Chronobiology, cognitive function and depressive symptoms in surgical patients

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STUDIES AND PAPERS INCLUDED IN THIS THESIS

STUDY 1 – Case-control study :
I.   Voigt HM, Rasmussen LS, Jespersgaard C, Rosenberg J, Gogenur I. There is no association between the circadian clock gene HPER3 and cognitive dysfunction after noncardiac surgery. Anesth Analg 2012;115:379-85.

STUDY 2 – The MELODY trial – an RCT:
II.   Hansen MV, Madsen MT, Hageman I, Rasmussen LS, Bokmand S, Rosenberg J, Gögenur I. The effect of MELatOnin on Depression, anxietY, cognitive function and sleep disturbances in patients with breast cancer. The MELODY trial: protocol for a randomised, placebo-controlled, double-blinded trial. BMJ Open 2012;2(1):e000647.

III.   Hansen MV, Andersen LT, Madsen MT, Hageman I, Rasmussen LS, Bokmand S, Rosenberg J, Gögenur I. Effect of melatonin on depressive symptoms and anxiety in patients undergoing breast cancer surgery: A randomized, double-blind, placebo-controlled trial. Breast Cancer Res Treat 2014;145:683-95.

IV.   Hansen MV, Madsen MT, Andersen LT, Hageman I, Rasmussen LS, Bokmand S, Rosenberg J, Gögenur I. Effect of melatonin on cognitive function and sleep in relation to breast cancer surgery: A randomized, double-blind, placebo-controlled trial (submitted)

STUDY 3 – A systematic review and meta-analysis:
V.   Hansen MV, Danielsen AK, Hageman I, Rosenberg J, Gögenur I. The therapeutic or prophylactic effect of exogenous melatonin against depression and depressive symptoms: a systematic review and meta-analysis (submitted)

INTRODUCTION

Chronobiology is defined as "The study of the effects of time and rhythmical phenomena on life processes" [1]. All living organisms are characterized by an endogenous ("built in") cyclic rhythmicity of a wide range of biological and behavioral processes [2]. These rhythmical phenomena oscillate from a time span of seconds, minutes, hours, days, and even years, leading to terms such as circadian (daily), menstrual (monthly) and circannual (yearly) [2].

A circadian rhythm is any biological process that displays an endogenous oscillation of about 24 hours. This endogenous rhythm is also entrained to the local environment by external clues, so called zeitgebers ("time cues"), such as light, food, physical and social activity, and temperature [2]. Circadian rhythms are controlled by a master biological clock, or central pacemaker, which in mammals is located in the suprachiasmatic nucleus of the hypothalamus [2]. At the subcellular level circadian rhythms are generated by transcriptional and translational feedback loops involving multiple clock genes [3]. These core circadian clock genes are defined as genes whose protein products are necessary components in the generation and regulation of circadian rhythms [3]. Furthermore, the endogenous hormone melatonin also plays an important role in circadian systems, especially the sleep-wake cycle [4, 5].

In humans, the sleep-wake cycle is the most obvious of all circadian rhythms. Sleep and the fundamental need for it remains an enigma in spite of much research on the topic and proof that even subtle alterations in the sleep-wake cycle can affect human health, performance and well-being [6-10]. There are many examples of this, i.e. shift work that involves circadian disruption has recently been classified as "probably carcinogenic to humans" [11], shift work is associated with an increased risk of vascular events [12], sleep deprivation and sleep fragmentation lead to a variable negative impact on cognitive performance [7], and insomnia is associated with a higher risk of developing depression [8].

In two specific groups of patients; namely surgical patients and patients with a cancer diagnosis, sleep and circadian disturbances are issues of much debate [13-16]. Circadian disturbances of the endogenous rhythms of hormone secretion,

core body temperature and autonomic nervous system tone are well-known in patients undergoing surgery [13]. Several closely intertwined cerebrally controlled functions, such as memory, concentration, pain, and mood, may arise as a consequence of the circadian disturbances and coexist with these. Cognitive dysfunction, sleep disturbances, fatigue, pain and the development of psychiatric symptoms such as depression and anxiety are prevalent in the perioperative period but are also found to overlap with symptoms that are predominant in relation to having a cancer diagnosis. Compared to other patients with cancer, patients with breast cancer have shown a particularly high risk of developing the above-mentioned symptoms in clusters consisting of symptoms such as depression [17], anxiety [18], disturbed sleep [19], fatigue [20], pain [20], and problems with memory and concentration [21, 22]. The consequences of these symptoms are an adverse effect on quality of life [17], reduced compliance to treatment [23, 24], lower patient satisfaction [25], and higher morbidity and mortality [18, 26-28].

To summarize, the overall topic of chronobiology, including circadian rhythm and the sleep-wake cycle, the hormone melatonin and on a cellular level, clock-genes, is prominent with regard to surgical and cancer patients in general, especially in patients with breast cancer. Due to the extent of the aforementioned symptoms and the far-reaching consequences, investigations into the genetic basis and possibilities of treatment or prophylaxis are necessary.

BACKGROUND

COGNITIVE FUNCTION IN RELATION TO SURGERY AND GENETICS

*Postoperative cognitive dysfunction (POCD)*
The brain is vulnerable during the perioperative period in patients of all ages and delirium and cognitive dysfunction are common complications in the postoperative period after both cardiac and non-cardiac surgery [29-33]. Postoperative cognitive dysfunction is characterized by more subtle changes than delirium, affecting mainly memory and concentration [29, 34]. Although many of the minor symptoms of this cognitive impairment are not readily apparent to other people, the patients themselves can sense them and a final consequence can be decreased quality of life, increased morbidity and mortality and an economic burden on healthcare resources [35-38]. In previous studies the incidence of POCD ranges from 7-41% one week after surgery to 6-14% three months after surgery [30-33, 35].

Advanced age, larger and more invasive operations, a lower educational level, pre-existing diseases, psychological factors and individual vulnerability of the brain are some of the possible risk factors for the development of POCD [29, 36, 39] (Figure 1). Genetic variations, such as the apolipoprotein E gene (APOE) and polymorphisms in genes encoding C-reactive protein, P-selectin, complement component 3, inducible nitric oxide synthase and cytochrome P450 have been studied, but the present conclusion is that no genomic predictors of POCD lasting more than three months have been identified [40].

The etiological factors contributing to the development of POCD are still being studied intensively, but many still remain unclarified. To date, some of the main proposed factors are the drugs used for anesthesia and analgesia, changes in cerebral blood flow, hypoxemia, microembolisms, and surgery-induced tissue damage and inflammation leading to elevated cytokines [29, 36, 39]. In relation to the circadian rhythm, periop-

erative sleep disturbances and changes in hormone secretion of melatonin [41, 42] and cortisol [43] have also been investigated as possible contributing factors.

FIGURE 1 – Possible etiology and risk factors for postoperative cognitive dysfunction
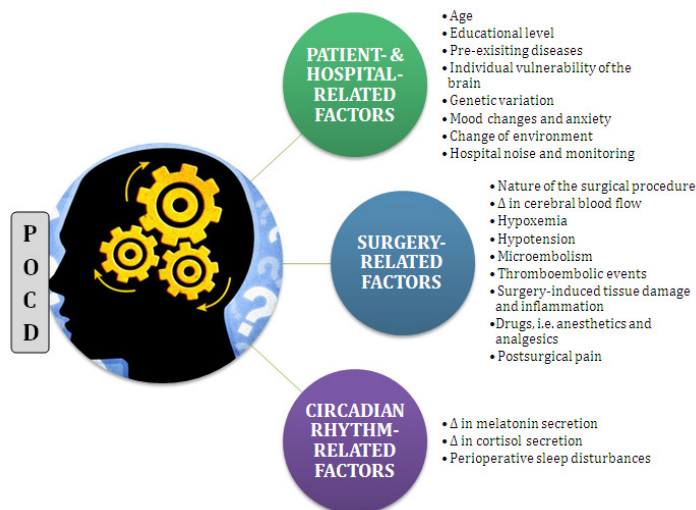
Clock-genes
The biological clock in mammals is an intricate process, consisting of both a central and several peripheral clocks, which regulate the timing of sleep and many other physiological processes [2]. The central human master clock resides in the suprachiasmatic nucleus of the hypothalamus [2]. The peripheral clocks reside in individual cells that have the ability to generate a self-sustained circadian rhythm through transcription and translation feedback loops of the expression of central clock genes, especially Clock, Bmal1/Arntl, Bmal2/Arntl2, Per1, Per2, Per3, Cry1 and Cry2 [3]. The peripheral clocks are coordinated by the central clock through both neural and humoral signals [44].

