB2-binding and DBD domains) in the *BRCA2* gene.[249,251]

Based on the different location of the mutation and the consequent classes of variants, the IARC guidelines define five principal *BRCA1/2* variants: pathogenic [C5], likely pathogenic [C4], benign [C1], likely benign [C2] and variants of uncertain significance [VUS; C3]. Furthermore, according to their predicted effects, each pathogenic variant could be further described as *loss of function variant* (LOF, which includes frameshift, nonsense, large deletion and spliceogenic variants, and *nonsynonymous variants* (including missense and small inframe deletion).[253] Through genome-wide association studies, it is possible to provide helpful stratification of risk to assist women in planning risk-reducing measures, childbirth, and other aspects of life.[238]

Slight histopathological differences between cancer derived from *BRCA1* mutation rather than *BRCA2* mutation are noticeable. Malignant primary BC of *BRCA1* mutation carriers is more likely to be high grade with medullary subtype features, and about 70% of BC do not express ER or PR or HER2 (triple-negative BC). The majority of *BRCA2*-associated tumours are high-grade invasive ductal, ER^+ and PR^+ and $HER2^-$.[238]

4.4.2. Numbers of cancer and penetrance rate related to *BRCA1* and *BRCA2* mutations

The mutation of the *BRCA1/2* genes is estimated to occur in 0.2%–0.3% of the general population (1 in 300–500 women), 2.1% of Ashkenazi Jewish women, 3% of women with BC (6% of women whose BC is diagnosed before age 40), and 10% of women with ovarian cancer. The frequency of the *BRCA1/2* mutations is even higher, and around 20% in families with multiple breast and/or ovarian cancers, especially early-onset cancers. Furthermore, Ashkenazi Jews represent high-risk families for these mutations; indeed, the prevalence of the gene is 10% in these families. However, unique founder mutations have been identified in different populations (e.g., in blacks, Hispanics and some families in the Netherlands, Iceland, Sweden, Hungary, French Canada, and Scotland), and, with the proceedings of screening and codifying the gene, it is supposed to find other new variants.[243,254] In conclusion, the attributable BC incidence and cases of germline mutations in the high-penetrance genes *BRCA1* and *BRCA2* can vary across different populations and world regions due to founder effects.[238]

Generally, women carriers of pathogenic variants (mutations) in *BRCA1* or *BRCA2* have an increased lifetime risk of BC (55% compared to 12% in the general population), ovarian cancer (20–60% for *BRCA1* carriers and 10–20% for *BRCA2* carriers), and other tumours (e.g. melanoma, prostate and pancreatic cancers associated with mutations in *BRCA2*).[238,255] The cumulative lifetime risk for BC varies depending on the study sample: it is around 80% in the study of high-risk families and around 40% in a population-based study.[255] The recent prospective study by Kuchenbaecker and colleagues (2017), including approximately 10.000 *BRCA1/2* pathogenic variant carriers, reports a likelihood for *BRCA1* of developing breast and ovarian cancer by the age of 80 of 72% (95% CI = 65–79%) and of 44% (95% CI = 36–55%), respectively; for *BRCA2* mutation carriers, the lifetime risk is 69% (95% CI = 61–77%) and 17% (95% CI = 11–25%) for breast and ovarian cancer, respectively.[256] *BRCA1/2* mutations could occur both in men and women, even though the latter is the most frequently screened

and diagnosed. Men carrying *BRCA1/2* mutations are at an increased risk of BC, especially *BRCA2* carriers whose risk is increased by 100-fold and the cumulative lifetime risk is 6%.[243]

In women carrying germline *BRCA1/2* mutations, cancer usually develops at a younger age than the sporadic one does. However, not all women affected by the mutation develop the pathology. The incomplete penetrance leads to the supposition that other factors than the genetic ones, such as environmental or metabolic factors, may modulate the penetrance. For example, obesity, high energy intake and life-long weight gain are associated with a higher *BRCA1/2* penetrance rate through several mechanisms, including low-grade inflammation, insulin resistance and IGF-1 regulation. Indeed, recent studies by Dumais and colleagues (2017) and Bruno and colleagues (2020) show that IGF-1, metabolic syndrome and fat mass are significantly associated with *BRCA*-related cancer and increase the *BRCA1/2* mutation penetrance.[257,258]

Furthermore, higher insulin levels are significantly and differently associated with the location of the mutation in *BRCA1/2* genes. This suggested a different association and altered response of the *BRCA1/2* protein with metabolic factors. From this point of view, *BRCA1/2* mutations are supposed to be more sensitive to the mitogenic effects of insulin, probably due to their lowered ability to repair the DNA damages.[259] Compared with carriers without cancer, *BRCA1/2* carriers with cancer frequently develop type-2 diabetes after the BC diagnosis, and the prediabetic condition can raise the risk of BC in these women.[257,260]

In addition to the previous factors, low serum adiponectin levels also increase the risk of *BRCA1/2*-associated cancer. Adiponectin is involved in several pathways able to modulate risk factors predisposing to BC. A recent study by Daniele and colleagues (2020) advances the hypothesis that increasing adiponectin levels may be a valid option to reduce the lifetime risk factors in women carrying *BRCA1/2* mutation, particularly in the presence of the T allele.[261]

4.4.3 Management of *BRCA1* and *BRCA2* mutation carriers

Firstly, the genetic-mutation diagnosis usually starts with a familial screening investigating the previous first-grade relative cancer/s, the relatives' age at the time of the first diagnosis, the typology and the features of the BC, etc. Secondly, the individuals undergo genetic testing, which could also be offered to all deemed suitable BC patients. If the subject is identified as a carrier of a *BRCA1/2* mutation, the main goal for managing the *BRCA1/2* mutation is to reduce the risk of developing cancer or, at least, to promote an early diagnosis and increase the chances of cure.[238]

In order to guarantee these goals, the oncologist request regular and careful screening, preventive and risk-reduction surgery and some protocol of chemoprevention. Regarding BC screening, *BRCA1/2* carriers should self-examine once a month, undergo clinical breast examinations twice a year, and magnetic resonance imaging (MRI) once a year starting at 25–30 years old. Yearly mammography starts at the age of 30, but some studies contrast this practice because the radiation could enhance the BC risk. Screening for genetic ovarian cancer is less accurate. Traditional methods, such as transvaginal ultrasound and serum measurement of CA 125, have not been proven to be sufficiently sensitive to substitute for surgery in women at increased genetic risk of ovarian cancer.[238]

The second option includes bilateral mastectomy and Salpingo-oophorectomy as risk-reduction surgery practices. Bilateral mastectomy is largely accepted as an option for women carrying mutation *BRCA1/2*.[238] Women who undergo prophylactic mastectomy show a reduction of 90% or more in the risk of subsequent BC than women of similar risk who do not undergo surgery. However, the reduction in BC incidence is not associated with a reduction in BC mortality.[262] Salpingo-oophorectomy is the most common practice in preventing genetic ovarian cancer due to insufficient screening effectiveness in the early diagnosis of the pathology. *BRCA1/2* mutation carriers are advised to undergo risk-reducing surgery after completing childbearing (35-40 years).

Nevertheless, advice may be different depending on diverse conditions. For example, a *BRCA1/2* mutation carrier who previously underwent bilateral mastectomy could delay the risk-preventive surgery Salpingo-oophorectomy. It should be taken into account that Salpingo-oophorectomy provokes premature and surgically induced menopause leading to an increased risk of cardiovascular disease, osteoporosis, vasomotor symptoms, sleep disturbances, mood swings, and sexual dysfunction and, thus, adversely affecting the quality of life. A possible new approach could be the salpingectomy with or without delayed oophorectomy as a risk-reducing strategy.[238]

The last preventive approach is represented by chemoprevention, including the use of oral contraceptives and tamoxifen. The first reduces ovarian cancer risk in the general and *BRCA1/2* mutation carrier populations. In contrast with this positive effect, oral contraceptives increase 2.5-fold the BC risk, particularly in *BRCA2*. The second is supposed to prevent primary BC in *BRCA1/2* carriers. Furthermore, evidence suggests that tamoxifen use could be more effective in preventing contralateral BC, thus in the setting of secondary prevention.[238]

4.4.4 Lifestyle options for *BRCA1* and *BRCA2* mutation carriers

The prophylactic and preventive treatments in women diagnosed with a *BRCA1/2* mutation are very invasive and somehow compromising in everyday life (e.g., the possibility of having a baby, living with a buttered body or the consequences of the surgical therapies). Women are demanding less invasive and non-surgical options for reducing their lifetime risk of developing breast or ovarian tumours. Furthermore, newly *BRCA1/2*-mutated diagnosed women are destined to be younger than before, and this condition increases the need to find alternative support in the *BRCA1/2* cancer-related treatment.[261,263]

In this context, dietary, PA, and evidence-based lifestyle intervention and counselling are increasingly considered and studied to give an alternative option to *BRCA1/2* mutation carriers. The

dietary intervention, focused not only on caloric, but also on protein restriction, underlines how the reduction in fat mass, insulin resistance, IGF-1 and metabolic syndrome factors are positively associated with the decreased risk of *BRCA1/2* -related cancer. [259,263]

Even though predominantly based on retrospective studies, PA is an effective tool in improving most of the metabolic, endocrine and hormonal pathways that can reduce the risk of developing breast or ovarian tumours in women carrying *BRCA1/2* mutations.[252,264]

The action of the lifestyle intervention is supposed to modulate the penetrance of *BRCA1/2*-related cancer by acting on the environmental factor related to tumour development.[261]

5. Physical activity and sleep in breast cancer pathology

Nowadays, many studies deal with the relationship between PA and BC. By reflecting on the evidence in healthy subjects, many (still not all) linkages between engaging in PA and reduced risk factors for the BC pathology have been highlighted. The importance of being physically active throughout the life course is now well established. PA also contributes to improving emotional aspects of daily life as well as psychological recovery after diagnosis and adjuvant treatments.[81]

In this context, during recent years, another risk factor has been described as a new environmental or lifestyle one: sleep.[265] The bond between sleep and BC could be described as a vicious circle in which the two main actors (sleep and BC) interact negatively with each other. On the one side, the pathology with its consequences (surgery, adjuvant treatments, anticipated or induced menopause, stress, pain, side effects, etc.) is recognized to adversely affect nocturnal sleep quality. On the other side, impaired sleep may be considered a predisposing factor for tumour development and a worse prognosis. Additionally, some compromising aspects of the disease are present at the onset of the pathology, making the BC patients defined as subjects with compromised sleep.[266]

PA is even more frequently considered a tool to improve sleep in both healthy and cancer patients. The pathways linking these three facets are still on the way to be explained, but to date, results are promising in improving sleep-health status in BC women through PA intervention.[267]

PA and sleep in the BC context are also emerging in the field of the genetic BC. Women with *BRCA1/2* germline mutations show considerably increased lifetime risks for BC. The incomplete penetrance and the interindividual differences of the mutation of these genes suggest that exogenous factors act together with polymorphisms in BC development. For example, the BC risk in *BRCA1/2* carriers is higher if their genotype is obese or physically inactive.[252] That is why it is crucial to elucidate the role of the lifestyle risk factors in modulating the penetrance of the mutations.

In the following paragraphs, PA and sleep effects, as well as their relationship, are described with reference to the BC patients and *BRCA1/2* carrier populations.

5.1 Physical activity and breast cancer

As previously seen in chapter 1, PA shows an anti-BC effect, explained by different biological mechanisms, including the increase in insulin sensitivity with the consequent reduction in IGF-1 levels as well as the reduction in fatty acid deposits and the active fraction of female sex hormones. These beneficial effects are partially mediated by improvements in body composition and reduction in body fat.[81] However, risk reductions in BC patients are also observed independently of the obesity status.[268] Recent studies investigate the PA anti-tumour effect in healthy women (aiming to assess the risk reduction), in women under chemo- or radio-therapy, in survivals (aiming to assess BC-recurrence and death-related risk reduction) and in metastatic BC.

Lynch and colleagues (2011) meta-analysis, conducted on 73 studies, reports a 25% average BC risk reduction amongst physically active women compared to the less active women. The risk

reduction is more evident when PA is a recreational activity, MVPA and performed regularly and over the lifetime.[269] The importance of a continuative PA practice is also highlight by Lope and colleagues (2017) in their epidemiological study on a cohort of Spanish women. The authors underline the value of being physically active in nulliparous women during the years before diagnosis and the PA beneficial effects on all BC subtypes.[270] Similar findings are present in the previous metanalysis by Wu and colleagues (2013) on 31 prospective studies. In this study, both occupational and non-occupational PA are significantly and linearly associated with reduced risk of BC. More specifically, the BC risk decreases by 2%, 3% and 5% for every 25 METs h/week increment in non-occupational PA, 10 METs h/week increment in recreational PA, and 2 h/week increment in MVPA, respectively. The authors conclude by stating that PA should be advocated in primary BC prevention.[271] A recent study on an Italian-Swiss cohort of BC cases shows that higher adherence to the WCRF/AICR 2018 recommendations, including PA, decreases BC risk independently of the menopausal status. Particularly for PA, a 1-point increment in the adherence score significantly decreases the BC risk.[272]

Being normal-weight or obese and in a pre- or post-menopausal situation represent an important condition to evaluate the PA effect on BC risk. Indeed, it seems the protective effect of PA against BC is more evident in normal-weight rather than in obese women during the premenopausal years.[269] The opposite situation is true for post-menopausal women when the PA role is more decisive in overweight or obese subjects.[273] This difference could be explained through the different female sex hormone sources between pre- and post-menopause. In the latter case, fat mass is the principal oestrogen source.[252] However, the role of weight and BMI is still debated, considering that body composition only partially mediate the association between PA and BC-related risk factors.[271] Apart from BMI, PA is essential in both pre- and post-menopause women; however, the latter seems to need a more intense PA to reach a protective role similar to that of the premenopausal situation.[270]

The mechanisms known to decrease the BC-related risk factors are supposed to be the same involved in BC recurrence prevention and mortality risk reduction.[274] In the latest years, the BC mortality rate has been decreasing, resulting in a high number of BC survivors.[275] These women usually suffer from adjuvant therapy-related symptoms, which may be present even after several years from the end of medical treatments.[276,277] In this context, PA is described as a beneficial tool in decreasing recurrence and mortality rate as well as side effects of the past treatments. PA should be taken into account both pre- and post-diagnosis. In their systematic review, Lahart and colleagues (2015) show that women practising more extensively both life-long and more recently pre-and post-diagnosis recreational PA have a lower risk of BC-related death and BC events. Additionally, the death-risk reduction is more evident in subjects with a BMI >25 kg/m^2 and showing ER$^+$/PR$^+$.[274]

In other words, being physically active or increasing the PA level is also beneficial after the diagnosis and helps in improving the prognosis. For example, Irwin and colleagues (2003) demonstrate that women who increase their PA level after diagnosis reduce the BC-related death risk by 45%, while, conversely, women who decrease their PA level after diagnosis show a four times greater risk of death.[278] In the same way, Holick and colleagues' (2008) study demonstrates that active women have a significantly better survival rate than sedentary women (with no distinctions in terms of age, state of disease, or BMI).