Interindividual differences in sleep-wake patterns and development of specific circadian rhythm sleep disorders, such as advanced or delayed sleep phase disorder, have been associated with specific clock-gene variations [44]. Moreover, different circadian rhythm phenotypes have been studied in relation to different clock-genes. Specifically, in PERIOD3 (PER3) a variable number tandem repeat polymorphism in which a 54-nucleotide sequence encoding 18 amino acid residues is repeated 4 or 5 times, has been coupled with several phenotypic variables, such as morning/evening preference and cognitive vulnerability in response to sleep deprivation [45]. In most populations approximately 10% of individuals are homozygous for the 5-repeat (PER3$^{5/5}$), 50% are homozygous for the 4-repeat (PER3$^{4/4}$) and 40% are heterozygous (PER3$^{4/5}$) [46, 47].

Vast amounts of research on both animals and humans have proven that sleep deprivation produces profound, negative neurocognitive consequences [7, 48, 49]. With regard to the PER3 genotypes, several studies have investigated the phenotype of behavioral and cognitive response to sleep loss and changes in circadian phase [50-52]. It can be concluded from these studies that carriers of the PER3$^{5/5}$ genotype have a higher vulnerability to sleep deprivation reflected in cognitive performance impairments and more pronounced effects of sleep deprivation on physiological markers of sleepiness.

BREAST CANCER, CIRCADIAN DISRUPTION, MOOD DISORDERS AND SYMPTOMS CLUSTERS

Symptom clusters

Although there is no clear consensus on exactly what defines a symptom cluster, it is normally described as three or more concurrent, related symptoms that form a stable group and may or may not share the same etiology [53]. The majority of research on pain, fatigue, depression, anxiety, cognitive disturbances and sleep disturbances associated with cancer focuses on one symptom, although patients rarely present with a single symptom. Furthermore, it is known that cancer symptoms are dynamic constructs and the symptoms may exist in a complex relationship in which they exacerbate each other's intensity, leading to an experience of the symptoms that varies and evolves over time [54, 55]. Identification of symptom clusters in patients with breast cancer has the potential of leading to more effective symptom assessment and management strategies than the traditional approach to individual symptoms [56]. It has been proposed that a common etiology, in particular cytokines, may be responsible for symptom clustering in cancer patients [57]. Hence, further investigation into the common etiology and the possibility of collective symptom management is necessary.

Depression, circadian disturbances and cancer

There is a multidirectional and multifaceted association between cancer, circadian disruption and mood disorders with all three closely intertwined by predisposing, contributing and etiological factors (Figure 2). Solely between mood disorders and circadian disturbances a bidirectional association exists, with one influencing the other and a reciprocal causal effect between the two, as not only does depression lead to circadian changes but circadian changes may also lead to depression [58]. Circadian disturbances and sleep disturbances are a prominent feature of depressive symptomatology and both the DSM-IV and the ICD-10 have disturbed sleep as one of the diagnostic criteria for major depression. Abnormal levels and rhythms of melatonin secretion have also been observed in depressed patients, underlining the circadian disturbances [58, 59].

Depression is an underrecognized and undertreated problem in women with breast cancer with up to 50% of women experiencing this problem within the first year after diagnosis [17, 60]. Depressive symptoms are shown to have a negative impact on quality of life [17], can reduce compliance to treatment [23, 24], lower patient satisfaction [25] and lead to higher morbidity and mortality [18, 26-28]. Recognition and early diagnosis of depression is warranted and more focus is needed to provide sufficient treatment and hereby prevent the negative consequences of this psychiatric disorder. Even more preferably, would be to identify women at risk of developing depression in order to prophylactically intervene. Antidepressants are frequently prescribed in this population [61] and many of the antidepressants used today have potentially clinically important anticancer-antidepressant drug interactions [62, 63], emphasizing the need for novel treatments.

FIGURE 2 – Multidirectional association between cancer, circadian disruption and mood disorders
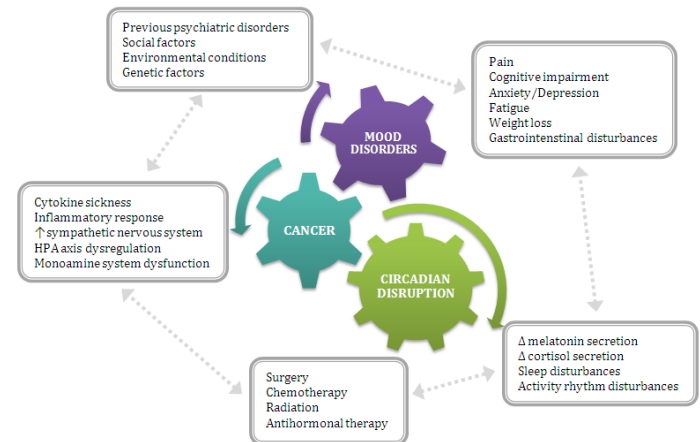
MELATONIN

Melatonin is a hormone which is secreted primarily by the pineal gland at night under normal conditions, although with great inter-subject and age-related variability [4, 5]. In the biosynthesis of the hormone, tryptophan is first converted to 5-hydroxytryptophan, which is then decarboxylated to serotonin and finally catalyzed to melatonin [5]. The endogenous rhythm of secretion is generated by the suprachiasmatic nucleus and entrained to the light/dark cycle, with secretion increasing soon after the onset of darkness, peaking between 2-4 a.m. and falling during the second half of the night [4, 5].

Melatonin is primarily known as a circadian hormone with hypnotic and chronobiotic effects. Moreover, in a variety of experimental and clinical studies it has also been found to have sedative [64], anxiolytic [64-66], analgesic [65, 67-69], antihypertensive [70, 71], anti-inflammatory [72], oncostatic [73-75], antioxidant [72], antidepressant [76-83] and beneficial cognitive effects [84-86].

The bioavailability of oral melatonin varies widely and doses of 1-5 mg produce serum melatonin concentrations that are 10 to 100 times higher than the usual nighttime peak within one hour after ingestion and decline to baseline values within four to eight hours [5].

Melatonin is relatively non-toxic and this has been confirmed by the absence of adverse events and side effects in many clinical studies performed on a wide variety of patients, for different therapeutic purposes and at a range of different doses. Several specific clinical safety studies have been conducted with doses of 10mg/day orally for 28 days [87], 300mg/day orally for four months [88], 50mg/kg orally single dose [89], and 300mg/day rectal for up to two years [90] without any toxic effect or serious adverse events.

Melatonin - sleep and mood

As mentioned previously, the bidirectional association and interaction between depression and sleep is well-known [10, 58, 59]. The most well-known and investigated mechanisms of melatonin are its hypnotic and chronobiotic effects, with the hypnotic effects arising with low, physiologically relevant doses (0.1-0.3 mg orally) whereas the optimum dose for the chronobiotic effects is not yet known, but studies have been conducted in ranges from 0.05-10 mg orally [91, 92]. The notion that depression is frequently associated with desynchronization of circadian rhythms, in-

somnia and sleep disturbances forms the basis that a drug such as melatonin which resets normal circadian rhythms and promotes sleep may have antidepressant potential.

Furthermore, rodent models have shown antidepressant effects of exogenous melatonin through an effect on several receptors and pathways; dopamine D1 and D2 receptors [80], peripheral benzodiazepine receptors, central serotoninergic neurotransmission and 5-HT$_{2A}$ antagonism, NMDA glutamate receptors, L-arginine-nitric oxide pathway, hypothalamic-pituitary-adrenal axis normalization, and restoration of corticosterone levels [76].

Regarding clinical studies, agomelatin, a novel drug that works on melatonergic (MT$_1$ and MT$_2$), 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors has proven effective as an antidepressant with less side-effects than selective serotonin reuptake inhibitors (SSRIs) but with no difference in efficacy compared to other available antidepressants [93-95].

The rationale for the choice of melatonin

The rationale for using melatonin in the MELODY trial (Study 2) is based on experimental and clinical studies on the various direct effects of melatonin, but also on studies evaluating circadian disturbances and changes in secretion of melatonin in surgical patients, patients with breast cancer, and depressed patients.

There is evidence showing disturbances in melatonin secretion after surgery in several populations [13, 96, 97], and specifically in patients with breast cancer [98]. Furthermore, disturbances in the circadian rhythm of patients with breast cancer are prevalent [99, 100], as they also are in patients with depression, alongside changes in melatonin secretion [58, 59, 101]. In addition, several of melatonin's proposed effects on promoting sleep and synchronizing circadian rhythm [91, 92, 102], and also melatonin's antidepressant [76-83], anxiolytic [64-66], analgesic [65, 67-69], anti-inflammatory [72], and beneficial cognitive effects [84-86], could have both direct and indirect effects on the symptoms investigated in the MELODY trial (Figure 3).

Our hypothesis for melatonin's effect on our primary outcome in the MELODY trial; depressive symptoms, is based on a mix of the hypnotic and chronobiotic effects, the direct antidepressant effect through various possible mechanisms, and last but not least, the parallel effects on pain, anxiety, general well-being and cognitive function which could have an influence on depressive symptoms as well.

HYPOTHESES
Based on the above, several hypotheses formed the basis of this thesis:

- The specific clock gene genotype PER3$^{5/5}$ is associated with cognitive dysfunction after noncardiac surgery
- Exogenous melatonin has a therapeutic and prophylactic effect on depression and depressive symptoms in adults
- Exogenous melatonin can decrease the risk of depressive symptoms in women with breast cancer in a three month period after surgery
- Exogenous melatonin can decrease anxiety and sleep- and cognitive disturbances in the immediate- and long-term postoperative period in women with breast cancer

FIGURE 3 – The effects of melatonin
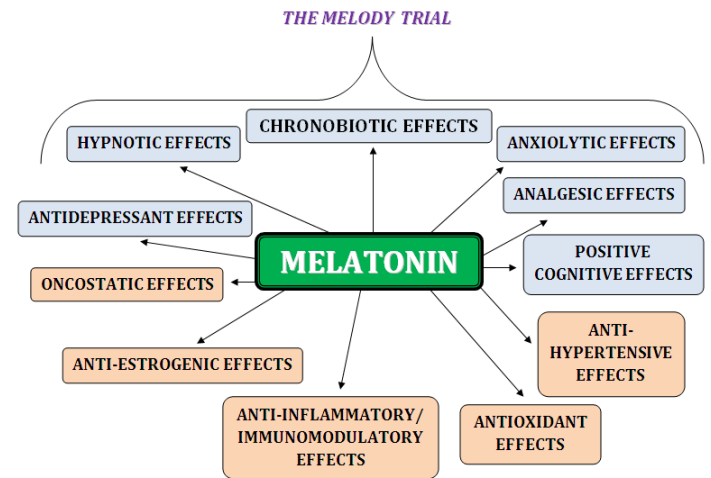
MATERIALS AND METHODS

To investigate the hypotheses, the following questionnaires, neuropsychological tests, devices and biochemical analyses were used.

MAJOR DEPRESSION INVENTORY (MDI)
Self-administered questionnaires require less resources than clinician-administered questionnaires, but it has been criticized that most self-report measures of depressive symptoms were developed before the release of DSM-III, DSM-IV and ICD-10 and do therefore not cover all of the symptoms included in the algorithms of major depression. With DSM-III, as with DSM-IV and ICD-10, the diagnostic criteria for depressive illness changed from an etiologic binarian principle (i.e. endogenous versus reactive depression) to a unitarian symptom-based principle. Consequently, a new self-rating depression scale was recently developed, the Major Depression Inventory (MDI – Appendix 1) [103, 104]. This scale is based directly on all of the depressive symptoms in DSM-IV and ICD-10. The individual items measure how much of the time the symptom has been present during the past 14 days and is scored on a six-point Likert scale, ranging from 0 (not present at all) to 5 (present all of the time). The MDI contains the 10 ICD-10 symptoms of depression and these symptoms are identical to the DSM-IV major depression symptoms except for low self-esteem, which is incorporated in the symptom of guilt in DSM-IV. Of the ten MDI items, items 8 and 10 regarding psychomotor activity and appetite respectively, are split into two sub-items, "a" and "b". On these items, only the highest score is included in the analysis.

The questionnaire includes a total of 12 questions and has been widely used in the Danish population and validated at both clinical and population levels [104-109]. With regard to the psychometric properties of the MDI, a high degree of internal validity, reliability and unidimensionality have been found in several previous studies [103, 106, 110]. Furthermore, a sensitivity (true positive rate) and a specificity (true negative rate) of >0.80 have previously been found when the MDI was tested for applicability and external validity when using SCAN (The Schedules for Clinical Assessment in Neuropsychiatry) as the gold standard [104]. In addition, the MDI correlates well with the

Hamilton Depression Scale (HAM-D$_{17}$) [106] and with the Zung Self-Rating Depression Scale (Zung-SDS) [103].

The MDI has a dual function and can be used both as a diagnostic instrument and a measuring instrument (rating scale) to indicate the severity of the depression (Appendix 1). The former uses a diagnostic algorithm based on either ICD-10 or DSM-IV criteria for major or moderate to severe depression. Using the diagnostic demarcation line shown in Appendix 1, core symptoms have to have been present "most of the time" during the past two weeks and accompanying symptoms "slightly more than half the time" for the last two weeks. The ICD-10 algorithm that follows is:

- Mild depression: 2 core and 2 accompanying symptoms
- Moderate depression: 2 core and 4 accompanying symptoms
- Severe depression: 3 core and 5 accompanying symptoms

The algorithm for DSM-IV major depression is: items 4 and 5 are combined and only the highest score is considered, leaving 9 items in total. To fulfill a diagnosis of DSM-IV major depression, at least one of the first two symptoms and in total at least five symptoms must be indicated.

The latter, when using the MDI as a rating scale, it is the sum of all questions ranging from 0 (no depression) to 50 (maximum depression) that counts:

- Mild depression: Total score from 21-25
- Moderate depression: Total score from 26-30
- Severe depression: Total score ≥31

THE INTERNATIONAL STUDY OF POSTOPERATIVE COGNITIVE DYSFUNCTION (ISPOCD)

Many different neuropsychological tests are available and they allow an assessment of different cognitive domains. There is no consensus on the best test, as this inevitably depends on the situation and the population being tested. Various brain areas are involved in the neurocognitive functions of POCD and the mechanisms behind POCD affect the brain in general, leading to impairment of several cognitive domains [39], necessitating the need for a test battery that is sensitive and suitable to detect these impairments in this specific population. As our study involved surgical patients, we chose the widely applied neuropsychological test battery used in the The First and Second International Studies of Postoperative Cognitive Dysfunction (ISPOCD 1 and 2) [30-33, 111]. These studies included more than 2500 patients from 25 centers in 12 countries and are therefore the largest studies on POCD to date.