PA practice in survivors is not only linked to BC recurrence or death risk reduction; in fact, active women also report improvements in several personal and physical spheres, such as in cardiorespiratory levels, pain, fatigue, early menopause symptoms, quality of life and symptom severity.[279–283] For example, the study by Chen and colleagues (2020) reports decreased severity of arm and shoulder symptoms on a cohort of women receiving axillary node dissection and surgery and performing 30 mins/day of arm-shoulder exercises. By improving symptom severity, PA enhances the

quality of life in these BC women.[284] Furthermore, different typologies of PA are needed in order to achieve a wide range of goals (e.g., pain, BMI, general health, vitality, flexibility, strength, etc.).[285]

The reduced risk of mortality through the action of PA is also assessed in metastatic BC to evaluate its influence on survival chance. In a prospective French study, PA at baseline (retrospective and self-assessed through questionnaire) is associated with more prolonged survival in women with advanced BC, and this association is more robust in women showing HER^{+}. More specifically, women practising MVPA before the pathology have higher survival probabilities than women practising light PA.[286]

Finally, in addition to BC-risk and BC-related death reduction, PA is suggested and also tested during adjuvant treatment. Being engaged in PA protocols during medical treatments brings several improvements in patients' health. Most of the time, outcomes depend on the patients' conditions and PA typology. For example, Courneya and colleagues (2007) compared the effects of aerobic and resistance PA protocols on 242 women under adjuvant treatment for 17 weeks. The PA interventions improve self-esteem, physical fitness, and body composition, whereas only the resistance training significantly improves the chemotherapy completion rate.[287] On the contrary, Mijwel and colleagues (2020) find no differences in chemotherapy completion rates between aerobic-HIIT and combined-HIIT groups. However, the combined-HIIT group shows a lower hospitalization rate and thrombocyte concentration. Furthermore, the authors advance the hypothesis that exercise during chemotherapy helps in maintaining the immune cells population.[288] In the CARE randomized trial, women practising a combination of aerobic and resistance exercises for 17 weeks experience a higher muscular strength both in upper and lower body regions compared to standard (25-30 mins) and higher dose (50-60 mins) of aerobic exercise.[289] Finally, light PA also helps maintain regular bone turnover in BC women treated with aromatase inhibitors, while less active women show enhanced bone turnover.[290]

5.1.1 Physical activity in *BRCA1* and *BRCA2* carrier women

Risk factors predisposing to sporadic BC are known to also enhance the BC risk in women at higher risk for hereditary BC, such as women carrying *BRCA1/2* mutations. Therefore, these women ask for treatments less invasive than surgery and able to decrease their BC risk. Studies and clinical trials evaluate PA as a suitable solution to fulfil this request and provide evidence-based proposals and solutions. In addition, also the control in body size through PA and diet is increasing attention.[252]

Preliminary evidence of the protective role of PA emerges from a few retrospective and questionnaire-based studies. One of the first studies assesses that a medium level of PA (during the ten years before the assessment) reduces the risk of BC among *BRCA1/2* carriers. Furthermore, in women active before age 30, an exercise dose-response trend is highlighted (i.e., the risk reduces with increasing MET-hour/week and number of active years); in contrast, no trend is visible after the age of 30.[291] A more recent study, conducted by Lammert and colleagues (2018), reveals a reduced premenopausal *BRCA1/2*-BC risk in women more active during adolescence. More specifically, the women in the highest interquartile during the age of 12-17 reduce their BC risk by 38% compared to women in the lowest interquartile. Together with Grill and colleagues' (2017) conclusions, these findings suggest an early-life protective role of PA during premenopausal years.[264,292]

Contrasting results are present in the recent publication on the *Prospective Family Study Cohort*, which, among a large cohort of women exposed to a high risk of familial BC, also includes premenopausal *BRCA1/2* women. Kehm and colleagues (2020) find a positive association between recreational PA during adulthood and lower BC risk. More specifically, meeting 10.75 METs/week reduces the BC risk by 20%. However, no association between recreational PA during childhood and decreased risk is significant.[293]

Collectively, the cited studies enhance the need to make adolescents, and in general, all women with a mutation diagnosis or familial history of BC, adopt a healthier lifestyle. Indeed, even

though aware of their mutation-BC risk, young females seem not to transpose this consciousness in healthy habits, including constant and regular PA practice.[294] Furthermore, as affirmed in the study from the *New York Site Breast Cancer Family Registry*, high-risk women with familial history for BC could reduce their mortality risk if they adhere to the ACS recommendations (BMI $\leq$25kg/m^2, one drink per day, 150 mins/day of light PA or 75 mins/day of MVPA). Notwithstanding the mortality risk reduction following a healthy lifestyle, only 28% of women adhere to all three guidelines and only 60% respect the guidelines to be physically active.[295]

Although the critical information obtained from the retrospective studies, prospective and controlled trials are necessary to understand the effects of PA during the adult or post-diagnosis life and understand the biological mechanism and biomarkers underlying the association between PA and the gene mutations. In this view, Wang and colleagues (2009) demonstrate a significantly higher level of *BRCA1*, p53 and ER mRNA expression in exercised rats compared to control (unexercised) rats. Furthermore, the former may lower their possibilities for neoplastic transformation due to a higher number of differentiated alveolar buds and lobules.[296]

However, these findings remain confined to the rat model, while Pettapiece and colleagues (2015) test the sedentary downside of *BRCA1* mRNA expression on a cohort of 50 women monitored with the actigraph. The results show a negative correlation between the longest sedentary time and *BRCA1* mRNA expression. The interesting point is that the negative correlation is significant only with the most prolonged period and not with the mean sedentary time; this means that uninterrupted sedentary time is more deleterious than shorter periods of sedentary time with frequent interruptions.[252] These findings align with the new WCRF/AICR 2018 guidelines, which suggest achieving the recommended PA mins/week and avoiding sedentary habits.[45]

Among the few clinical trials on *BRCA1/2* women, the recent randomized study by Schmitz and colleagues (2015) evaluates the role of PA on steroid hormones in a cohort of 139 women. After

five menstrual cycles of aerobic PA intervention, the low- and high-dose intervention groups report a breast tissue less sensitive to oestrogen stimulation compared to the control group. This study demonstrates the dose-response effects of aerobic PA on oestrogen hormones, diminishing the level of one of the principal BC-related risk factors.[297] Similar findings are already present in Kossman and colleagues' (2011) study with a study protocol similar to the previous one. This last study, conducted on a small sample of seven women, reports both the decrements in oestrogen and progesterone hormones, reducing total exposure to them by 18.9% and 23.7%, respectively.[298]

Even though considered separately from PA, body size and weight could also impact *BRCA1/2* breast-associated cancer. Also in this case, studies are still retrospective and self-reported. For example, Kotsopoulos and colleagues (2005) report on a cohort of 1,073 matched pairs, the association between weight loss of at least 10 pounds (4.5 Kg) and a 34% reduction in BC risk. After stratifying by age and mutation type, the protective effect of weight loss is limited to women diagnosed before age 40 and with a *BRCA1* mutation.[299]

To date, the only worldwide prospective randomized lifestyle intervention trial in *BRCA1/2* mutation carriers is *LIBRE-1*. The multicentre study entails a lifestyle intervention (physical activity and Mediterranean diet) involving German *BRCA1/2* mutated women and aims to assess whether a long-term multifactorial lifestyle intervention can reduce the BC incidence and mortality in *BRCA1/2* mutation carriers.[300] In 2017, Kiechle and colleagues publish the first results of the pilot study, including data about adherence and feasibility. The one-year intervention protocol shows positive effects on BMI, Mediterranean diet patterns and stress levels; 81% of participants complete the study; adherence rate is higher (73%); no adverse effects have been registered; the majority of the women are satisfied with the study protocol and some women included in the control group even complain and ask to be inserted in the intervention group.[301]

5.2 Sleep and breast cancer

Sleep is an essential contributor to the quality of life and the maintenance of good health status.[302] Despite this, sleep problems and, more generally, sleep deficiency are becoming an issue for contemporary society in different fields, including BC.[265,303,304] The construct "*sleep deficiency*" incorporates the multiple dimensions of sleep (duration, deprivation, insomnia, latency, hygiene, quality, disorders, impairments, problems, etc.), and it is universally utilized to define a:

"*deficit in the quantity or quality of sleep obtained versus the amount needed for optimal health, performance, and well-being*."[305]

Sleep deficiency is connected to BC in a bi-directional way, and it is supposed to play an incisive role at different pathology times. Indeed, on the one hand, some studies evaluate sleep deficiency as a risk factor for BC, thus triggering or contributing to some alterations or pathological pathways leading to BC. On the other hand, the diagnosis, the pathology and the related medical treatments could predispose the subject to develop sleep deficiency and/or exacerbate the pre-existing ones.[266]

Some mechanisms explaining this relationship have already been advanced, while others are still under evaluation. Furthermore, some studies are discordant in affirming the reciprocal influences of sleep and BC. These differences and sometimes inconclusive results may be reconducted to the study protocol, instruments used (actigraphy or questionnaires), cohorts, pathology characteristics, and, generally, the heterogeneity of the studies.[306] However, sleep deficiency is becoming more and more frequent among BC patients.[307]

5.2.1 Sleep deficiency as a risk factor for breast cancer

There is a body of evidence that supports the association between sleep deficiency and enhanced BC risk. The recent conclusions by Hurley and colleagues (2020) on a cohort from the *California Teachers Study* illustrate a significant and positive correlation between sleep deficiency and most of the sleep dimensions with BC risk.[308]

Sleep duration is probably one of the most investigated sleep variables concerning BC, and several studies find an increased BC risk depending on different sleep duration.[309,310] Considering 7-8 hours as an optimal sleep duration in adults, the study by Shen and colleagues (2019) shows a 1.87-fold increment (95% CI = 1.01 – 3.45) for BC risk in women sleeping less than six hours per night, while the prospective analysis in the review by Lu and colleagues (2017) reports an estimated risk of 1.11 (95% CI = 1.03–1.19) in >10 hours sleepers.[265,311] Similarly, the increased BC risk when sleep duration is longer than recommendations is also reported by Richmond and colleagues (2019), whose results derive from Mendelian randomization and include chronotype evaluation.[312] Sleep duration could probably exert a different role depending on the tumour characteristics. For example, longer sleep duration is positively associated with higher ER^+ or ER^+/PR^+ rather than ER^-/PR^-.[308,311] However, attributing increased BC risk only to the duration aspect of sleep could not be sufficient to explain the elevated BC risk or incidence in concomitance to bad sleep deficiency.[313]

Sleep deficiency also includes sleep disorders, of which one of the most frequent respiratory manifestations is *obstructive sleep apnoea* (*OSA*). Two recent studies investigating the relationship between OSA and BC risk demonstrate the incremented BC risk in women suffering from OSA.[314,315] In particular, Gao and colleagues' (2020) study, based on Mendelian randomization, points out a causal relationship between higher BC risk and OSA: women who are more genetically predisposed to OSA are also at higher risk for BC.[314] The most accredited interpretation of this negative influence is traceable in intermittent hypoxia conditions developed during the sleep apnoea episodes. The intermittent

hypoxia could favour BC onset and proliferation by facilitating cell migration.[316,317] Furthermore, HIF-1α, one of the two primary hypoxia markers, increases the number of adipocytes, known to enhance BC progression.[318]

Excluding OSA, different potential mechanisms are advanced to explain the linkage between sleep deficiency and BC or cancer in general, including inflammation, endocrine function, oestrogen secretion, obesity and melatonin. Sleep indirectly impacts the adaptive and innate immune responses by influencing the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system, the two primary immune system regulators. Thus, sleep deficiency, particularly sleep deprivation and short sleep duration, impairs immune responses and increases inflammation, predisposing to cancer.[319] Sleep curtailments, even lasting for short periods, alter the metabolic and endocrine function, resulting in a decreased carbohydrate tolerance, leptin levels and increased cortisol levels, which, together, predispose to insulin resistance and obesity or weight gain.[320,321]

Another likely mechanism by which sleep deficiency represents a risk for BC is reducing melatonin secretion. Indeed, sleep deficiency leads to decreased *urinary 6-sulfatoxy melatonin levels*, directly reflecting a decrement in melatonin secretion. Melatonin, a hormone secreted by the pineal gland, is known to promote sleep, and be inversely associated with BC risk. In fact, melatonin is also capable of regulating the onset and progression of BC; thus, conditions that can decrease its expression indirectly enhance BC risk.[322–325] More specifically, melatonin is recognized as anti-aromatase and anti- desmoplastic reaction (the reaction by which adipose fibroblasts increase oestrogen biosynthesis in the breast tissue) properties.[326]

5.2.2 Sleep deficiency during breast cancer treatments and in survivors

Sleep deficiency is emerging as a long-term, side-effect treatment in BC women. In fact, chemotherapy and radiation therapy are two contributing factors in developing sleep deficiency either

during or after treatments. In women with BC, sleep deficiency or insomnia are approximately 20-70%, with a higher range than the general population.[266] Sleep deficiency also continues after medical treatments end: the review by Budhrani and colleagues (2015) states that sleep problems are still present and sleep does not improve after one year from the end of therapy.[327] Furthermore, in women already suffering from sleep problems, BC diagnosis could exacerbate them.[266]

In women starting adjuvant treatments, sleep deficiency is higher than in the general population, and women report more sleep problems, as stated in the German study by Kreutz and colleagues (2021) on a cohort of women selected from the BENEFIT study.[328] Also, Rades and colleagues (2021) and Li and colleagues (2019) find increased sleep deficiency in BC patients starting radiotherapy and during the first week of chemotherapy, respectively; furthermore, sleep deficiency is associated with both emotional/psychological and physical problems.[329,330] The same sleep deficiency condition was reported previously by Liu and colleagues (2013) in a study involving women at the onset of chemotherapy treatment. In addition to having sleep problems before the chemotherapy onset, BC women face the worsening of their quality of life in concomitance to the increase of their sleep deficiency.[331]

Another cluster symptom that needs attention concerning sleep is C-RF, which is reported by up to 99% of BC patients.[332] In their cross-sectional study, Fox and colleagues (2020) stratify the participants based on reported symptoms and show that higher self-perceived fatigue is usually associated with worse sleep quality. The most reported fatigue at the start of medical treatment is the emotional one. During the therapy course, sleep worsens, and all types of fatigue increase; simultaneously, the *Minimal symptoms group* collects lower subjective sleep deficiency and less self-perceived fatigue.[333]

Sleep deficiency represents a crucial issue because its worsening is associated with increased comorbidities, recurrence and mortality risk in BC women.[334,335] In the study by Phipps and colleagues (2016), women with a sleep duration <6 hours before the diagnosis are at a significantly higher risk

of poorer cause-specific survival compared to normal sleepers (7-8 hours). Furthermore, if short sleep duration is associated with snoring, also the risk of a poor prognosis is higher (HR = 2.14, 95% CI = 1.47–3.13).[336] These results are in line with those of Palesh and colleagues (2007), who describe actigraphy-based sleep efficiency as a positive predictor of six-year overall survival (HR = 0.94, 95% CI = 0.91–0.97) among women with advanced BC.[337]