The ISPOCD test battery used in Study 1 and Study 2 consisted of four neuropsychological tests:

- Visual Verbal Learning Test
- Concept Shifting Test
- Stroop Color-Word Interference Test
- Letter-Digit Coding Test

Seven variables from these four tests were used in the analysis:

- Cumulative number of words recalled in three trials, and the number of words at delayed recall from the Visual Verbal Learning Test
- The time and number of errors in part C of the Concept Shifting Test
- The time and error scores from the third part of the Stroop Color-Word Interference Test

- The number of correct answers from the Letter-Digit Coding Test.

The advantages of this test battery are that the baseline performance of the patient and the general variability of a control population are taken into account in the way the outcome (Z-score) is calculated. In the way cognitive dysfunction is defined (see definition below), it also takes into account both the overall deterioration across all tests and the specific impairment in an individual test. The control group we used for the MELODY trial was normative data from 133 females aged 40-60 years collected in a previous study [33]. The practice effect is minimized by using parallel, instead of identical versions of the test and by subtracting the average learning effect in the calculation of the Z-score. A composite Z-score is defined as the sum of the seven Z-scores and normalized using the standard deviation for that sum in the controls. We tried to minimize variability by making test conditions as similar as possible, only using three qualified examiners, and by trying to avoid distractions in the test situations. We chose our intervals between test sessions to be as similar as possible to previous studies [30-33, 35] to provide a basis for comparability.

Calculation of the Z-score:

$$Z = \frac{(\text{Postop score} - \text{preop score}) - (\text{average learning effect from controls})}{\text{Standard deviation for change from baseline in the controls}}$$

Calculation of the composite Z-score:

$$Z_{comp} = \frac{(Z1 + Z2 + Z3 + Z4 + Z5 + Z6 + Z7)}{\text{Standard deviation for } (Z1 + Z2 + Z3 + Z4 + Z5 + Z6 + Z7) \text{ in the controls}}$$

Definition of POCD:

- A composite Z-score >1.96
  OR
- A Z-score >1.96 in at least two of the 7 subtests

The exact value of 1.96 has been chosen as only 2.5% of the controls would have a Z-score >1.96 by chance and the value 1.96 can therefore be interpreted as a substantial deterioration.

PERIOD3 GENOTYPING

The PER3 genotyping was done on a blood sample which had been stored at -20°C on filter paper. DNA was extracted from the filter paper and exon 18 and the adjacent introns were amplified using primers as described by Ebisawa et al. [112]. The polymerase chain reaction fragments were detected and then interpreted by analysis software. Patients were classified in one of three genotype categories depending on the length of the PERIOD3 allele; PER3$^{4/4}$, PER3$^{4/5}$, PER3$^{5/5}$.

ACTIGRAPHY

An actigraph is a wrist-worn mini-computer which objectively measures sleep, circadian rhythm, activity rhythm and activity in general [113]. This low-cost, non-invasive method compared to the gold standard of polysomnography, has been used for several years; both in the perioperative setting in general [114] and in patients with breast cancer [99, 100, 115, 116]. A major advantage compared to the use of polysomnography is the possibility of measuring and recording the sleep-wake cycle for several weeks [113]. When comparing with polysomnography in the

ability to detect sleep and wake, high levels of sensitivity and specificity have been found in the perioperative period in patients with breast cancer (unpublished data).

SLEEP DIARY
The sleep diary is regarded as the gold standard for subjective sleep assessment, although lack of a standardized and widely used sleep diary has compromised the ability to fully interpret and integrate results from previous studies [117]. The questions in the sleep diary we used in the MELODY trial are in overall accordance with a newly defined "Core Consensus Sleep Diary" [117].

The sleep diary we used obtained patient-reported information on "what time did you go to bed", "what time did you try to fall asleep", "how long did it take to fall asleep", "how many times did you wake up in the night", "how long were these awakenings", "what time did you wake up", "what time did you get out of bed" and "did you take any naps during the day and how long did they last". These data were used to estimate sleep latency in minutes, number of awakenings, total sleep period in minutes, and sleep efficiency in percent. Total sleep period (TSP) was defined as time in bed trying to sleep and sleep efficiency was defined as (TSP-sleep latency-minutes awake)/(TSP).

VISUAL ANALOGUE SCALE (VAS)
A Visual Analogue Scale (VAS) is a measurement instrument that tries to measure a characteristic or attitude that is believed to range across a continuum [118]. To capture this continuum we interpreted VAS as continuous data, and not ordinal data, even though there is no consensus on this topic [119].
There is sufficient evidence that brief symptom rating scales such as the VAS are useful in patients with cancer [120]. Therefore, in the MELODY trial (Study 2) anxiety, sleep quality, pain (at rest), fatigue, and general well-being were assessed quantitatively by patient-reported scores, measured by VAS. To ensure uniformity and continuity for the patients when completing the scales, the VAS were all horizontal and had the same direction of increasing severity. A 0-100 mm VAS was used with the best statement placed at 0 mm (= no anxiety, best possible sleep, no pain, no fatigue, best general well-being), ranging to the worst possible statement at 100 mm (= worst possible anxiety, worst possible sleep, worst possible pain, worst possible fatigue and worst possible general well-being).

Small differences in VAS scores may be statistically significant but the question is whether the difference is clinically relevant. The minimal clinically important differences for VAS on sleep quality [121], pain [122] and fatigue [123] have previously been reported to be in the range of 8-13 mm for other patient populations.

KAROLINSKA SLEEPINESS SCALE (KSS)
The Karolinska Sleepiness Scale was used to quantify levels of sleepiness in the MELODY trial (Study 2). The KSS measures the subjective level of sleepiness and is a 9-point scale ranging from 1 (extremely alert) to 9 (extremely sleepy – fighting sleep), where a score of 7 (sleepy – but no difficulty remaining awake) or more reflects EEG (electroencephalogram) and EOG (electro-oculogram) verified changes with increased levels of energy [124].

ETHICAL CONSIDERATIONS

For Study 1, approval was obtained from the Danish National Committee on Biomedical Research Ethics (H-3-2010-056) and the Danish Data Protection Agency (HEH.afd.D.750.89-5). The study was also registered on Clinicaltrials.gov (NCT01088100). The requirement for written informed consent was waived by the Danish National Committee on Biomedical Research Ethics, as written informed consent was obtained in the parent studies [31-33].

For Study 2 approval was obtained from the Danish National Committee on Biomedical Research Ethics (H-4-2011-007), the Danish Medicines Agency (EudraCT nr. 2010-022460-12) and the Danish Data Protection Agency (2007-58-0015/HEH.750.89-12). Written informed consent was obtained from all patients and the trial was registered on Clinicaltrials.gov (NCT01355523). The Good Clinical Practice Unit at Copenhagen University monitored the trial.

STATISTICAL CONSIDERATIONS

For all studies IBM SPSS Statistics for Windows, Version 18.0 or 20.0 (IBM Corp., Armonk, NY, USA) were primarily used, supplemented in special circumstances with other statistical software specified below. In general a two-sided p-value ≤ 0.05 was considered statistically significant.

Paper I:
Assuming 25% having the PER3$^{5/5}$ genotype in the group with POCD and 10% in the group without POCD, a sample size of the 93 patients with POCD was to be matched in a 1:2 ratio with 186 controls, accepting a Type I error of 5%, a power of minimum 80% and 10% missing samples. The χ^2 test was used to investigate the relationship between POCD (+/-) and PER3 genotype. The Nur-minen-Miettinen method (http://phsim.man.ac.uk/risk/Default.aspx) was used to calculate the risk difference between POCD –(-POCD) with 95% confidence intervals. 1-way ANOVA was used to analyze the three genotypes (nominal data) and the specific Z-scores for the seven subtests, applying a Bonferroni correction due to multiple comparisons. The only significant result was then tested separately with a Jonckheere Terpstra test, which was chosen due to it having more statistical power than a Kruskal Wallis test, when there was an a priori ordering of the genotypes (ordinal data). 1-way ANOVA was also used to analyze the three genotypes and the total Z-score from the first postoperative test.

Paper II:
The primary outcome was depressive symptoms measured by the MDI. The sample size estimation was based on a conservative estimate of the incidence of depression of 30% [60] with a reduction to 15% with melatonin treatment. The study was powered at 80% with a risk of a Type I error of 5%. All other statistical considerations are described in the paper. These are the statistical analyses we had a priori planned to do but as the trial was terminated prematurely some changes occurred and will be described below in the associated paper.

Paper III:
The primary outcome was the incidence of depressive symptoms of any severity (mild, moderate, severe) at one point in the study after baseline measured by the MDI. The definition of mild de-

pression was an MDI score of at least 21. Our secondary outcomes were the subjective parameters of anxiety, sleep, general well-being, fatigue, pain and sleepiness.

Normality of the data was tested with the Shapiro-Wilk test and non-parametric statistics were applied according to the non-normal distribution of most of the data. Data are presented as frequencies or median and interquartile (IQR) range. χ^2 test or Fishers exact test were used to compare baseline, perioperative and demographic characteristics, our primary outcome, dropout rates and side-effects. Both intention-to-treat (ITT) and per protocol (PP) analyses were completed for the primary outcome. For "depression at one point" relative risk, number need to treat, relative risk reduction, and absolute risk reduction were calculated with 95% confidence intervals. Supplementary analyses where missing data were replaced in the ITT analysis with either "YES" or "NO" for depression were conducted. To analyze time without depression, the Kaplan-Meier method was used and the Mantel-Cox Log Rank Test was used for comparison. Area under the curve (AUC) was calculated for the subjective parameters using the following formula:

$$AUC_{A-Z} = (0.5xA)+(B+C+D+...)+(0.5xZ)$$

Last observation carried forward (LOCF) was used to fill out single missing data. The two groups were compared using Mann-Whitney test for AUC data on subjective parameters and MDI scores at the five measuring points. A Bonferroni correction was applied for multiple comparisons.

Paper IV:
Our primary outcome was cognitive function two weeks after surgery and our secondary outcomes were cognitive function three months after surgery, sleep diary data and sleep quality. Normality of the data was tested with the Shapiro-Wilk test and non-parametric statistics were applied according to the non-normal distribution of most of the data. Data are presented as frequencies or median and interquartile range, except for the results of the bootstrapping. All analyses were completed as per protocol analyses, as it is recommended not to substitute missing data in the neuropsychological testing [125]. The analysis of the neuropsychological test data were completed according to the formulas written above for Z-score and composite Z-score. Outcomes were the incidence of POCD in % at approximately 2 and 12 weeks postoperatively with 95% confidence intervals calculated at http://www.graphpad.com/quickcalcs/confInterval1/. Incidence of POCD between the 2 groups was compared by Fishers exact test. The seven variables of the four neuropsychological tests are reported in median and IQR for each group at each of the three test sessions.

Data from the sleep diary were calculated as a median for each patient in the two time periods. Instead of simply reporting medians for the two groups and a p-value using Mann-Whitney, the bootstrapping method was used as a resampling method providing the possibility of calculating a confidence interval for the mean difference between the two groups. Bootstrapping using the "smean.cl.boot" function in the "Hmisc" library in R version 3.0.1 (R Foundation for Statistical Software, Vienna, Austria) was performed with 10000 bootstrap samples and p-values were calculated by an unpaired t-test.

Paper V:
Our primary outcome was the measurement of depression or depressive symptoms with a validated clinician-administered or self-rating questionnaire. Our secondary outcome was adverse events.

The are several methodological considerations when performing meta-analyses, including types of data and effect measures in the studies, but also the study design and possible unit-of-analysis issues. For the meta-analyses, data were extracted as mean and standard deviation, or otherwise converted using SD = SEM x √n. Using one of the approaches suggested by The Cochrane Handbook for Systematic Review of Interventions [126] for including a cross-over trial in a meta-analysis, measurements from the two groups were analyzed as if the trial was a parallel group trial, even though we are aware of the unit-of-analysis issue and the more conservative estimate. If we had had many more studies it would be more appropriate to do sensitivity analyses by producing separate meta-analyses for randomized controlled trials (RCTs) and cross-over trials respectively, to clarify the differences in effect of the study designs.

As we had continuous data and our effect measure was mean difference, we chose the method of inverse variance and random-effects. Inverse variance has the advantage of giving larger studies with smaller standard errors more weight than smaller studies with larger standard errors [126]. Heterogeneity is the observed intervention effects being more different from each other than expected by random error (chance) alone and it is a consequence of clinical (participants, interventions, outcomes) and methodological (study design, risk of bias) diversity in the studies [126]. A random-effects method incorporates heterogeneity among studies and even though our I^2 values were 0% and 44%, which may not at all be important, we chose the more conservative estimate of the random-effects method. A meta-analysis with an I^2 of 0%, as in our second meta-analysis, gives the same result for both fixed- and random-effects.

OBJECTIVES

This thesis is based on three studies resulting in five papers and the specific objectives were:
- ➢ To examine whether the occurrence of postoperative cognitive dysfunction was associated with the PER3 genotype in patients undergoing noncardiac surgery (Paper 1)
- ➢ To clinically investigate the effect of melatonin on depression, anxiety, cognitive function and sleep disturbances in patients with breast cancer (Paper 2, 3 and 4)
- ➢ To systematically review the literature on the therapeutic and prophylactic effect of melatonin against depression and depressive symptoms (Paper 5)

PAPER PRESENTATION

PAPER 1: THERE IS NO ASSOCIATION BETWEEN THE CIRCADIAN CLOCK GENE HPER3 AND COGNITIVE DYSFUNCTION AFTER NON-CARDIAC SURGERY

Aim:
In this case-control study we aimed to investigate whether patients with the specific clock-gene PER3$^{5/5}$ genotype would have an increased risk of postoperative cognitive dysfunction one week after noncardiac surgery.

Methods:

From an original study of 976 patients, this study included a subgroup consisting of the 93 patients with POCD in the original study, who were matched with 186 patients without POCD in a 1:2 ratio. Blood samples previously collected were analyzed by polymerase chain reaction analysis of DNA to genotype the PERIOD3 gene: $PER3^{4/4}$, $PER3^{4/5}$, and $PER3^{5/5}$. The patients were aged 40 years and older, had undergone noncardiac surgery and been tested with a neuropsychological test battery consisting of seven subtests preoperatively, one week and three months postoperatively. The study was registered on www.clinicaltrials.gov (NCT01088100).

Results:
Due to missing samples and missing PCR products a total of 89 patients with POCD and 182 patients without POCD were genotyped. The distribution of the three genotypes was 11.8% $PER3^{5/5}$, 41.7% $PER3^{4/5}$ and 46.5% $PER3^{4/4}$. There was no significant difference in the distribution of PER genotype according to POCD one week after surgery (p=0.677). The absolute risk differences of incidences between the groups with and without POCD were -6% (-18% to 7%) for $PER3^{4/4}$, 5% (-8% to 17%) for $PER3^{4/5}$, and 1% (-7% to 10%) for $PER3^{5/5}$. There was no significant difference in the individual Z-scores for the seven subtests or the total Z-scores from the first postoperative session among the three genotypes.

Strengths and limitations:
No gold standard for neuropsychological testing of POCD exists and therefore various tests have been used in previous studies but the major strength of this study is the use of the ISPOCD battery which has been extensively used in surgical patients through the last 15 years [30-33;111]. The benefits of this neuropsychological test method are that it takes into account: the baseline performance of the individual, variability in a control population and practice effects. In addition, the results are constructed to depict both general deterioration and substantial deterioration in only some tests.