Sleep deficiency or insomnia risks are incremented in BC patients for various reasons. Some are dependent on the tumour and the symptoms of its invasion process, such as pain, fatigue, nausea, and pruritis. Others are related to the treatments, such as hormonal fluctuations, medication use (narcotics, neuroleptics, sympathomimetics, sedatives, antidepressants, etc.), and the induced menopause consequent to drugs used in cancer treatments. Finally, also environmental factors and psychological distress can ruin sleep quality. The former could also be related to hospitalization conditions, which could be characterized by an unfamiliar situation in the bedroom with disturbing light, noise, temperature, etc. The second is linked to the anxiety, stress and worries due to cancer.[266,306,329,335]

5.2.3 Sleep deficiency in *BRCA1* and *BRCA2* carrier women

Studies aiming at investigating sleep quality or deficiency in *BRCA1/2* women are still in their infancy, and data are still few. The first study reporting sleep analysis in asymptomatic or unaffected *BRCA1/2* is by Shochat and Dagan (2009) on a cohort of Israelian women. This cross-sectional study evaluates sleep and other burden symptoms in three groups: the first includes *BRCA1/2* carriers, the second women tested for *BRCA1/2* mutations but resulted as non-carriers, and the third healthy women (control group). In both PSQI and actigraphy sleep assessment, the *BRCA1/2* group results as the most sleep deficient group; indeed, it is classified as a bad sleeper, with the most prolonged sleep latency and the shortest sleep duration. The authors advance the hypothesis that the mutation diagnosis, associated with psychological distress, could represent a stressor negatively impacting

sleep. In support of this hypothesis, the data referring to the *BRCA1/2* group show poor sleep related to psychological distress more prominently than the other groups and the highest levels of cancer-related worry.[338]

Despite the stress due to the mutation diagnosis, other factors following the diagnosis, the treatments and the surgery could compromise sleep in these women. In addition to being more predisposed to sleep deficiency than men, perimenopausal women suffer most of the time from vasomotor symptoms, which compromise sufficient sleep duration and quality. *BRCA1/2* women are suggested to undergo risk-reducing bilateral salpingo-oophorectomy that induces anticipated and surgical menopause, which is known to predispose to more pronounced and severe side-effects, including sleep deficiency.[339] Indeed, a menopause-induced subsample of the *Korean Genome and Epidemiology Study* shows 2.1 folds (95% CI = 1.055–4.303) of suffering from insomnia compared to the subsample experiencing natural menopause.[340] Sleep deficiency has already been assessed in *BRCA1/2* women who underwent prophylactic Salpingo-oophorectomy by Campfield Bonadies and colleagues (2010). More than 40% of the sample reports sleep deficiency after surgery. The study aims to raise consciousness about sleep deficiency after surgery in these women and include it in the most common post-operative symptoms list.[341]

Physical aspects of menopausal symptoms are not the only possible explanation for the worse sleep quality after surgery. The *Study of Women's Health Across the Nation* associates the severity of vasomotor symptoms to more depressed mood and explain this relationship through sleep deficiency. Furthermore, also anxiety appears as one of the predictors of sleep deficiency. In other words, vasomotor symptoms could lead to depression and anxiety, provoking sleep deficiency.[342] However, the bi-directional relationship between sleep and anxiety/depression could be feasible. Thus, sleep deficiency, consequently to hot flashes, could be the cause of anxiety and depression.[339]

5.3 Rest-Activity circadian rhythm and breast cancer

Sleep deficiency or, even worse, sleep disruption (such as insomnia) could disrupt the Rest-Activity circadian Rhythm (RAR), the expression of 24 hours of locomotor activity. RAR is usually considered a marker of the human circadian timing system, whose alterations, in addition to having been found in various natural conditions (such as ageing), are also reported in cancer, including BC.[172,208,343]

Particularly for BC cohort women, previous studies assess RAR at all stages of the pathology, i.e. pre, during, and after surgery or chemotherapy.[344–349] As reported by Ancoli-Israel and colleagues (2006) in their study investigating sleep, fatigue and circadian rhythms before BC surgery and chemotherapy, women reporting RAR alterations and delayed acrophase are more prone to experience daily dysfunctions.[345] Furthermore, all-day-long reduced activity levels (probably caused by sleep deficiency and increasing fatigue sensation) are noticeable before BC treatments and RAR disruptions are associated with flattened diurnal cortisol rhythms.[344,346]

During medical treatments, chemotherapy negatively impacts all the rhythmometric parameters of RAR, showing a decreased midline estimating statistic of rhythm (MESOR) and amplitude and a delay in the acrophase, which persist even after one year since the end of chemotherapy.[348–350] More specifically, Savard and colleagues (2009) record the worst RAR parameters during the first week of the chemotherapy cycle; they slowly recover the baseline values during the second and third weeks. However, a sufficient recovering in RAR parameters is not visible during the fourth chemotherapy cycle, indicating the progressive alteration of RAR and decrease of daytime activity levels as medical treatments progress.[351] In concomitance with RAR alterations, fatigue, depression, and lower morning energy are worsened, especially in women with increased BMI.[348,349]

A highly marked RAR disruption co-occurs with the poor functional and physical quality of life in advanced BC patients.[352] Furthermore, sleep disorders and fatigue reported before

chemotherapy are exacerbated by the rhythm disruption during chemotherapy, generating depression and mood disorders.[345,353,354] A less robust RAR during and after treatments could be associated with more distressing fatigue and depression and accompanied by desynchronized cortisol, melatonin, and serotonin secretion rhythms. Altogether, these alterations may lead to more severe chemotherapy-related symptoms and higher mortality rates in BC patients.[355,356]

Based on the study explained previously and also referring to other studies involving different types of cancer, it is possible to affirm that RAR and cancer are intercorrelated and linked in a bidirectional way. On the one hand, the disrupted RAR is able to increment the cancer incidence, while, on the other hand, chemotherapy and the physiological aspects of the tumour could disrupt RAR and accelerate cancer progression.[208,343,357–360] Indeed, RAR worsens during the cycles of chemotherapy, explaining why chemotherapy could be considered one of the factors implicated in the deterioration of RAR.[335] Additionally, BC-related psychological distress is a strong predictor of the disruption of the sleep-wake circadian rhythm.[361]

The evidence of the association between altered RAR and increased cancer incidence stems from studies involving shift workers and, more specifically, shift workers with only one-night shift or constantly working during the night.[307,362] In these workers, the night-at-light exposure leads to a misalignment between the internal and external clock (particularly the internal and external night), which causes the suppression of the melatonin secretion. Melatonin is recognized for anti-carcinogenesis properties that decrease along with melatonin suppression and, consequently, increase the risk for cancer in night-shift workers.[322,363] The decrease in melatonin secretion in BC patients is also reported by Li and colleagues (2019), who observe a decrease in the morning evaluations of *urinary 6-sulfatoxymelatonin* in concomitance with the decrease of sleep duration.[330] The recent review by Fagundo-Rivera and colleagues (2020) sustains the hypothesis that the decreased melatonin secretion

may also alter oestradiol and insulin levels and give rise to a greater amount of oestrogen secreted by the ovaries.[364] These are all predisposing factors to BC.

Finally, genetic makeup represents another link between alterations in the circadian rhythms (even though not specifically for RAR) and BC. Although longitudinal and large sample size studies are still ongoing, the study on single-nucleotide polymorphisms by Lesicka and colleagues (2019) points out the association between some circadian rhythm gene variants and the increase for BC.[365] Previously, other associations between BC and the CLOCK genes (e.g., PER2 and Cryptochrome 2) have been advanced.[366–368]

5.4 Effects of physical activity on sleep behaviour in breast cancer patients

In cancer patients, together with fatigue and depression, sleep deficiency is one of the three major distressing symptoms, which are usually addressed as a *symptom cluster*.[369,370] Also considering that BC patients and survivors are constantly increasing, it is necessary to find a solution to alleviate sleep deficiency in this population. One of the main features of this solution is that it has to avoid the onset or establishment of other side effects symptoms (such as headache, dizziness, and seizures), as many pharmacological approaches do.[266,371] Thus, researchers are looking for risk-free and non-pharmacological interventions, including PA, which is established as a safe non-pharmacological intervention in cancer patients.[267,370,371]

Several studies have approached a PA intervention protocol on BC patients during recent years.[370] Notwithstanding the promising results, conclusions are sometimes discordant depending on the duration of the PA intervention, the type, the intensity, the frequency and the modality of the proposed PA, the phase of the medical treatments during which the PA is planned, the patients'

baseline condition and the modality of sleep assessment (objectively or subjectively – mainly through the PSQI).[267,370,372]

The meta-analysis by Yang and colleagues (2021) takes into consideration six studies that assess sleep through PSQI administration after a PA intervention. The authors conclude that BC patients and survivors could benefit from PA intervention, because PA may improve sleep quality (weighted mean difference = .22, 95% CI .04–.40, p = .018), even though differences in the seven PSQI subscales are not significantly different between the intervention and the control groups.[372] Kreutz and colleagues (2019) drew similar conclusions, stating the efficacy of both PA and body-mind exercise in improving subjective sleep quality, but not in the objective assessment.[267]

The lack of agreement between the subjective and objective sleep assessment is also reported by Rogers and colleagues (2017), in whose study, sleep improvements are significant for perceived sleep quality, sleep disturbances and daytime dysfunctions. Notwithstanding the absence of significant sleep improvements through objective assessments, subjective sleep improvements persist after three and six months since the PA intervention.[373]

In contrast with Kreutz and colleagues (2019), another systematic review by Tang and colleagues (2019) delines a superior effect of walking in improving sleep rather than practising yoga (an exercise modality included in the body-mind exercise category).[267,374] Possible explanations could be the higher home-based walking feasibility, the more straightforward standardization of walking compared to yoga and the easiest applicability of walking protocols during medical treatments.[374]

In the sleep improvements assessments, several aspects need to be taken into consideration, for example, the PA typology or amount. Aerobic PA is usually the most common involved with BC patients;[370] however, a combination of aerobic and resistance exercise and MVPA seem to apport more significant sleep improvements.[375,376] Regarding the amount of PA, passing from 25-30 minutes to 50-50 minutes thrice a week of aerobic PA apports superior improvements in global and subjective sleep

quality and sleep latency (PSQI assessment) in Canadian women trained during and after chemotherapy.[375] Additionally, the individualization of the PA intervention is also receiving attention. For example, due to the possible relationship between obesity and sleep deficiency, obese women need a protocol, including the weight reduction goal.[267]

To date, the mechanism, or more probably the mechanisms, underlying how exercise could affect sleep is still unclear. One of the linking features of BC, PA and sleep is chronic low-grade inflammation and the pro-inflammatory cytokines and mediators. Indeed, chronic inflammation is recognized as playing a crucial role in BC development and is usually reported in BC patients.[10] At the same time, sleep deficiency is associated with increases in markers of systemic inflammation, such as C-reactive protein, IL-6, and TNF-α.[377] PA has already recognized anti-inflammation properties able to decrease the level of IL-2, IL-8, IL-6, and TNF-α, and, consequently, the chronic low-grade inflammation.[377] Due to all these considerations, it is possible that improvements in inflammation can mediate the effects of PA on sleep quality in BC patients.[372] Other sleep-promoting mechanisms are represented by the facilitation of the temperature cool-down after PA sessions and the ability of PA in reducing stress and depression.[154,166]

Second Part

Studies

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

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IF: 2.877; Rank: Q2

Aim of the study

To date, several studies have investigated and highlighted the potential role of physical activity in breast cancer prognosis, also including RAR analysis. However, few focused on breast cancer survivors, especially in long-survivors, such as 5-year after the primary diagnosis.

The present study aims to actigraphically monitor RAR in a population of 5-years breast cancer survivors since the primary diagnosis and compares their characteristics with healthy controls.

The results could help plan physical activity programs to increase daytime activity levels in these women and improve their RAR.

Methods

Participants

The study sample was composed of 28 women: 15 BC survivors at five years after primary diagnosis (BC-group) and 13 healthy controls (Ctrl-group). The two groups were matched for age and BMI. The women showing the following inclusion criteria were recruited at *IRCCS Istituto Nazionale dei Tumori di Milano*: for the BC-group (i) mastectomy or conservative surgery for any type of primary invasive breast cancer and within the previous five years, (ii) no history or objective evidence of recurrent ipsilateral or contralateral breast cancer, (iii) not scheduled for and not currently undergoing chemotherapy, (iv) no other cancer diagnosis within the last ten years, (v) age 35–60 years at recruitment, (vi) BMI <30 Kg/m2; for the Ctrl-group (i) no history or objective evidence of breast cancer diagnosis, (ii) no other cancer diagnosis or pathology, (iii) age 35–60 years at recruitment, (iv) BMI <30 Kg/m2.

The study was carried out in accordance with the tenets of the 1964 Declaration of Helsinki and approved by the Ethical Review Board of the Istituto Nazionale dei Tumori di Milano. The guidelines required by the journal (*Chronobiology International*) were accomplished either for the study protocol or during the procedures.[1]

Study design

All the 28 participants underwent a medical examination for the following assessments:

- height and body mass to calculate BMI (kg/m2);

- systolic and diastolic blood pressure (mmHg), using an electronic sphygmomanometer (DynaPulse 5000A Pulse Metric, Inc., Vista CA, USA) with the cuff placed on the left arm and the subject supine after 10 min of rest;
- blood collection after fasting overnight to assess glycemia (mg/dl), total cholesterol (mg/dl), high density lipoproteins (HDL, mg/dl), low density lipoproteins (LDL, mg/dl), and triglycerides (mg/dl).

Furthermore, during the medical visit, women were instructed to undergo continuous 7-day actigraphic monitoring to detect RAR. During the actigraph monitoring, the actigraph (MotionWatch 8®, CamNtech, Cambridge, UK) was worn on the non-dominant wrist and removed when swimming or bathing. Additionally, subjects filled in a diary with their bed and get up time, hours of naps and without wearing the actigraph (on average, once a day for 30 minutes; period not included in the data analysis) and the number of nocturnal awakenings. The actigraphic monitoring started at the first clinical meeting. During the actigraphic monitoring, subjects performed their usual daily activities, not inviting them to increase their physical activity.

Statistical analysis

The anthropometric, cardiovascular, and blood chemistry parameters are reported as mean ± SD, while the rhythmometric parameters are expressed as mean with 95% CI. The normal distribution of each parameter was verified using Shapiro-Wilk's test. To compare anthropometric, cardiovascular, and blood chemistry data between the two groups, the unpaired Student t-test was used. Firstly, the RAR parameters were assessed using the single cosinor analysis; after that, rhythmometric parameters of BC- and Ctrl-group were compared using the Hotelling T2 test. The Hotelling T2 test is a generalization in the multivariate field of Student's t-test and is based on the hypothesis that the distance

between the two samples' mean vectors is null. Pearson's Correlation Coefficient (*r*) investigated the association between anthropometric and rhythmometric parameters.

The used software were Expert Soft Technology (version 6.0, Esvres, France) for the cosinor analysis and JMP® (version 14.0.0, 2018 SAS Institute Inc., Cary, NC, USA) for all the remaining statistical analyses. Statistical significance was set at $p < .05$.