Another strength is the diversity of our population with regard to both age and type of surgery as it makes our findings more generalizable. Although we did not find the association we hypothesized, we have for the first time presented the distribution of this genotype in a surgical population and this can now provide the basis for future studies, including sample size calculations in this field.

Selection bias is to be considered as the original studies, providing our sample of 976 patients, consist of elective, surgical patients who voluntarily chose to participate and who all completed the neuropsychological testing. These factors could be a source of selection bias as patients not choosing to participate or dropping out early and not completing the testing could have been those with the $PER3^{5/5}$ genotype, major postoperative sleep disturbances and associated POCD. Ultimately, this could have led to an underestimate of patients with $PER3^{5/5}$ and POCD.

Our study is based on the fact that we "only" had 93 patients with POCD in the original study, hereby setting the limit for the possible size. These patients were matched as stringently as possible with a control group for age, sex, type of surgery, educational level and ASA (American Society of Anesthesiologists) score. Optimally, we could have performed propensity matching but as it can be seen in Table 1 of the paper, the characteristics were similar for patients with POCD, the controls and the entire cohort, proving that our matching had been sufficient with respect to the criteria we chose. Although, it cannot be completely ruled out that a negative confounder could be an explanation for our negative findings in the association between $PER3^{5/5}$ genotype and POCD.

In designing the study, a limitation was our assumption that 25% would have the $PER3^{5/5}$ genotype in the group with POCD. As no previous studies had been made regarding the PER3 genotype and POCD, our assumption of the 25% with the $PER3^{5/5}$ genotype in the POCD group was an estimate. Ending up with 89 patients in the POCD group and 182 in the control group, we had a statistical power of just below 85% to find the a priori assumed difference of 25% having the $PER3^{5/5}$ genotype in the group with POCD and 10% in the group without POCD. In this relatively large sample we have not detected an association, although we cannot exclude that low statistical power, a Type II error, could be an explanation. We are aware that we were unable to exclude an association, but we are confident that the difference in the occurrence of the $PER3^{5/5}$ genotype according to POCD (if any) is small and most likely not clinically important.

A significant, although inevitable limitation due to the retrospective design, is that we did not have data on postoperative sleep architecture or subjective sleep quality. Without these data we are missing the biological link between PER3 genotype and POCD and cannot determine whether patients with specific genotypes actually had disturbed sleep or not. The overall hypothesis of this pilot study was to investigate whether there was a link between POCD and genotype of the PER3 gene. The ideal setting would certainly have been a study with sleep data. Accordingly, this limitation warrants the need for future studies with PER3 genotype, sleep quality and quantity and POCD to entirely rule out an important association between PER3 genotype and POCD after non-cardiac surgery.

Conclusion:
This study did not show an association between genotype of the PER3 gene and POCD after noncardiac surgery, though low statistical power could be an explanation. If $PER3^{5/5}$ is associated with a deterioration in cognitive performance, the difference in the incidence of developing POCD versus not developing POCD in this group of patients is less than 10%.

PAPER 2: THE EFFECT OF MELATONIN ON DEPRESSION, ANXIETY, COGNITIVE FUNCTION AND SLEEP DISTURBANCES IN PATIENTS WITH BREAST CANCER. THE MELODY TRIAL: PROTOCOL FOR A RANDOMISED, PLACEBO-CONTROLLED, DOUBLE-BLINDED TRIAL.

Aim:
The main aim of publishing our trial protocol was to promote transparency, as well as preventing unnecessary duplication of research and at the same time inform patients, the public and the ethics review boards of this planned trial [127]. The protocol was also used as a document for applications to the local Ethics Committee, the Danish Medicines Agency and to apply for funding and grants and used for reference by the Good Clinical Practice Unit who were to monitor the trial.

Methods:
To prospectively write a document (although the actual protocol article was published after recruitment was started) with details on the rules and intended methods of conducting, analyzing and reporting the trial before recruiting patients. This document was also designed to provide sufficient detail to enable understanding of the background, rationale, objectives, study population, inter-

vention, methods, ethical considerations and statistical analyses [128]. A timeline of the trial can be seen below in figure 4.

FIGURE 4 – Timeline of the MELODY trial
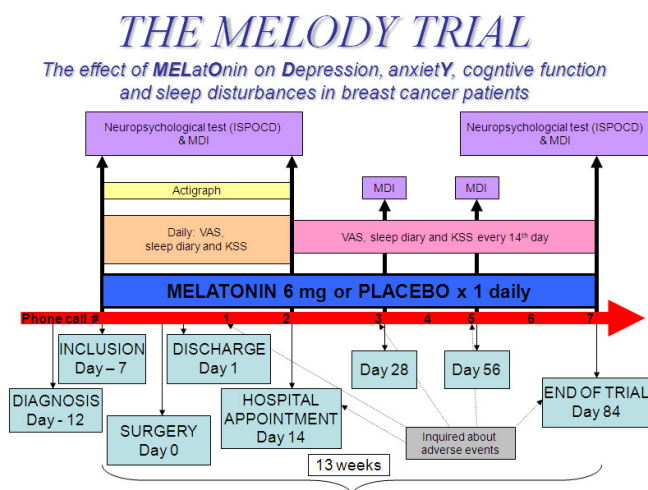
Strengths and limitations:
In general the main strength of writing a protocol article is that it promotes transparency and restricts the likelihood of posthoc changes to the trial methods and hereby selective reporting. Unnecessary duplicates of the trial are avoided and both the general public and all health professionals are informed of the trial.

As the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) 2013 [128], a guideline for the minimum content of a clinical trial protocol was not available at the time of writing this protocol article, the content is not in full accordance with this and some limitations of the protocol exist. Firstly, all outcomes, especially our primary outcome should have been specified in much more detail. This specification should have included the specific measurement variable, analysis metric (e.g. change from baseline, final value, time to event), method of aggregation (e.g. median or proportion) and time point for each outcome [128]. Especially for our primary outcome, we should have specified whether it was MDI total score or ICD-10 classification prevalences. For all VAS outcomes we should have specified the analysis metric i.e. AUC, but also the time point for the outcomes. In addition the statistical analyses for the all outcomes should have been more detailed and subgroup analyses should have been mentioned. We should not have included intragroup comparisons as this is not what an RCT is designed to test. With regard to completing both intention to treat and per protocol analyses, a plan for how to handle missing data should have been described. In retrospect, we should also have included a paragraph about stopping prematurely and if doing so, which types of analyses we would have expected to be able to conduct.

PAPER 3: EFFECT OF MELATONIN ON DEPRESSIVE SYMPTOMS AND ANXIETY IN PATIENTS UNDERGOING BREAST CANCER SURGERY: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

Aim:
We aimed to investigate whether melatonin could lower the incidence of depressive symptoms during a three-month period after surgery in women with breast cancer. In addition, we investigated the effect of melatonin on the subjective parameters of anxiety, sleep, general well-being, fatigue, pain and sleepiness.

Methods:
The design was a randomized, double-blind, placebo-controlled trial, originally intended to include 2x130 patients. Eligible women were aged 30-75 years, undergoing a lumpectomy or mastectomy for breast cancer and without signs of depression on the Major Depression Inventory (MDI). We included patients approximately one week before surgery and randomly assigned them to receive 6 mg oral melatonin or placebo for three months. The incidence of depressive symptoms measured by MDI ≥21 was the primary outcome. Secondary outcomes were anxiety, sleep, general well-being, fatigue, pain, and sleepiness measured by VAS or KSS. AUC was calculated for the subjective parameters (anxiety, sleep, general well-being, fatigue, pain, and sleepiness) in the short-term perioperative and the long-term postoperative period. The trial was registered on www.clinicaltrials.gov (NCT01355523) and the trial was reported according to the CONSORT (Consolidated Standards of Reporting Trials) guidelines [129].

Results:
The trial was terminated prematurely due to restructuring and lack of funding. 703 patients were screened for eligibility; 432 excluded due to not meeting the exclusion criteria, 70 declined to participate and 147 could not be included due to logistical problems (Figure 5). Fifty-four patients were randomized to melatonin (n=28) and placebo (n=26), respectively. Baseline characteristics were similar between the two groups. A total of 11 patients withdrew from the study; 10 in the placebo group and 1 in the melatonin group (p=0.002).

A significant difference between the groups was found for the incidence of depression at any point in the study. 45% (9/20) of patients had depressive symptoms at any time point compared to 11% (3/27) in the melatonin group. The relative risk was 0.25 [95% CI 0.076-0.80], number needed to treat 2.95 [95% CI 1.703-11.024] and absolute risk reduction 0.34 [95% CI 0.083-0.559]. An effect of the intervention was also illustrated by a Kaplan-Meier curve (p=0.007). The analyses where missing data were filled out with "YES" or "NO" for depression also showed a significant difference, p=0.002 and p=0.035, respectively.

No significant differences were found between any of the subjective parameters in the two groups, neither in the short-term perioperative or long-term postoperative period. The groups did not differ with respect to side effects either (p=0.78).

FIGURE 5 – CONSORT diagram

ITT = Intention to treat, PP = Per protocol, MDI = Major Depression Inventory
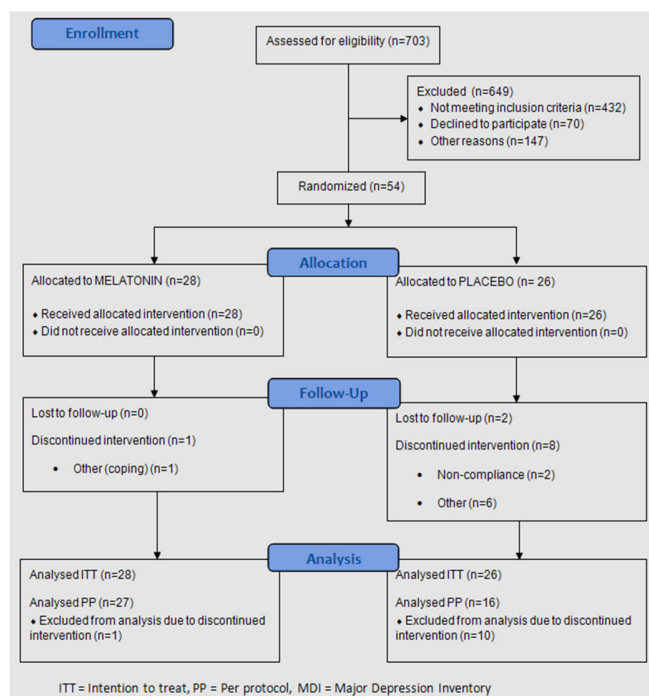
General strengths and limitations:

A major limitation was the premature termination of the study. Having a sample size of 54, the statistical power was 26% as we aimed at detecting a difference in the incidence of depressive symptoms between 30% and 15% (significance level 5%) (http://www.stat.ubc.ca/~rollin/stats/ssize/b2.html). If the MELODY trial was to be viewed as a pilot study and give rise to further investigations, a sample size calculation using the incidences of depression of 45% and 11% in the placebo and melatonin group respectively, a power of 80% with a risk of a Type I error of 5% and a Type II of 20%, 32 patients should be included in each group.

Since we were not able to perform the statistical analyses stated in the protocol due to a smaller sample size, we compared multiple categories of depressive symptoms at any time instead of specific measurement time points of varying severity of depression. We are aware that this might have increased the risk of a Type I error, but we found it acceptable with respect to the smaller sample size. On the contrary, an even larger effect of melatonin might be present as a placebo effect is most likely in a study like this [130, 131]. It would have been interesting if we had included a control group without any intervention to follow the natural course of depressive symptoms using MDI.

Regarding the low external validity and generalizability of the study, we have retrospectively looked at the reasons for only including approximately 8% of the screened patients and two of the main reasons are the strict exclusion criteria and patients declining to participate. The former was mainly based on the summary of product characteristics (SPC) for melatonin from Pharma Nord (Vejle, Denmark) and future investigations could be done to find out whether these need to be as strict in future studies. The latter is a well-known problem that including patients with cancer in clinical trials is difficult [132]. It has been shown that women with newly diagnosed breast cancer have less favor-able attitudes toward randomized trials and were twice as likely to decline to participate compared with women with a benign diagnosis [133]. We acknowledge that the time of inclusion is vulnerable as the patients had just been diagnosed with cancer, however our proactive (approaching patients in person), instead of reactive (letter or email) method of inclusion has previously been shown to increase the chance of recruitment [134] and the design of our study and hypotheses of melatonin's prophylactic effect limited the possible time span for inclusion.

The unequal distribution of dropouts in this study is an interesting issue, which we have chosen to see as another strength, or at least as a positive, unexpected finding. We interpret this as an indirect effect of the melatonin treatment. We hypothesize that it either is due to a decrease in depressive symptoms alone or to some undescribed mechanism that melatonin may have enabled them to complete the trial. Confirmation of this theory is also seen in the fact that three women in the placebo group dropped out of the study right after they scored ≥ 21 on the MDI, whereas this did not happen in the melatonin group. This leads us to hypothesize that the depressive symptoms could be part of the reason for dropping out. If we had assessed whether participants were able to ascertain their treatment assignment (efficacy of blinding) and if we had also collected data on whether dropouts developed a depression, it could have supported our post-hoc hypothesis.

In RCTs, ITT analysis is recommended, as it reflects the clinical situation and avoids bias arising with the non-random loss of participants [129], however we also chose to perform PP as we had to deal with the issue of missing data. To underline our main result and as an extra strength, we chose to perform worst and best case scenario analyses [135] where missing data were filled out with "YES" or "NO" for depression and both were significantly in favor of melatonin. It is a limitation that we did not perform an analysis of differences in variables between dropouts and completed patients. It could have been interesting to see if there was a difference in variables known to predict outcome, i.e. adjuvant treatment, age, menopausal status, job status, relationship status.

The internal validity of the study is based on the extent to which the design and conduct of the trial eliminate the possibility of bias. Using the Cochrane risk-of-bias tool [136] critically on our study, it can be noted that there is a low risk of selection, performance and detection bias. Furthermore, even though we have incomplete outcome data and we find it to be adequately addressed, it can still be discussed whether there is a risk of attrition bias. With regard to selective reporting, our protocol was not explicit enough and our sample size was not reached. Therefore, there is not total adherence to the specified statistical plan and outcomes, leading to a high risk of reporting bias.

Specific strengths and limitations with regard to outcomes:

The use of the Major Depression Inventory can retrospectively be seen as both a strength and a limitation of the MELODY trial. The main strengths are that it has the quality of being both a screening/diagnostic instrument (as we used it at inclusion) and being able to monitor depression as a measuring instrument (as we did for the following assessments). A priori, we hypothesized that many women might already have a depression measured by the MDI at the time of diagnosis and inclusion, so we conducted a pilot study on 21 patients (unpublished data) and only one woman had a mild depression, rated diagnostically. This result led us to believe that the MDI was applicable in the context in which we

wished to use it, it was brief (takes about 5 minutes), and easy to understand.

However, it could have been more informative and could have provided greater external validity and comparability if we had used another more widely used self-rating questionnaire with known psychometric properties such as the Beck Depression Inventory, the Center for Epidemiological Studies Depression Scale, the Hospital Anxiety and Depression Scale or the Zung Self-Rating Depression Scale. If a clinician-administered scale had been logistically and financially possible, the Hamilton Depression Scale could have been a worthy alternative together with a self-rating scale, especially when considering comparability with other trials on antidepressants. In a broader perspective, using the more widely-used rating scales would also have made the data more accessible for systematic reviews and meta-analyses in comparison with other antidepressants.