Results

The anthropometric, cardiovascular, and blood chemistry parameters for the BC- and Ctrl-group are shown in Table 1. No parameters show significance.

Table 1 Characteristics of the participants, stratified into the BC-group and Ctrl-group.

Characteristics of the experimental groups		
	BC-group (n = 15) Mean ± SD	Ctrl-group (n = 13) Mean ± SD
Age (years)	56.7 ± 6.6	54.4 ± 7.2
Weight (Kg)	64.8 ± 9.4	68.3 ± 7.8
BMI (kg/m2)	24.5 ± 3.8	25.2 ± 2.8
Systolic Blood Pressure (mmHg)	124.0 ± 12.2	129.1 ± 13.2
Diastolic Blood Pressure (mmHg)	76.8 ± 11.2	77.1 ± 12.0
Glycemia (mg/dL)	95.6 ± 13.2	97.1 ± 10.5
Total Cholesterol (mg/dL)	215.3 ± 22.2	222.3 ± 18.7
HDL (mg/dL)	67.3 ± 21.3	59.5 ± 14.3
LDL (mg/dL)	127.5 ± 24.1	119.6 ± 24.5
Triglycerides (mg/dL)	105.5 ± 54.2	106.7 ± 39.9

The results obtained with the cosinor analysis are shown in Table 2 and Figure 1. Both the MESOR and the amplitude are statistically lower in the BC-group compared to the Ctrl-group (both $p < .001$). Acrophase shows no statistically significant difference.

Table 2 *Results of the actigraphy-based analysis of RAR in BC-group and Ctrl-group by the population mean cosinor.*

Rhythmometric analysis in the BC-group and the Ctrl-group					
Group	PR (%)	p-value	MESOR (a.c.) (mean ± 95% CI)	Amplitude (a.c.) (mean ± 95% CI)	Acrophase (hr:min) (mean ± 95% CI)
BC-group n = 15	48	<.001	192.0 ± 12.4**	167.0 ± 38.4 •	15:09 ± 01:14
Crtl-group n = 13	47	<.001	276.4 ± 19.2**	222.6 ± 26.8 •	15:01 ± 00:45

PR: percentage of rhythm. MESOR (activity counts): Midline Estimating Statistic of Rhythm. Amplitude (activity counts): half the difference between the highest and the lowest points of the cosine function best fitting the data. Acrophase (hr:min) indicates when the highest values fall; the values are referred to local midnight. (**p < .001; •p < .001).

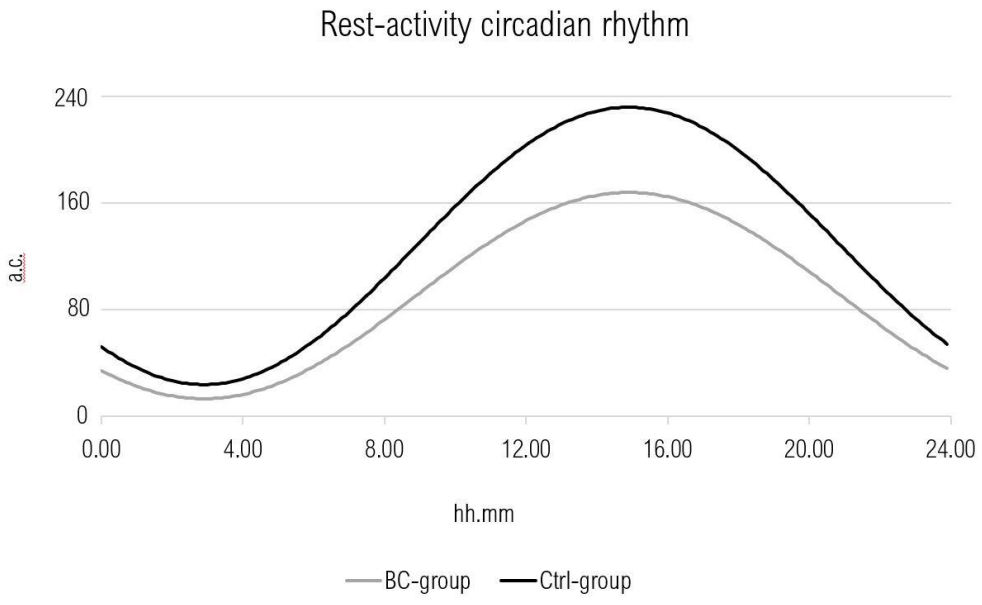

Figure 1 *Rest-activity circadian rhythms: data collected by the actigraph in BC-group (grey line) and Ctrl-group (black line).*

Figure 2 shows a visual comparison of the MESOR and amplitude parameters for the two groups. In this figure are represented two scatterplots of amplitude vs MESOR data, one for each group. For what concerns the BC-group, the reduction in both the MESOR and the amplitude translates into a downward and leftward shift of the BC-group scatterplot compared to the Ctrl-group one. Two regression lines overlay the two scatterplots. These two regression lines are forced to pass through the origin; thus, the two slopes measure the average ratio between the amplitude and the MESOR separately for the two groups. Also considering the two respective equations, when the group amplitude values of the BC- and Ctrl-group are expressed relative to the respective MESOR, the relative amplitude of the breast cancer patients is larger (amplitude 87% of MESOR) than that of the Ctrl subjects (amplitude 77% of the MESOR).

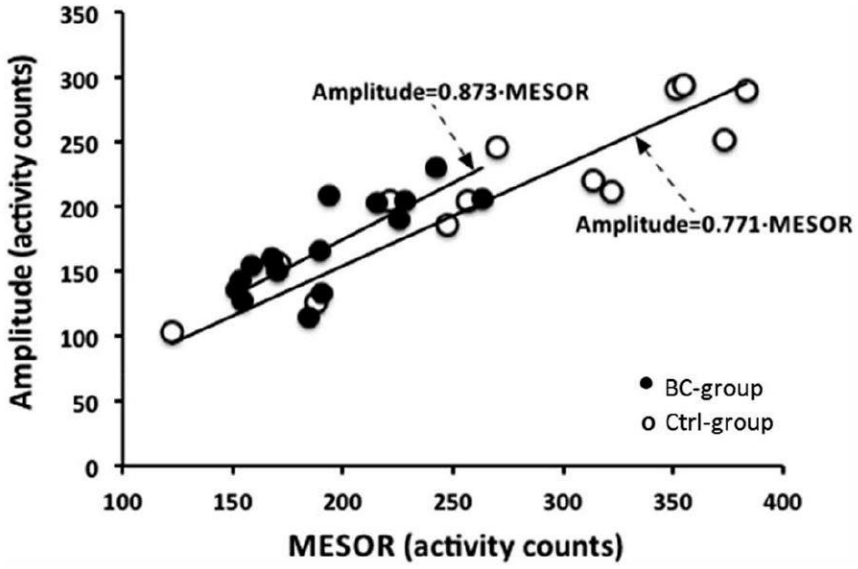

Figure 2 A visual comparison of the MESOR and amplitude parameters of BC- and Ctrl-group. For each group, it is represented a scatterplot of amplitude vs MESOR data, two superimposed regression lines and two respective equations. The two slopes measure the average ratio between the amplitude and the MESOR in the two groups.

About the correlation analysis, no correlations between the anthropometric and rhythmometric parameters are significant.

Discussion

The present study reports the actigraphically-monitored RAR in breast cancer survivors (BC-group) and healthy control groups (Ctrl-group). The principal outcome is that RAR differs between the two groups, with BC-group showing a lower MESOR and amplitude than Ctrl-group. These results are the first experimental evidence for RAR alteration (specifically for MESOR and amplitude parameters) in breast cancer survivors at five years after the primary breast cancer diagnosis. Conversely, acrophase is similar for both groups, suggesting that a previously reported delayed acrophase may occur only in the early stages of the clinical pathway.[2] Moreover, since the rhythm (T = 24 h) is statistically significant in all subjects, we can affirm that RAR in breast cancer survivors is not de-synchronized.

The deterioration in RAR has already been reported from breast cancer diagnosis up to one year after chemotherapy,[2–7] but our data show that RAR alterations persist even after five years since the primary diagnosis and in patients free of disease. The progressive reduction of 24 h activity levels in breast cancer women is likely to lead to RAR alterations, which are maintained for several years after primary diagnosis. We could make some speculations about the persistent RAR alteration in breast cancer survivors. Some disease-specific neuropsychological factors could alter RAR; for example, breast cancer diagnosis and related treatments are associated with increased psychological distress and risk of depression and diminished quality of life and physical activity levels.[8–13] Concerning physical activity, consistent with our results that show a lowered MESOR and amplitude compared to healthy subjects, Emery and colleagues (2009) report a gradual decrease of the physical activity levels in breast cancer survivors, even though they assess physical activity with a self-report questionnaire.[14]

In this context, the role of physical activity in the long-term monitoring of breast cancer patients is becoming increasingly important. Studies have highlighted the protective effect of physical activity in several aspects of breast cancer survivors, including reduced risk breast cancer-related and all-cause mortality.[15–19] Two meta-analyses, including randomized trials testing the effect of supervised

physical activity interventions during adjuvant therapy for breast cancer, conclude that physical activity benefits fatigue and quality of life.[20,21] Regarding the last point, Cheng and colleagues (2017) also report the positive effect of physical activity on quality of life after home-based multidimensional programs in women within ten years of completion of surgery or adjuvant cancer therapy for breast cancer.[22]

Notwithstanding the importance of these conclusions, to date, no study has used the rhythmometric RAR analysis to assess the physical activity levels in BC survivors. Moreover, the relationship between RAR and breast cancer is limited to the time at diagnosis. This, in addition to our results, suggests the rationale for planning physical activity interventions to improve RAR in breast cancer survivors, which is a marker of daily spontaneous activity. Indeed, due to the consideration that the two groups had similar jobs (i.e., teaching, office, or housework), the differences in MESOR and amplitude and, generally, in RAR synchronization may be mainly related to breast cancer survivors' daily activity.

It could be supposable that differences in MESOR and amplitude between breast cancer survivors and healthy controls might be related to disparities in sleep quality. In order to be sure that RAR differences are mainly due to daily activities, we also assessed the sleep parameters (sleep start and offset, sleep duration, sleep latency, fragmentation index, immobile time, and sleep efficiency) for all participants. Since no differences are significant in all parameters between the two groups, we can assume that sleep is not an influent factor in decreasing MESOR and amplitude in breast cancer survivors, whose RAR decrement could be related only to activity level.

The present study shows strengths and limitations. The strengths are represented by the methodology, i.e., the measurement of RAR by actigraphy and the population, i.e., breast cancer survivor women at five years after the primary diagnosis. However, the sample size is small; there is no

longitudinal evaluation of RAR parameters, which could have allowed us to evaluate changes in RAR over time.

Conclusions

Our study may produce new findings with a novel potential tool in managing breast cancer survivors: physical activity.

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

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IF: 2.877; Rank: Q2

Aim of the study

Since the cosinor analysis left unexplained variance of the data, the present study aims to increment the previous analysis in breast cancer survivors at five years since the diagnosis by implementing the analysis with the non-parametric parameters of the cosinor analysis. As a consequence, the present study detects whether breast cancer survivors and healthy subjects show differences in Mean (M), Variance (VAR), Interdaily Stability (IS), Intradaily Variability (IV), nocturnal activity (L5),

and daily activity (M10).[1–3] It could be assumed that an additional approach could bring new information on RAR and sleep in these women.

Methods

Since the sample is the same as the previous work, I reported the description of the participants and study design similar to those of the previous study.

Participants

The study sample was composed of 28 women: 15 BC survivors at five years after primary diagnosis (BC-group) and 13 healthy controls (Ctrl-group). The two groups were matched for age and BMI. The women showing the following inclusion criteria were recruited at *IRCCS Istituto Nazionale dei Tumori di Milano*: for the BC-group (i) mastectomy or conservative surgery for any type of primary invasive breast cancer and within the previous five years, (ii) no history or objective evidence of recurrent ipsilateral or contralateral breast cancer, (iii) not scheduled for and not currently undergoing chemotherapy, (iv) no other cancer diagnosis within the last ten years, (v) age 35–60 years at recruitment, (vi) BMI <30 Kg/m2; for the Ctrl-group (i) no history or objective evidence of breast cancer diagnosis, (ii) no other cancer diagnosis or pathology, (iii) age 35–60 years at recruitment, (iv) BMI <30 Kg/m2.

The study was carried out in accordance with the tenets of the 1964 Declaration of Helsinki and approved by the Ethical Review Board of the Istituto Nazionale dei Tumori di Milano. The guidelines required by the journal (*Chronobiology International*) were accomplished either for the study protocol or during the procedures.[1]

Study design

All the 28 women, during the medical visit, were instructed to undergo continuous 7-day actigraphic monitoring to detect RAR. During the actigraph monitoring, the actigraph (MotionWatch 8®, CamNtech, Cambridge, UK) was worn on the non-dominant wrist and removed when swimming or bathing. Additionally, subjects filled in a diary with their bed and get up time, hours of naps and without wearing the actigraph (on average, once a day for 30 minutes; period not included in the data analysis) and the number of nocturnal awakenings. The actigraphy monitoring started at the first clinical meeting. During the actigraphic monitoring, subjects performed their usual daily activities, not inviting them to increase their physical activity.

Analysis of the non-parametric parameters

The analyzed non-parametric indices are: M and VAR (the two major descriptors of a time series); IS (ranging between 0 = lack of synchronization and 1 = perfect synchronization); IV (ranging between 0 = less fragmented rhythm and 2 = more fragmented rhythm); L5, and M10.

Statistical analysis

The non-parametric parameters are reported as mean ± SD with 95% CI and expressed in activity counts (a.c.). The normal distribution of each parameter was verified using Shapiro-Wilk's test. To compare data between the two groups and depending on the normality or not of the data distribution, the unpaired Student t-test or Mann-Whitney U test were used. Pearson's Correlation Coefficient (*r*) investigated the association between non-parametric and parametric parameters of RAR.

A stepwise binary logistic regression was run to fulfil the aim of identifying the more suitable characteristics of RAR to assess the change in RAR in individuals with breast cancer. BC-group (1)

and Ctrl-group (0) were the dependent variables, whereas MESOR, Amplitude, M10, L5, M, and VAR were set as the independent variables.

The statistical analysis was made using *R statistics* software (Version 3.6.0). Statistical significance was set at $p < .05$.

Results

Table 1 shows the non-parametric indices of the two groups. BC-group shows significantly lower M, VAR, L5 and M10 than Ctrl-group, while IV is significantly higher than Ctrl-group.

Table 1 Non-parametric analysis of RAR in BC survivors and healthy subjects.

Group	Mean (a.c.) (mean ± SD)	Variance (a.c.) (mean ± SD)	IS (a.u.) (mean ± SD)	IV (a.u.) (mean ± SD)	L5 (a.c.) (mean ± SD)	M10 (a.c.) (mean ± SD)
BC-group n = 15	189 ± 35.7§	81358 ± 24954•	0.61 ± 0.09	0.86 ± 0.18*	11.27 ± 5.00*	326.82 ± 58.14#
Crtl-group n = 13	268 ± 81.8§	134428 ± 100499•	0.56 ± 0.10	0.65 ± 0.14*	34.41 ± 17.63*	428.07 ± 117.74#

IS: Interdaily Stability. IV: Intradaily Variability. L5: the least active 5-h period obtained from the average 24-h profile. M10: the most active 10 hr period obtained from the average 24-h profile. a.c.: activity counts; a.u.: arbitrary units.
§ $p < .001$ for Mean (Student's t-test), • $p < .05$ for Variance (Student's t-test), * $p < .01$ for IV and L5 (Student's t-test), # $p < .0001$ for M10 (Mann–Whitney U).

The significant correlations in the total sample between non-parametric indices (M, VAR, L5, or M10) and parametric indices (MESOR or Amplitude) are shown in Figure 1. Significant correlations are reported between Amplitude and MESOR ($p < .0001$), L5 and MESOR ($p < .0001$), L5 and Amplitude ($p < .02$), M10 and MESOR ($p < .0001$), M10 and Amplitude ($p < .0001$), M10 and L5 ($p < .002$), M and MESOR ($p < .0001$), M and Amplitude ($p < .0001$), M and L5 ($p < .0001$), M and M10

(p < .0001), VAR and MESOR (p < .0001), VAR and Amplitude (p < .0001), VAR and L5 (p < .001), VAR and M10 (p < .0001) and VAR and M (p < .0001).

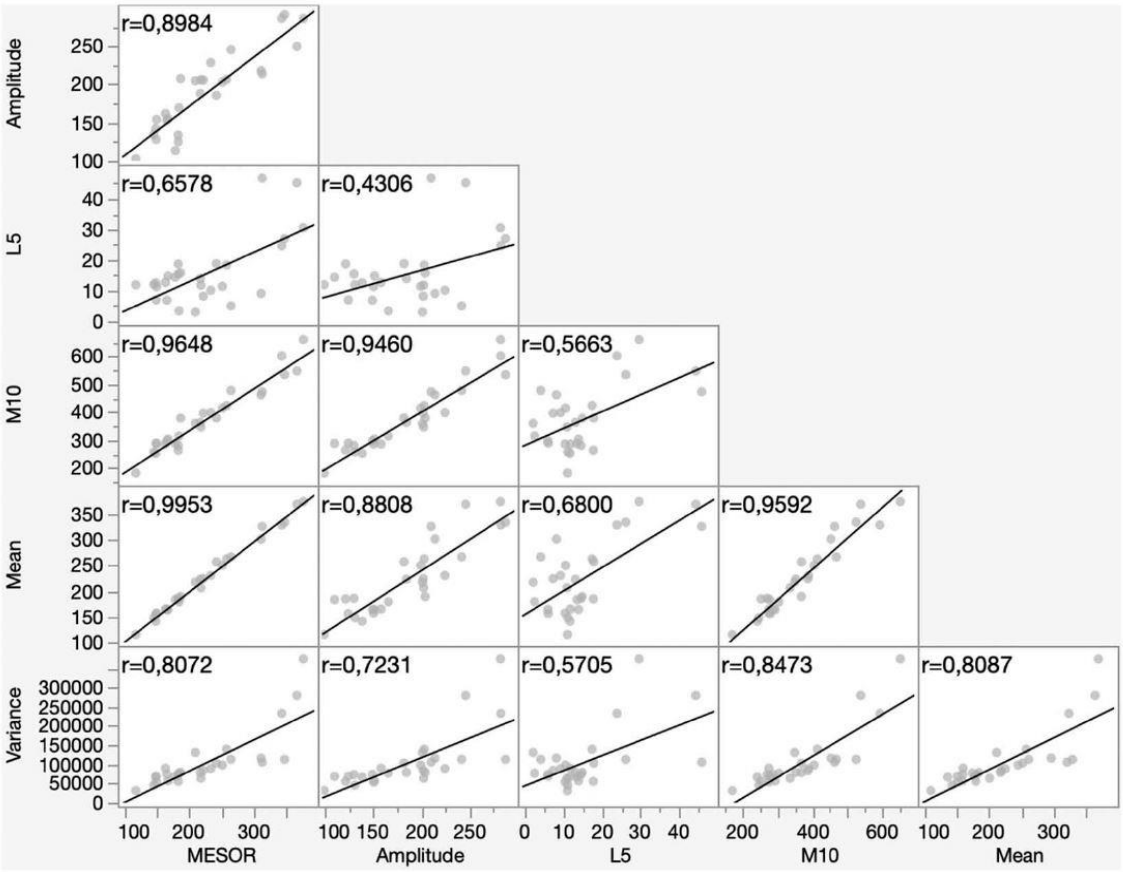

Figure 3 *Correlation analysis in the total sample between non-parametric indices (Mean, Variance, L5 or M10) and parametric indices (MESOR or Amplitude).*

Finally, the binary logistic regression for each independent variable shows statistical significance for MESOR (p < .019), Amplitude (p < .038), M10 (p < .024), L5 (p < .042) and M (p < .012), but not for VAR. Then, the analysis ends with a stepwise binary logistic regression analysis (dependent variables: BC-group and Ctrl-group; independent variables: MESOR, Amplitude, M10, L5, M, and VAR) that finds MESOR as the most sensitive variable (p < .012).

Discussion

The present study aims to deepen the RAR analysis by using non-parametric indices in breast cancer survivors and healthy control groups. The results, particularly IV, provide new and interesting information about breast cancer survivors, which show a higher IV value, thus, a more fragmented RAR over the 24 hours activity. A possible explanation of this increased fragmentation could be associated with reductions or alterations of the diurnal activity level, i.e., more inactive periods, as supported by lower M10 values. Previous studies report that reduced physical activity in breast cancer survivors could be traced to incremented psychological distress and risk of depression, which together can diminish the quality of life.[4–6]

Referring to the least active hours and keeping in mind that the previous analysis of sleep behaviour revealed no significant differences between the BC- and Ctrl-group, the L5 value is better in breast cancer survivors than controls, showing less movement-activity level during the night-time. We advance the hypothesis that the cancerous pathology does not deteriorate sleep quality during the last five years compared to unaffected and well-matched by age and sex subjects.

The present study results corroborate the previous findings showing reduced MESOR and Amplitude in breast cancer survivors compared to healthy controls. We may speculate that, particularly during the daytime, breast cancer survivors have an overall lower 24 h level of activity. It could be possible to conclude that M and VAR reflect the previously highlighted differences in MESOR and Amplitude by parametric analysis.[7]

Altogether, these data support the hypothesis that the progressive reduction in daily activity levels provoked by breast cancer disease is present also after several years from the diagnosis and not only in the first stages.[8–11] In support of our hypothesis, previous evidence supports the association between lower M10 values and motor inactivity or daily activity reduction.[12] The improvements of daytime activity level (i.e., M10) are correlated to a better quality of life (Goncalves et al. 2015), and this

association could also be translated in breast cancer survivors, as previously reported by several authors.[13–15] Furthermore, increased daytime activity levels are associated with better markers of breast cancer risk, while their decrement is considered a factor able to negatively influencing the breast cancer prognosis.[16–19]

Based on the present and previous findings, we suggest the need for constant assessment of RAR in breast cancer patients at all stages of the pathology, including several years after the end of treatments. This represents a valuable strategy aimed at developing new approaches for health protection and maintenance, for example, structured and tailored physical activity programs, which can improve circadian activity level and, consequently, raise the quality of life in breast cancer survivors.

To reach this goal, it should keep in mind that the MESOR, the most appropriate parameter to assess changes in RAR in subjects with breast cancer, could not be able to identify small activity fluctuations. Thus, as happened in our sample, the non-parametric analysis may be considered more descriptive of the situation and able to detect either daytime or night-time activities over the 24 hours.

In this context, future studies must consider administering a more detailed daily diary to collect information as accurately as possible, making possible a richer comparison between subjects.

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8. Rest-activity circadian rhythm, physical activity and sleep behaviour in women carrying BRCA1/2 mutations

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Physical activity and sleep behaviour in women carrying BRCA1/2 mutations

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Aim of the study

Since the breast cancer and *BRCA1/2* mutation diagnosis could lead to RAR disruption and sleep deficiency and data referring to *BRCA1/2* women are still missing, we want to assess these variables in a cohort of Italian *BRCA1/2* carrier women.

Moreover, taking into consideration the benefits of physical activity on modifiable sporadic breast cancer risk factors and sleep, we perform a cross-sectional analysis to explore the relationship between sleep quality and physical activity in women *BRCA1/2* mutations.

Methods

Participants

Our cross-sectional study analysed a sample of 68 *BRCA1/2* mutation carriers enrolled in the Italian multicentre, prospective, randomized controlled trial, registered at www.clinicaltrial.gov (reference number: NCT03066856) at the *IRCCS Istituto Nazionale dei Tumori of Milan* (Italy). This paper analysed only participants recruited into the trial between October 2018 and June 2019 because they also had complete sleep and physical activity data.

Extensive descriptions of the trial design and main results have already been widely described and reported.[1–6] In brief, the trial aims to test if a dietary intervention based on the Mediterranean diet with a moderate protein restriction significantly reduces potential modulators of *BRCA1/2* penetrance such as body weight, IGF-I, insulin, and main factors of Metabolic Syndrome.

The inclusion criteria were: (i) Diagnosis of *BRCA1/2* mutations; (ii) age between 18-70 years; (iii) with or without a previous diagnosis of breast or ovarian cancer; (iv) without clinical evidence of metastases. Women who underwent preventive, bilateral prophylactic mastectomy without previous breast or ovarian cancer diagnosis were excluded from the study.

The Ethics Committee of Fondazione IRCCS Istituto Nazionale dei Tumori di Milano approved the study (approval number: INT106/13).

Study design

The 68 women included in the present analyses were asked to:

- fill in a questionnaire on their medical history, including questions on principal female cancers risk factors (e.g., menstrual and reproductive history and health behaviours);
- attend a clinic examination for anthropometric measurements (weight and height – wearing only light clothes, and waist and hip circumferences – using a professional measuring tape) and waist to hip ratio and body mass index (BMI, weight (kg) / height (m2)) calculation. Blood pressure was measured using an electronic sphygmomanometer;
- undergo a body composition evaluation (fat mass and fat-free mass) using impedance balance. Women were fasted and did not drink during the four previous hours and did not practice physical activity within 24 hours;
- donate a 20 mL blood sample for metabolic (serum IGF-1 and insulin, plasma glucose, total cholesterol, HDL, LDL, and triglycerides) and hormonal assays. The blood sample was collected after 90 minutes of a standard meal;
- undergo continuous 7-day actigraphic monitoring to detect RAR. During the actigraph monitoring, the actigraph (MotionWatch 8®, CamNtech, Cambridge, UK) was worn on the non-dominant wrist and removed when swimming or bathing. Additionally, subjects filled in a diary with their bed and get up time, hours of naps and without wearing the actigraph (on average, once a day for 30 minutes; period not included in the data analysis) and the number of nocturnal awakenings. The actigraphy monitoring started at the first clinical meeting. During the actigraphic monitoring, subjects performed their usual daily activities, not inviting them to increase their physical activity.

- fill in the PSQI to assess sleep quality and habits during the previous 30 days. This is a retrospective self-report questionnaire, based on 19 items that evaluate seven sleep components: (i) perceived sleep quality, (ii) sleep latency, (iii) sleep duration, (iv) sleep efficacy, (v) sleep disturbances, (vi) use of sleep medications, (vii) day-time dysfunctions. The score for each question ranges between 0 (no problem at all) and 3 (several problems). The sum of the seven components returns a final score from 0 to 21, with lower scores indicating a better sleep quality. The cut-off value of 5 divides participants into *good sleepers* (0-5) and *bad sleepers* (6-21). In addition, the numbers of hours spent in bed and sleeping, and the sleep efficiency can be obtained from the questionnaire;[7,8]
- fill in the GSL-TPAQ to assess how much physical activity the women had conducted during the last seven days. Three questions investigate the frequency (number of times on a week) for three physical activity intensities (heavy, moderate and light), which are associated with specific *Metabolic Equivalent of Task* (*MET*) amount. The final score is obtained by multiplying the number of times on a week*METs of each intensity and summing the three results together (MET/week). If the final score is < 24, the subject is classified as *inactive*; in the opposite case, the person is described as *active*.[9]

Statistical analysis

The statistical analyses were carried out using R Statistics (version 3.6.0, R Development Core Team, 2011), STATA 14 (StataCorp–College Station, TX, USA) and IBM Statistical Package for the Social Sciences - SPSS Statistics (version 27 IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp). Descriptive data of the sample are shown using mean (± SD), frequencies and percentage.

The RAR parameters were assessed using the single cosinor analysis; after that, rhythmometric parametric parameters of *affected BRCA1/2* women and *unaffected BRCA1/2* women were compared using the Hotelling T2 test. The Hotelling T2 test is a generalization in the multivariate field of Student's t-test and is based on the hypothesis that the distance between the two samples' mean vectors is null. To compare non-parametric parameters (IS, IV, L5, M10) between the two groups and depending on the normality of the data distribution, the unpaired Student t-test or Mann-Whitney U test was used.

Actigraphic sleep assessment included Time in Bed, Assumed sleep, Actual wake time, Actual sleep time, Sleep efficiency, Sleep latency, Immobile minutes, Moving Minutes and Fragmentation index. The parameters were compared between *affected* and *unaffected BRCA1/2* carrier women.

Regarding the anthropometric, body composition, metabolic data, and questionnaire results, we compared the data of affected and unaffected women before performing the statistical analysis. Based on the normal distribution results (Shapiro Wilk test), a t-test or Mann-Whitney U-test was used. Since the two groups showed no statistically significant differences, we decided to analyse the sample altogether.

Based on BMI categorization, the sample was divided into normal weight (BMI <25 kg/m2) and overweight (BMI >25 kg/m2). Related to GSL-TPAQ, we considered both the total score and the results for each intensity (heavy, moderate, light) separately in all the analyses.

Using the ANCOVA analysis (adjusted by age and BMI categorization), we compared all the continuous variables between *good* and *bad sleepers* (based on the PSQI results) and *active* and *inactive* subjects (based on GSL-TPAQ results). Then, we conducted a correlation analysis (Spearman coefficient) among all the continuous variables.

Finally, to satisfy the principal aim of the study, we built a binomial regression model including age at recruitment (in tertiles) and BMI (in tertiles) as covariates to evaluate the prevalence ratios (PRs)

and 95% confidence intervals (CI) of being a good sleeper by increasing the METs/week. In this view, all the physical activity intensities were considered, and the sample was divided based on the median value for each intensity.

A p-value of <.05 was taken as significant; CI were set at 95%.

Results

63 women with *BRCA 1/2* mutations [mean age, 47.6 ± 12.4 years; n = 42 (66.7%) affected, n = 21 (33.3%) unaffected] were included in the present investigation. 27 women (19 *affected* and 8 *unaffected*) completed the actigraphic monitoring. Women had a PSQI score of 7 ± 3.6, a GSL-TPAQ score of 22.8 ± 18.3 and a BMI of 24.4 ± 4.2 kg/m2.

RAR and sleep parameter evaluation

The rhythmometric analysis on RAR parametric (Figure 1) and non-parametric parameters does not show any differences between the *affected* and *unaffected BRCA1/2* carrier women (Table 1).

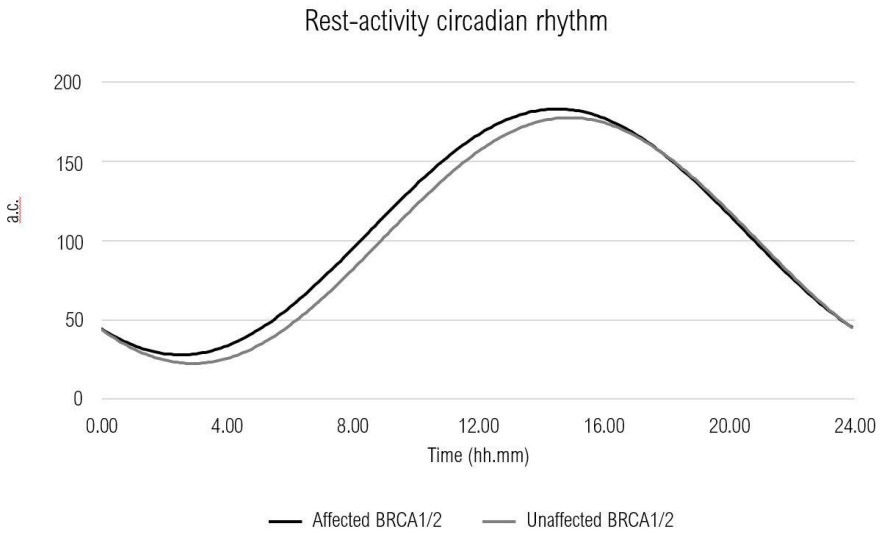

Figure 1 *Rest-activity circadian rhythms: data collected by actigraphy in affected BRCA1/2 (black line) and unaffected BRCA1/2 group (grey line).*

Table 1 Parametric and non-parametric parameters of RAR stratified for presence (affected BRCA/2) and absence (unaffected BRCA1/2) of a previous cancer diagnosis.

Rest-activity circadian rhythm parameters			
	Affected *BRCA1/2* (n=8) Mean ± SD	Unaffected *BRCA1/2* (n=19) Mean ± SD	*p*
MESOR (a.c.)	250.3 ± 40.3	228.5 ± 46.3	n.s.
Amplitude (a.c.)	176.7 ± 42.1	176.1 ± 28.0	n.s.
Acrophase (hh:mm)	14:10 (13:16-15:01)	14:14 (14:07-15:05)	n.s.
IS (a.u.)	0.52 ± 0.2	0.56 ± 0.1	n.s.
IV (a.u.)	0.80 ± 0.2	0.86 ± 0.1	n.s.
L5 (a.u.)	16.9 ± 15.3	33.1 ± 56.4	n.s.
M10 (a.u.)	356.5 ± 80.4	387.5 ± 133.8	n.s.

MESOR (activity counts): Midline Estimating Statistic of Rhythm. Amplitude (activity counts): half the difference between the highest and the lowest points of the cosine function best fitting the data. Acrophase (hr:min) indicates when the highest values fall. IS: Interdaily Stability. IV: Intradaily Variability. L5: the least active 5-h period obtained from the average 24-h profile. M10: the most active 10 hr period obtained from the average 24-h profile. a.c.: activity counts; a.u.: arbitrary units.

Table 2 Actigraphic sleep parameters referred to the smaller sample (n=27) who underwent the seven-day actigraph monitoring.

Sleep parameters	
	Mean ± SD
Time in Bed (hh/mm)	7:55 ± 0:56
Assumed sleep (hh/mm)	7:24 ± 0:53
Actual Wake Time (hh/mm)	0:54 ± 0:18
Actual sleep time (hh/mm)	6:29 ± 1:00
Sleep efficency (%)	81.8 ± 7.9
Sleep latency (hh/mm)	0:27 ± 0:22
Immobile minutes (n°)	390.0 ± 57.6
Moving Minutes (n°)	54.7 ± 17.6
Fragmentation index	26.0 ± 9.2

The sleep parameters recorded by the actigraph are presented in Table 2. The sample (n=27) slept the 7/8 recommended hours per night (Assumed Sleep value); however, it does not get the 85% of the sleep efficiency that is considered a cut-off point between a good and bad sleep quality.[10,11]

Table 3 shows data and comparison results for the stratification between *affected* and *unaffected BRCA1/2* women.

Table 3 Actigraphic sleep data stratified for presence (affected BRCA1/2) and absence (unaffected BRCA1/2) of a previous cancer diagnosis with related p-values obtained with t- or Wilcoxon-test.

Rest-activity circadian rhythm parameters			
	Affected *BRCA1/2* (n=8) Mean ± SD	Unaffected *BRCA1/2* (n=19) Mean ± SD	*p*
Time in Bed (hh/mm)	7:43 ± 0:47	8:20 ± 1:07	n.s.
Assumed sleep (hh/mm)	7:14 ± 0:47	7:46 ± 1:01	n.s.
Actual Wake Time (hh/mm)	0:56 ± 0:19	0:50 ± 0:17	n.s.
Actual sleep time (hh/mm)	6:17 ± 0:55	6:55 ± 1:04	n.s.
Sleep efficency (%)	81.1 ± 8.7	83.1 ± 6.3	n.s.
Sleep latency (hh/mm)	0:25 ± 0:18	0:31 ± 0:30	n.s.
Immobile minutes (n°)	377.3 ± 50.2	415.6 ± 65.7	n.s.
Moving Minutes (n°)	56.8 ± 17.2	50.4 ± 18.7	n.s.
Fragmentation index	26.5 ± 9.2	25.0 ± 9.8	n.s.

No statistically significant differences emerge from the analysis. However, it is possible to make some consideration: from a visual data inspection, it would appear that the unaffected *BRCA1/2* group present a tendency of longer (Assumed sleep values) and better sleep (Sleep efficiency, Immobile minutes and Fragmentation index values).

Comparison between *good* and *bad* sleepers

Table 4 shows the baseline anthropometric/metabolic characteristics and physical activity levels of the 63 *BRCA1/2* women stratified by *good* (n = 26, 41,3%) and *bad sleepers* (n = 37, 58,7%).

Table 4 Descriptive data in good and bad sleepers.

Anthropometric, metabolic and physical activity parameters		
	Good sleepers (*n*=26) Mean ± SD	*Bad sleepers* (n=37) Mean ± SD
Weight (kg)	61.1 ± 10	64.9 ± 10
BMI (kg/m²)	23.5 ± 4	25.1 ± 4.2
Waist circumference (cm)	71.6 ± 9.2	75.5 ± 11
Hip circumference (cm)	95.8 ± 8.1	98.5 ± 8.4
Waist/Hip ratio	0.74 ± 0.6	0.76 ± 0.7
Systolic pressure (mmHg)	120.5 ± 11	122.4 ± 14.2
Diastolic pressure (mmHg)	82 ± 12.1	82.3 ± 7.2
Insulin (µU/ml)	19 ± 19.7	24.6 ± 25
Glycemia (mg/dL)	95.6 ± 23.7	99 ± 25.1
Total cholesterol (mg/dL)	187.1 ± 28.8	195 ± 35.6
HDL (mg/dL)	64.6 ± 18.8	62 ± 17
Triglycerides (mg/dL)	81 ± 43.8	102.7 ± 43
Fat mass (%)	27.8 ± 8.2	31.7 ± 7
Fat mass (kg)	17.6 ± 8	21.8 ± 10
GSL-TPAQ score	32.2 ± 19.8	16.3 ± 13.9*
GSL-TPAQ heavy intensity	8 ± 11.2	1.9 ± 6°
GSL-TPAQ moderate intensity	14.4 ± 12.1	8 ± 10.1°
GSL-TPAQ light intensity	9.8 ± 8.4	5.8 ± 6.6#

*$p < .001$, °$p < .01$, #$p < .05$.

The two groups do not differ either in anthropometric or metabolic parameters, even though *good sleepers* show a slightly better metabolic/anthropometric condition than *bad sleepers*.

The two groups report a statistically significant difference in the GSL-TPAQ score, with the *good sleepers* more active than the *bad sleepers* ($p < .05$). Furthermore, the physical activity intensity (heavy, moderate, light) is significantly higher in *good sleepers* than *bad* sleepers ($p < .01$; $p < .01$; $p < .05$, respectively).

Comparison between *active* and *inactive* women

Table 5 shows results obtained from the comparison between *active* (n = 22, 34,9%) and *inactive* (n = 41, 65,1%) women referring to the anthropometric, body composition, metabolic, hormonal and sleep parameters.

In general, *active* women show better anthropometric and metabolic parameters compared to *inactive* women. However, only insulin levels are significantly lower in *active* compared to *inactive* women ($p < .05$).

Sleep quality is significantly better in *active* women than *inactive* women, which reach a higher PSQI score (5 ± 3.1 vs 7.8 ± 3.7, respectively; $p < .01$). Even though not significant, *active* women tend to sleep longer and better than *inactive*.

Table 5 *Descriptive data in active and inactive women.*

Anthropometric, metabolic and sleep parameters		
	Active (*n*=22) Mean ± SD	*Inactive* (*n*=41) Mean ± SD
Weight (kg)	60.1 ± 8.6	65 ± 10.5
BMI (kg/m²)	22.9 ± 3.4	25.2 ± 4.4
Waist circumference (cm)	70.4 ± 9.7	75.7 ± 10.4
Hip circumference (cm)	94.1 ± 7	99.2 ± 8.5
Waist/Hip ratio	0.75 ± 0.6	0.76 ± 0.7
Systolic pressure (mmHg)	118.2 ± 14.8	123.5 ± 11.5
Diastolic pressure (mmHg)	79.6 ± 10.9	83.6 ± 8.4
Insulin (µU/ml)	12.4 ± 9.3	27.6 ± 26.2#
Glycemia (mg/dL)	94.3 ± 22.7	99.3 ± 25.3
Total cholesterol (mg/dL)	191.7 ± 32	191.8 ± 33.8
HDL (mg/dL)	67.1 ± 17.5	60.9 ± 17.6
Triglycerides (mg/dL)	77.9 ± 28	102.3 ± 49.2
Fat mass (%)	27.2 ± 7.6	31.7 ± 7.4
Fat mass (kg)	16.8 ± 6.8	21.8 ± 10.2
PSQI score	5 ± 3.1	7.8 ± 3.7°
Hours spent in bed (hr:mm)	08:04 ± 00:37	07:52 ± 01:16
Slept hours (hr:mm)	06:47 ± 00:56	06:21 ± 01:17
Sleep efficiency (%)	84.2 ± 10.7	80.7 ± 14.8

°p <.01, #p <.05.

Correlation analysis

The PSQI score correlates with triglycerides levels (r_s = 0.29, p <. 05).

GSL-TPAQ score is inversely correlated with weight (r_s = -0.27, p < .05), BMI (r_s = -0.32, p < .05), waist circumference (r_s = -0.27, p < .05), hip circumference (r_s = -0.31, p < .01), insulin (r_s = -0.35, p < .01), triglycerides levels (r_s = -0.28, p < .05), fat mass in % (r_s = -0.36, p < .01), fat mass in kg (r_s = -0.31, p < .05), and PSQI score (r_s = -0.41, p < .01).

Similarly, heavy-intensity physical activity level is inversely correlated with weight (r_s = -0.33, p < .01), BMI (r_s = -0.32, p < .01), waist circumference (r_s = -0.41, p <.01), hip circumference (r_s = -0.34, p < .01), waist/hip ratio (r_s = -0.32, p < .05), systolic (r_s = -0.31, p <.01) and diastolic blood pressure (r_s = -0.43, p < .01), triglycerides levels (r_s = -0.25, p < .05), fat mass in % (r_s = -0.47, p < .01) and fat mass in kg (r_s = -0.36, p < .01). Even though not significant, heavy-intensity physical activity levels show a tendency of being inversely correlated with with PSQI score (r_s = -0.23, p = .07)

Moderate-intensity physical activity level is inversely correlated with PSQI score (r_s = -0.34, p < .01). Light-intensity physical activity level is inversely correlated with waist circumference (r_s = -0.28, p < .05), but shows no significant correlation with PSQI score (r_s = -0.15, p = .23).

Binomial regression analysis

The Prevalence Ratio (PR) of being a *good sleeper* by increasing the METs/week is shown in Table 6.

Table 6 Evaluation of the Prevalence Ratio (PR) of being a good sleeper.

	PR to be a *good sleeper* PR (95% CI)
GSL-TPAQ score < 16	1
GSL-TPAQ score ≥ 16	2.20 (1.20-4.02 - 9.11) (p < .01) °
GLS-TPAQ heavy intensity < 9	1
GLS-TPAQ heavy intensity ≥ 9	2.01 (1.05 – 3.73) (p < .03) °
GSL-TPAQ moderate intensity <10	1
GSL-TPAQ moderate intensity ≥10	2.10 (1.29 - 3.39) (p < .01) °
GSL-TPAQ light intensity < 6	1
GSL-TPAQ light intensity ≥ 6	1.38 (0.78- 2.39) (p = .27)

Prevalence Ratio (PR, ± 95% CI) adjusted for age and BMI of being a good sleeper. °p <.01.

Practising more than 16 METs/week of physical activity, 9 METs/week of heavy-intensity physical activity (corresponding to 1 session/week of heavy physical activity), and 10 METs/week of moderate-intensity physical activity (corresponding to 2 sessions/week of moderate physical activity), significantly increases of 2.20, 2.01, and 2.10 respectively the prevalence of being a *good sleeper* ($p < .01$, $p < .03$, $p < .01$, respectively). Practising more than 6 METs/week of light-intensity physical activity (corresponding to 2 sessions/week of light physical activity) does not significantly increase the prevalence of being a good sleeper ($p = .27$).

The last analysis is a regression analysis to evaluate the PR of being a *good sleeper* by increasing the physical activity practice or combining the physical activity intensities (Table 7). Compared to women who do not practice any physical activity, the PR of being a *good sleeper* rises in concomitance with increased physical activity.

Table 7 Evaluation of the Prevalence Ratio (PR) of being a good sleeper by increasing physical activity level.

	PR to be a *good sleeper* PR (95% CI)
None physical activity	1
GSL-TPAQ light intensity ≥ 3	1.27 (0.37 – 4.35)
GSL-TPAQ moderate intensity ≥ 5 or GSL-TPAQ heavy intensity ≥ 9	2.13 (0.84- 5.41)
2 or more combined physical activity (light + moderate or light + intense or moderate + intense)	3.06 (1.34 - 7.01)

Prevalence Ratio (PR, ± 95% CI) adjusted for age and BMI of being a good sleeper. p for trend < .01.

Discussion

This cross-sectional analysis of 63 women sheds light on a possible association between physical activity and sleep in *BRCA1/2* mutation carriers. To our knowledge, this is the first study assessing the potential and reciprocal influences of sleep and physical activity in this population.

Good sleepers BRCA1/2 women show higher GLS-TPAQ scores, suggesting that they are more active than *bad sleepers*. The probability of being a *good sleeper* is increased by 2.20 folds when the physical activity level is higher than 16 METs/week. Furthermore, the prevalence of being a *good sleeper* is significantly enhanced with the increment of either the intensity or sessions/week of physical activity. Indeed, the prevalence of being a *good sleeper* is significantly strengthened when women are habitually engaged in heavy- and moderate-intensity physical activity or combine different types of physical activity.

This direct association between physical activity and sleep and the probable protective role of physical activity has already been advanced in previous studies involving sporadic breast cancer patients at different stages of breast cancer pathology. In these studies, the improvement of sleep quality happens in concomitance with the increase of physical activity.[12–16]

Breast cancer diagnosis and treatments usually reflect on sleep as a long-term and side-effect symptom, and patient's quality of life usually decreases together with sleep.[17] Thus, upgrading sleep quality in breast cancer patients is essential for their quality of life and represents a challenging point for researchers.[18,19] Bad sleep quality could be provoked by distress and anxiety of the consequences of the diagnosis; pain and hot flashes consequent to surgery or treatments; fatigue and not being sufficiently active.[19,20] In line with these theories, *inactive* women of the present study sleep significantly worse than *active* ones.

In addition to the quality of life, sleep is associated with both breast cancer risk and survival. Hurley and colleagues (2020) recently concluded with a significant and positive correlation between

sleep deficiency and breast cancer risk.[21] In the same way, Phipps and colleagues (2016) state that women sleeping <6 hours before the diagnosis have a significantly higher risk of poorer cause-specific survival compared to normal sleepers (7-8 hours).[22] Still, Palesh and colleagues (2007), on a cohort of women with advanced breast cancer, describe the actigraphy-based sleep efficiency as a helpful predictor of six-year overall survival (HR = 0.94, 95% CI 0.91–0.97).[23] Unfortunately, no data are available about the effect of physical activity on sleep parameters in *BRCA1/2* mutation carriers.

The evidence stemming from several studies makes supposing sleep as one of the environmental factors involved in the modulation of *BRCA1/2* penetrance. Indeed, sleep deficiency is recognized to increase inflammation, impair the immune response, diminish melatonin secretion (which, in turn, enhance oestrogen levels), alter insulin/IGF-I signalling, carbohydrate tolerance, and leptin and cortisol levels, thus modifying insulin sensitivity and appetite.[24–30] All these factors are considered potential modulators of penetrance in women with *BRCA1/2* mutations and, by improving sleep, it could be possible to diminish their penetrance.

This cross-sectional analysis also suggests that, as previously seen in sporadic breast cancer, physical activity may be a valuable means for enhancing sleep quality in *BRCA1/2* women. Recently, in the breast cancer field, modifying daily habits favouring a more active lifestyle has been among the leading research goal for researchers. Notwithstanding the recent promising results, the mechanisms of this linkage are not yet fully understood; some supposition could be possible by observing reactions in healthy subjects. Indeed, physical activity could benefit sleep through body temperature cool down, energy conservation, decrement in anxiety, stress, endocrine function, obesity, and immune-inflammation response.[31–35]

Beyond sleep improvements, body weight, BMI and other anthropometric parameters, insulin and IGF-1 levels, adipokines balance and sexual hormones secretion in breast cancer women could benefit from a regular and constant physical activity practice.[36] Accordingly, weight, BMI, waist

circumference, hip circumference, fat mass and insulin levels of the present study sample inversely correlate (thus decrease) with either total physical activity or heavy-intensity physical activity levels, suggesting a protective association of physical activity and anthropometric parameters also in *BRCA1/2* women.

Despite this crucial first evidence, *BRCA1/2* carriers struggle to adopt a healthy lifestyle and adhere to the American Cancer Society recommendations (BMI ≤ 25kg/m2, one drink per day, 150 mins/day of light physical activity or 75 mins/day of moderate-to-vigorous physical activity).[37] In fact, *BRCA1/2* women of the present study are mainly *inactive*. However, findings from the diet trial revealed that women carrying *BRCA1/2* mutations could substantially improve their dietary habits, with significant modulation in IGF-I, body weight, and markers of insulin resistance.[1–4,6] Following these results, the *LIBRE-1* pilot study suggests the feasibility of a regular physical activity intervention protocol in *BRCA1/2* mutation carriers.[38] More physical activity interventions are necessary for these women to reduce their breast and/or ovarian cancer risk and improve sleep and other aspects of their quality of life.

The results of the present analysis should be seen given its limitations and strengths. Limitations may be attributable to the limited number of participants, the lack of an objective assessment, the cross-sectional design, and the majority of affected women, which could have determined the low baseline physical activity levels. The strength of the present study is the originality, because sleep data in relation to physical activity are somehow unexplored in this population.

Give an interpretation of the association between sleep, and physical activity is beyond the aim of the present study. This paper intends to describe the sleep and physical activity data of the *BRCA1/2* carriers who met our dietary intervention trial. However, these preliminary findings suggest the usefulness of specific healthier and more active lifestyle recommendations. Future investigations involving *BRCA1/2* women in structured physical activity are necessary.

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9. Conclusions and future perspectives

The two first studies on sporadic breast cancer underline how long-cancer survivors seem to be primarily affected in the daily activity levels, whose decrement could affect RAR rhythmicity. Indeed, a decreased MESOR, M10 and, generally, more inactive periods could favour the RAR variability during the 24 hours. Breast cancer survivors are not sufficiently active even after the end of the therapies, and they need interventions, advice, and coaching to improve their activity levels during the day. It could be supposed that if women practice more physical activity or succeed in being more active during the day, they could improve the synchronization of their RAR and avoid depressive, anxiety, and stressful situations. Furthermore, given the potential role of physical activity in cancer tertiary prevention, more active breast cancer survivors could find an ally to avoid cancer recurrence.

The analysis on women carrying *BRCA1/2* mutations lets us conclude that more physical activity is also needed and useful in this population to help it, firstly, improve their sleep. Sleep is a fundamental aspect of human wellbeing and quality of life. We might assume that, by improving sleep, we could help these women improve their quality of life and cope with the obstacles posed by the mutation diagnosis.

Current preventive options for *BRCA1/2* mutation carriers include *ad hoc* screening programs, risk-reducing surgery (bilateral prophylactic mastectomy and Salpingo-oophorectomy), while non-surgical options for prevention are not yet firmly established. Data regarding the relations between

"lifestyle" exposures and *BRCA1/2*-associated cancers come from the retrospective case-control analyses in mutation carriers, and the results are almost always distorted by bias in the clinical setting, as is survival. Validation of these findings in well-designed large prospective studies complemented by mechanistic analyses is now essential and urgent.

Our future plans are to involve all these women in a physical activity intervention, a project that the pandemic situation has highly hampered. However, our principal intention is to produce tangible changes in the daily habits of these women. Thus, we do not want only to prove that physical activity could help breast cancer women; we want to make physical activity a constant constitutor of the daily routine in this population. In this view, women will need assistance to become aware of the importance of physical activity and coaching and support strategies will be put in place to change the daily routine.

Physical activity should become an all life-long characteristic, and future studies aim to instruct breast cancer women to remain active even after the end of the study protocol.

The evaluation along time of the clinical, metabolic, hormonal, dietary and physical activity data, and their change with the lifestyle interventions, will allow studying the "environmental" modulators of *BRCA1/2* penetrance and their impact on the progression of the disease.

10. Other articles

In this chapter, the published and the submitted articles are briefly reported. During my PhD, I actively worked and collaborated in their data collection, data analysis, and writing.

The articles are divided into four sections based on the topic of the articles. They are presented with a background, methods, results and discussion paragraph and briefly resume the published article. Furthermore, are reported the authors and publication information.

10.1 Studies in collaboration with the Technische Universität – Dortmund

Sleep problems and their interaction with physical activity and fatigue in hematological cancer patients during onset of high dose chemotherapy

Castelli L., Elter T., Wolf F., Watson M., Schenk A., Steindorf K., Bloch W., Hallek M., Joisten N., Zimmer P. (2021). ***Supportive Care in Cancer***. *Doi: 10.1007/s00520-021-06377-5.*

IF: 3.603; Rank Q1

Background Sleep problems reported by haematological cancer patients have received less attention than solid cancers. Sleep problems are described as one of the five symptoms affecting these patients and linked to higher cancer-related fatigue (C-RF) levels. C-RF also prevent haematological cancer patients from being physically active. Indeed, C-RF and physical activity (PA) act in a vicious circle in these subjects. The present study assessed the differences between good and bad sleepers and investigated the relationship between sleep, PA, and fatigue.

Methods 58 newly diagnosed haematological cancer patients (47.1 ± 15.4 yrs; 51.7% males) completed questionnaires assessing sleep (PSQI), PA (visual analogue scale), C-RF (MFI-20), anxiety, depression (HADS), and quality of life (EORTC QLQ-C30) within two weeks from starting treatment. ANCOVA analysis investigates the differences between good and bad sleepers, while Mediation analysis describes the relation between sleep, PA, and fatigue.

Results Haematological cancer patients resulted sleep-impaired than a healthy German sample. The classification as good (ca 25%) or bad sleepers (ca 75%) showed less frequent PA ($p = .04$), higher fatigue ($p = .032$), anxiety ($p = .003$), depression ($p = .011$) and pain ($p = .011$) in bad sleepers. The mediation analysis revealed significant indirect effects of sleep on fatigue through PA habits

Conclusions This study highlighted the combined action of sleep problems and PA on fatigue during the onset of induction chemotherapy. These two parameters could represent meaningful intervention targets to improve a patient's status during chemotherapy.

Interactions between sleep, fatigue and cardiovascular performance in healthy young adults

Castelli L., Walzik D., Joisten N, Roveda E., Montaruli A., Watson M., Oberste M., Zimmer P.

Submitted to: *International Journal of Sports Medicine*

Background Physical performance could be negatively affected by sleep deficiency (sleep restriction or bad sleep quality) and fatigue. Previous studies mainly focused on males and athletic samples. The present study assessed the role of sleep quality, fatigue, and motivation on cardiovascular performance (VO_{2max}) in a mixed sample of active young subjects.

Methods 96 subjects (males 54.2%; 21.5 ± 2.9 yrs), recruited at the German Sport University of Cologne, completed an incremental test at the bicycle ergometer, the Pittsburgh Sleep Quality Index and two visual analogue scales to assess sleep, fatigue, and motivation, respectively. Separately for male and female samples and based on VO_{2max} categorization, one-way ANOVA compared mean values for sleep, fatigue, and motivation, while regression analysis defined the predictors of VO_{2max}.

Results In the male sample, the VO_{2max} fair-average category showed worse sleep quality and the lowest motivation than the VO_{2max} excellent category ($p = 0.02$, $p = 0.05$, respectively). Female VO_{2max} categories showed no difference in sleep quality, but VO_{2max} fair-average category reported higher fatigue than VO_{2max} good and excellent categories (both $p = 0.001$). Regression analysis indicated sleep ($\beta = -0.3$, $p = 0.03$) and fatigue ($\beta = -0.4$, $p = 0.01$) as the predictors of VO_{2max} in males and females, respectively.

Conclusions Sleep is a relevant factor for physical performance, particularly cardiovascular performance, and it accounts for 20% of variance in males. Physical performance seems more affected by fatigue in females; however, more standardized study protocols are needed to take into account the menstrual phase cycle.

10.2 Actigraphic and muscular evaluation on shift nurses

Differences in daytime activity levels and daytime sleep between night and day duty: an observational study in Italian orthopaedic nurses

Roveda E. & **Castelli L.**, Galasso L., Mulè A., Cè E., Condemi V., Banfi G., Montaruli A., Esposito F. (2021). **Frontiers in Physiology**, 26(12):628231. Doi: 10.3389/fphys.2021.628231

IF: 4.566; Rank: Q1

Background Risk of sleep deficiency is often reported in association with shift work. Night shift workers are prone to accumulating sleep debt, which they recover by sleeping during the day. The effect of this habit on daytime activity levels is still unknown. The present study aimed to actigraphically assess whether daytime sleep could affect daytime activity levels of shift worker nurses, resulting in an accumulation of their activity debt.

Methods 37 (41.7 ± 9.1 years) orthopaedic nurses working on a rotating schedule, including either a night shift (NS) or only day/afternoon shift (DS), composed the study sample. They were continuous 5-day actigraphically monitored for one entire work shift (five days), including both the working (three days) and the rest (two days) periods.

Results NS nurses recorded the highest daytime activity levels during the working period, while daytime sleep was similar during the two periods. DS nurses showed higher daytime activity levels and shorter daytime sleep during the working period. During the working period, NS nurses tended to nap longer and were less active than DS nurses. During the rest period, daytime activity levels for both groups were decreased. NS nurses recorded better sleep parameters during the rest period. During the working period, NS nurses slept worse than the DS nurses.

Conclusions The two groups tended to accumulate a daytime activity debt during the rest period, probably due to the longer daytime sleep. Thus, daytime sleep may be an excellent way to counteract sleep debt, but it makes nurses less active.

Effect of shift work in a sample of orthopaedic nurses: analysis of rest-activity circadian rhythm

Galasso L., Mulè A., **Castelli L.**, Cè E., Banfi G., Roveda E., Montaruli A., Esposito F. (2021).

International Journal for Environmental Research and Public Health. Doi: 10.3390/ijerph18168378.

IF: 3.390; Rank Q2

Background The temporary misalignment between the working hours and the internal time experienced by shift workers could promote circadian desynchronization. This desynchronization could alter physiological and behavioural functioning, resulting in compromised health (including increased cardiovascular risk), insomnia, sleep deficiency and reduced ability to work. The present study evaluated the effects of shift work on the rest-activity circadian rhythm (RAR) in a cohort of Italian orthopaedic nurses.

Methods 59 nurses (44 night-shift nurses – NS - and 15 day-shift nurses - DS) composed the study sample. They were continuous 5-day actigraphically monitored for one entire work shift (five days), including both the working (three days) and the rest (two days) periods. Additionally, the two groups were stratified by median body mass index (<25 kg/m2 = normal weight; >25 kg/m2 = overweight). The rhythmometric analysis was carried out through the Cosinr method analysis using the single cosinor test and the Hotelling test.

Results During the working period, the NS had a significantly lower amplitude than the DS nurses ($p < .001$), and the acrophase was significantly different between the two groups ($p < .01$). Similarly, normal weight and the overweight NS nurses reported a significantly lower amplitude during the working period ($p < .01$ and $p < .001$, normal weight and overweight, respectively).

Conclusions The current findings indicated alterations in RAR and activity level in nurses working the night shift. The lower activity levels could be due to different leisure time activity habits, or the daytime spent sleeping during the work shift. The altered RAR in night shift nurses suggests further studying the relationship between activity levels and shift work.

Reduced neuromuscular performance in night-shift orthopaedic 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***, 30(11):693. Doi: 10.3389/fphys.2020.00693.

IF: 4.566; Rank: Q1

Background Shift nurses offer an attractive model to study the effect of sleep-wake rhythm disruption on neuromuscular control and muscle fatigue. An objective assessment of this paradigm is missing in literature. The present study investigated neuromuscular activation and muscle contraction capacity at sub maximum and maximum levels with the simultaneous analysis of surface electromyographic (sEMG) and force signals in nurses working two night-shift schedules and compared them to levels in nurses working entirely in day shifts. Sleep quality and activity levels were also actigraphically assessed.

Methods 71 nurses working three different shift work schedules (night shift for five days (NS5, n = 46), night shift for ten days (NS10, n = 9), and only day/swing shift (DS, n = 16)) were measured in the finger flexor muscles for maximum voluntary contraction (MVC) force and muscle activation, neuromuscular control, and muscle fatigability. All the assessments were carried out before and after the shift-work cycle. A wrist actigraphy recorded the activity level and sleep quality during the shift-work cycle.

Results MVC and muscle activation and neuromuscular control significantly decreased after the work shift in all shift schedules ($p < 0.001$; $p = 0.007$); the NS5 and the NS10 group increased the muscle fatigability after the work shift ($p = 0.006$). Similarly, the NS5 and the NS10 groups reported a lower sleep quality after the work shift ($p < 0.001$), while the activity level for the three groups was similar.

Conclusions The night shift nurses reduced neuromuscular control and increased muscle fatigue after the work shift. These findings may be helpful for work schedule planning or recommendations for formulating new recovery strategies to counteract neuromuscular alterations in night shift nurses.

10.3 Studies involving chronotype assessments

Biological rhythm and circadian phenotype: New perspectives in health

Montaruli A., **Castelli L.**, Mulè A., Scurati R., Esposito F., Galasso L., Roveda E. (2021).

Biomolecules. *Doi: 10.3390/biom11040487.*

IF: 4.879; Rank: Q2

Background The circadian rhythms are essential in controlling biological functions, sleep-wake cycle, body temperature, hormonal secretion, food intake, and cognitive and physical performance. Chronic disease and sleep deficiency could stem from alterations in circadian rhythms. In humans, the phenotype expression of circadian rhythmicity is expressed through the concept of morningness-eveningness or chronotype. It represents the individual's preferred period of the day to be more active or sleep over a 24-h period. Three chronotypes are distinguished: Morning, Neither, and Evening-types, and their interindividual expression could lead to adverse effects of circadian rhythms disruptions on health.

Methods The present review included 217 articles focused on circadian rhythms, chronotype, rest-activity, sleep, chronic disease, health, and melatonin. The article research has been run on three electronic databases (Medline, Google Scholar, and Pubmed) until the 15$^{th\ of}$ January 2021. The rest-activity circadian rhythm, sleep-wake cycle, and chronotype have been investigated in chronic pathologies.

Results and discussions Older subjects, cancer patients, subjects with neurodegenerative and cardiovascular diseases, and those suffering from eating and metabolic disorders face rest-activity circadian rhythm and sleep-wake cycle alterations. The relationship between circadian rhythms alteration and chronic disease could be bidirectional. Chronotype is able to influence either the physiological or the psychological functioning, and the misalignment between the internal and external time could lead to adverse effects on health.

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: 10.1007/s11332-019-00573-x

Background Sleep is essential for human beings, and it could be influenced by physical activity and chronotype. Regular physical activity could positively influence sleep, while Morning-type, usually more aligned with social schedule, report less sleep problem than evening-type. Sleep could be negatively influenced by ageing; older people usually report more sleep problems than young people. The present study assessed the role of physical habits and chronotypes on sleep patterns in a sample of Italian elderly population.

Methods The study sample consisted of 100 Italian elderly (50 males; mean age±SD 70.9±6.3 years). They filled in the Morningness-Eveningness Questionnaire (MEQ), the International Physical Activity Questionnaire-Short Form (IPAQ-SF) and the Pittsburgh Sleep Quality Index (PSQI) and evaluated in the lipid profile (Total Cholesterol, triglycerides, HDL, LDL values, Total cholesterol/HDL and LDL/HDL ratios).

Results Based on IPAQ-SF, active subjects (AS) reported better PSQI values (5.3 ± 3.1 vs 6.2 ± 3.4), fewer sleep disturbances ($p < .003$) and daytime dysfunctions ($p < .006$), used fewer sleep medications ($p < .003$), and had higher HDL level ($p < .005$) compared to inactive subjects (IS). In general, Morning-types (MT) showed higher PSQI scores than Neither types (NT). The same situation was reported for the female sample, in which active NT collected a lower PSQI value than active MT (3.9 ± 3 vs 6.2 ± 3.5 respectively; $p < .05$). Active females MT used fewer sleep medications ($p < .02$) and had less sleep dysfunction ($p < 0.03$) compared to inactive females MT, while active females NT reported fewer daytime dysfunctions ($p < .03$) than inactive females NT. Focusing on males, active MT had fewer disturbances compared to inactive NT ($p < .05$).

Conclusions NT showed better sleeping habits compared to MT. Generally, IS had worse global sleep quality, more sleep disturbances and use more sleep medications than AS did. An active lifestyle could be suggested during ageing to promote good sleep quality and reduced sleep problems.

Effect of chronotype on academic achievement in a population 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):1482-1495Doi: 10.1080/07420528.2019.1652831.

IF: 2.877; Rank: Q2

Background The expression of circadian rhythms differs among individuals, distinguishing three chronotypes: Morning- [MT], Evening- [ET], and Neither- [NT] type. Considering the academic performance, that of ET could be compromised by university class schedules because they conflict with ET's circadian preferences; MT students, being more aligned with their daily schedule, might be more advantaged in their mental performance; NT students have been little considered. The present study explored the relationship between chronotype and academic performance in a sample of Italian university students of the Motor Science Faculty in Milan.

Methods 423 university students (290 males and 133 females) were categorized by chronotype according to Morningness-Eveningness Questionnaire (MEQ) scores. Theoretical and practical grades were recollected and mediated with the proper CFU amount to obtain a theoretical and practical grade.

Results MT showed the highest mean grades in the theoretical (MT vs ET $p < .002$; MT vs NT $p < .04$) and practical exams (MT vs ET $p < .0006$; MT vs NT $p < .003$). For the theoretical exams, the NT students had lower mean grades (24.8 ± .1) than either the MT (26.3 ± .4) or the ET (25.3 ± .2) students; on the contrary, for the practical exams, the ET (26.6 ± .2) performed worse than either the MT (27.8 ± .2) or the NT students (26.9 ± .1). For males, the differences were significant for the theoretical (MT vs ET $p < .006$; MT vs NT, $p < .002$) and the practical exams (MT vs ET $p < .004$; MT vs NT, $p < .01$); for females no significant differences were visible.

Conclusions The present results indicated overall better academic achievement by the MT students, whereas the NT and ET performed worse in the theoretical and practical subjects, respectively. We could speculate that the supposed higher ET intelligence might favour them to compensate for the misalignment between circadian preferences and university class schedules on theoretical but not practical exams.

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

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

Background Athletic performance and perceived exertion could be influenced by chronotype and time of day in which the effort is executed. Indeed, morning (MT) and evening chronotype (ET) fell more active and performant at different times of the day, and their athletic performance could be differently affected. The study evaluates the effect of chronotype on aerobic performance, heart rate (HR) and Rating of Perceived Exertion (RPE) in young expressing different chronotypes.

Methods 22 participants (11 MT, 11 ET) of the School of Motor Sciences, University of Milan, composed the study sample. They were classified through the Morningness-Eveningness Questionnaire (MEQ) administration and performed the Cooper test and the Borg Scale CR 0-10 at 9:00 a.m. and at 5:00 p.m. to investigate the effect of chronotype on aerobic performance, HR and RPE.

Results The two-way ANOVA revealed an interaction between chronotype, time and RPE. In particular, MT perceived less effort in the 9:00 a.m. compared to the 5:00 p.m. session ($p < .05$), both before (CR-10: 1.1 ± 0.8 vs 2.5 ± 1.3) and after exercise (CR-10: 7.4 ± 1 vs 8.6 ± 1). Conversely, ET felt more fatigued in the 9:00 a.m. than in the 5:00 p.m. session ($p < .05$), both before (CR-10: 2.4 ± 1.4 vs 1.1 ± 1.1) and after exercise (CR-10: 8.4 ± .6 vs 7.5 ± .7). Moreover, in the 9:00 a.m. session, ET had a greater perception of the effort (CR-10: 2.4 ± 1.4 vs 8.4 ± 0.6) than MT (CR-10: 1.1 ± .8 vs 7.4 ± 1). Instead, in the afternoon session, MT showed higher RPE values (CR-10: 2.5 ± 1.3 vs 8.6 ± 1) than ET (CR-10: 1.1 ± 1.1 vs 7.5 ± .7). Neither differences nor interactions were visible in Cooper Test and HR and between chronotype, daytime and performance or HR, respectively.

Conclusions A higher effort was perceived in the afternoon and the morning session by MT and ET, respectively. Coaches should take into account this information to plan tailored training programs.

Effect of chronotype on specifically motor skills related to soccer in adolescent players

Roveda E., Mulè A., Galasso L., **Castelli L.**, Scurati R., Michielon G., Esposito F., Caumo A. (2020). *Chronobiology International* *37(4):552-563, Doi: 10.1080/07420528.2020.1729787.*

IF: 2.877; Rank: Q2

Background Circadian rhythms influence lots of daily physiological functions and physical performance. The phenotype expression of circadian rhythm differs among individuals and results in three chronotypes: Morning, Neither, and Evening types (MT, NT, and ET). The chronotype changes with age; indeed, it is possible to observe a progressive shift toward eveningness during adolescence, responsible for a misalignment between the morning society schedules and the internal time. This condition could lead to a deterioration in physical performance. Soccer is widely practised among adolescents, usually after school in the afternoon or evening. The present study investigates the effect of chronotype on motor skills specific to soccer, specifically whether agility, aerobic endurance, and explosive power differ among the three chronotypes with the time of day.

Methods 141 Italian adolescent soccer players filled in the Morningness-Eveningness Questionnaire (MEQ) to assess chronotype. 75 subjects (25 MT, 25 ET, and 25 NT, performed the Sargent Jump Test - SJT, Illinois Agility Test - IAT, and 6-Minutes Run Test - 6MRT in the morning and evening training session (9:00 am and 6:00 pm). To test the interactions between chronotypes, physical performance, and time the Mixed ANOVA was used.

Results MT collected better performance during the morning than the evening session for all physical tests ($p < .05$); ET performed better in the evening than in the morning session ($p < .05$); NT showed differences in the tests.

Discussion These results highlighted the necessity of a proper chronobiological approach to sports training. To enhance performance, coaches should schedule training sessions according to an athlete's circadian preferences.

10.4 Evaluations in subjects with Metabolic Syndrome and Binge Eating Disorders

Sex differences in rest-activity circadian rhythm in patients with metabolic syndrome

Mulè A., Bruno E., Galasso L., **Castelli L.**, Caumo A., Esposito F., Pasanisi P., Montaruli A., Roveda E. (2021). ***Frontiers in Physiology***, 18(12):641461, Doi: 10.3389/fphys.2021.641461.

IF: 4.566; Rank: Q1

Background Rest-Activity circadian Rhythm (RAR) could be treated as a marker of the circadian timing system, whose irregularities are risk factors for hypertension, obesity, and dyslipidaemia. These pathologies are correlated to the Metabolic Syndrome (MS), a cluster symptom of metabolic risk factors that increases the risk of several cardiovascular and metabolic diseases. This cross-sectional analysis actigraphically explores the RAR characteristics in subjects with MS.

Methods 100 subjects with MS were recruited at *IRCCS Istituto Nazionale dei Tumori di Milano*; underwent an anthropometric and metabolic evaluation and completed seven-day actigraphic monitoring. The analyses have been performed stratifying the sample by sex and MS parameters and using parametric and nonparametric approaches.

Results Female participants exhibited higher values in the Midline Estimating Statistic of Rhythm (MESOR) (243.3 ± 20.0 vs 197.6 ± 17.9 activity count), and Amplitude (184.5 ± 18.5 vs 144.2 ± 17.2 activity count) than male participants, which measured the most active 10-h period (M10) (379.08 ± 16.43 vs 295.13 ± 12.88 activity count). This indicated a higher daily activity level in women, who also had lower Intradaily Variability (IV) than male participants (0.75 ± .03 vs 0.85 ± .03 activity count), indicating a more stable and less fragmented RAR.

Discussion The present study provided preliminary data and the first experimental evidence of a difference in RAR parameters between male and female subjects with MS. The sex differences in RAR characteristics could represent a helpful tool to improve the daily level of activity and set up personalized activity programs based on each person's circadian activity profile.

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(12), Doi: 10.3390/nu12123622. IF: 5.717; Rank: Q1

Background Lower physical activity levels associated with lower exercise capacity are frequent characteristics of binge eating disorder patients. Proper physical activity is already reported to assure overall beneficial results on general eating disorders, depression, and body mass index in bulimia, but research in binge eating disorder (BED) is scarce. The present study investigates the effects of specific physical activity training as an additional tool to the conventional treatment on eating disorder symptoms, anthropometric characteristics, and physical performance.

Methods The study sample was composed of 19 women with BED and in therapy with a diet and Cognitive-Behavioral Therapy program. The sample has been randomized in a Combined Aerobic and Anaerobic Exercise Training added to the conventional treatment group (CAAET group, 10 women) and in a conventional treatment alone group (CTRL group, 9 women). All the measurements were assessed before and after 6 months of treatment.

Results After a six-month intervention, we observed a significant decrease in binge episodes, weight, body mass index, and increased exercise capacity in both groups. Moreover, the CAAET group shows a more significant aerobic performance improvement than the one observed in the CTRL group.

Discussion Even though on a small sample, these results suggest that both interventions are similarly able to improve BED symptoms. The constant physical activity practice could be crucial in the long-term maintenance of both weight loss and reduction in binge episodes in BED patients.

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). *Chronobiology International*, 36(10):1311-1315. Doi: 10.1080/07420528.2019.1650059.

IF: 2.877; Rank: Q2

Background Rest-activity circadian rhythm (RAR) significantly differed in women with Binge Eating Disorder (BED) compared to the control (healthy) group. More specifically, patients with BED show significantly reduced levels of MESOR and Amplitude than the control group. Conversely, no differences in sleep parameters are visible by comparing the two groups. The present study enlarges the previous RAR analysis to the non-parametric parameters of the cosinor analysis.

Methods 28 volunteered women, 14 BED women, and 14 control (Ctrl) were recruited and underwent a 5-day long actigraphic monitoring to detect the rhythmometric parameters, Interdaily Stability, Intradaily Variability, L5, M10, and sleep parameters. During the monitoring, BED's women group followed an individual multidisciplinary therapy lasting five weekly days (outpatient care from 8:00 a.m. to 5:00 p.m.) and consisting of a cognitive-behavioural therapy and nutritional program.

Results The sleep quality of the BED group was significantly higher compared to Ctrl. The non-parametric indexes were higher in the BED group than the Ctrl group, indicating a better synchronization of the rest-activity circadian rhythm in the BED group. Furthermore, Interdaily Stability was significantly correlated to sleep efficiency.

Discussion Since these results, it is possible to conclude that maintaining a regular lifestyle, such as that imposed by the multidisciplinary therapy, is important to avoid alterations in the sleep-wake cycle, particularly in patients with BED.

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