A strength of choosing to calculate AUC for the subjective parameters (anxiety, sleep, general well-being, fatigue, pain and sleepiness) and using LOCF for the very few missing values was that we were able to use most of our data from both the daily and fortnight monitoring and give an overall picture of the two time periods. Conversely, a clear limitation is that more specific detail is lost and we combined both preoperative and short-term postoperative data together and could hereby have lost an effect of melatonin in one of these time periods. Equally, our use of VAS for most of the measurements could have been too imprecise to detect an effect of melatonin. Furthermore, had there been a statistically significant difference between the two groups, it would not have been possible to conclude whether it was clinically meaningful in respect to the existing literature on the topic, as AUC values cannot be directly compared with that purpose.

Regarding the specific analgesic and anxiolytic effect of melatonin, most studies have been done in the perioperative period [65, 67, 68]. An ameliorating effect on anxiety in the perioperative period has been found [65], as have clinically relevant analgesic effects [67, 68]. The 6 mg melatonin given in our study was similar to doses given in other analgesic and anxiolytic studies [65]. An explanation for why we did not find any effect on pain or anxiety could be a simple Type II error because of the small patient sample, or it could be explained by our longer measuring period of two days preoperatively till eight days postoperatively and calculation of AUC, as we could have overlooked the effect of melatonin in the very short perioperative period.

Conclusion:
In women with breast cancer, the risk of depressive symptoms was significantly reduced by 6 mg oral, daily melatonin in a three-month time period after surgery and the treatment was well tolerated. No effect of melatonin on the subjective parameters of anxiety, sleep, general well-being, fatigue, pain, and sleepiness was found.

PAPER 4: EFFECT OF MELATONIN ON COGNITIVE FUNCTION AND SLEEP IN RELATION TO BREAST CANCER SURGERY: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

Aim:
We aimed to assess the effect of melatonin on cognitive function and sleep in a three month period after breast cancer surgery.

Methods:
As this study reports on secondary endpoints from the aforementioned RCT focusing on depressive symptoms, the design, inclusion/exclusion criteria and the intervention were the same. Cognitive function was evaluated with a neuropsychological test battery and POCD was defined on the basis of a Z-score >1.96. Sleep diary recordings were used to assess sleep latency, number of awakenings, total sleep period and sleep efficiency. Subjective sleep quality was assessed by VAS. The trial was registered on www.clinicaltrials.gov (NCT01355523).

Results:
A total of 54 patients were randomized to melatonin (n=28) or placebo (n=26) and 11 patients dropped out of the study (10 placebo and 1 melatonin, p=0.002). Two weeks postoperatively the incidence of POCD was 0% (0/20) [95% CI 0.0%;16.8%] in the placebo group and 0% (0/26) [95% CI 0.0%;13.2%] in the melatonin group (p=1.00). Three months postoperatively the incidence of POCD was 6.3% (1/16) [95% CI 0.0%;30.2%] in the placebo group and 0% (0/26) [95% CI 0.0%;13.2%] in the melatonin group (p=0.38).

Sleep efficiency in the perioperative period was significantly greater in the melatonin group than the placebo group with a mean difference of 4.28% [95% CI 0.57;7.82] (p=0.02). Total sleep period was significantly greater in the melatonin group than the placebo group in the long-term postoperative period with a mean difference of 37.0 min [95% CI 3.6;69.7] (p=0.03). Subjective sleep quality measured by VAS did not differ at any time between the two groups.

Strengths and limitations:
The major strength of this study was the use of the widely used ISPOCD neuropsychological test battery with its advantages mentioned above in the materials and methods section. In spite of these advantages, some aspects deserve mentioning, especially since we found a lower incidence of POCD than expected.

Firstly, the baseline measurement of neuropsychological testing should represent an optimum; otherwise it can be more difficult to detect a deterioration. As our baseline measurement was close to the time point when the patients were diagnosed with cancer and close to their day of surgery, this could influence mood and anxiety, which has been shown to have a negative impact on motivation and performance [125]. Secondly, the intervals between the test sessions may have an influence, as our first postoperative test was possibly performed too late to detect an early deterioration. Thirdly, variability is a known problem in neuropsychological testing and may be reflected in the results, especially when repeated testing is done to detect minor changes in cognitive function [137]. It is impossible to avoid small differences in motivation and mental well-being in a course like this from cancer diagnosis, through surgery and oncological treatment. In this respect, we acknowledge that it is a limitation that we tested on days where preoperative anxiety, thoughts about a cancer diagnosis and treatment and side effects from chemotherapy could have influenced the results. However, this is the set-up that was possible in the clinic and these confounding factors are the same for all patients.

It is known that dropouts constitute a substantial methodological limitation in testing for POCD, as POCD may be more common in patients unwilling or unable to undergo testing [138]. In our case with the uneven distribution of dropouts, this could have led to an underestimation of POCD. As excluding missing data from the analysis is the preferred method

[125], we only did PP analyses and not ITT. It is interesting to note, that if we hypothesized that dropouts had POCD and completed an ITT with the same approach as used in Study 3, it seems that melatonin tended to decrease the incidence of POCD (p-values of 0.135 and 0.004 at the first and second postoperative test session, respectively).

It could have been interesting if we had measured perceived cognitive function as well as objectively assessed cognitive function, as a previous study in patients with breast cancer has shown that these two do not necessarily coincide [21].

A limitation of our reporting of the sleep diary and sleep quality data is that a specific value for each parameter in each group with an associated statistical test, i.e. median and IQR, tested by Mann-Whitney is missing. This could have been useful to portray any clinical relevance of the calculated mean differences.

Conclusion:
POCD was not a prevalent problem in this study measured by the ISPOCD neuropsychological test battery; neither two weeks nor three months after surgery. Melatonin increased sleep efficiency perioperatively and total sleep time postoperatively after lumpectomy or mastectomy for breast cancer compared to placebo. Subjectively assessed sleep quality did not show any differences between the two groups at any time point and no differences in side effects were found.

PAPER 5: THE THERAPEUTIC OR PROPHYLACTIC EFFECT OF EXOGENOUS MELATONIN AGAINST DEPRESSION AND DEPRESSIVE SYMPTOMS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Aim:
The aim was to quantify the existing evidence on the prophylactic or therapeutic effect of melatonin against depression or depressive symptoms in adult patients and to assess the safety of melatonin.

Methods:
This review and meta-analyses were performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [139] and used the Cochrane risk-of-bias tool [136]. A literature search was performed on November 14th 2013 in The Cochrane Library, PubMed, EMBASE and PsychInfo with the following search strategy:

> **((melatonin) AND (depression OR depressive disorders OR mood disorders OR depressive symptoms) AND (therapeutics OR treat* OR effect*))**

We included RCT or crossover trials, reported in English, in which patients with a depression or who were predisposed to developing a depression or depressive symptoms were included. Our main outcome was the measurement of depression or depressive symptoms by a validated clinician-administered or self-rating questionnaire.

Results:
Out of 304 screened records, we included 10 studies with data from 486 patients (Figure 6). Four of the studies with data from 148 patients were included in two meta-analyses; prophylactic and therapeutic effect respectively. Two studies were therapeutic (patients were depressed at the time of inclusion), three studies

were prophylactic (patients were not depressed at the time of inclusion) and the five remaining studies included a mixture. The dose of melatonin given ranged from 0.5-6 mg daily and the length of follow-up varied from 2 weeks to 3.5 years. A significant prophylactic effect of melatonin compared to placebo was found in one study and a significant treatment effect was found in another study. Improvement in depression scores in both the melatonin and placebo groups were found in six studies, but without a significant difference between the groups. No significant effect of melatonin was found in either of the two meta-analyses. No serious adverse events were reported.

FIGURE 6 – PRISMA flow diagram
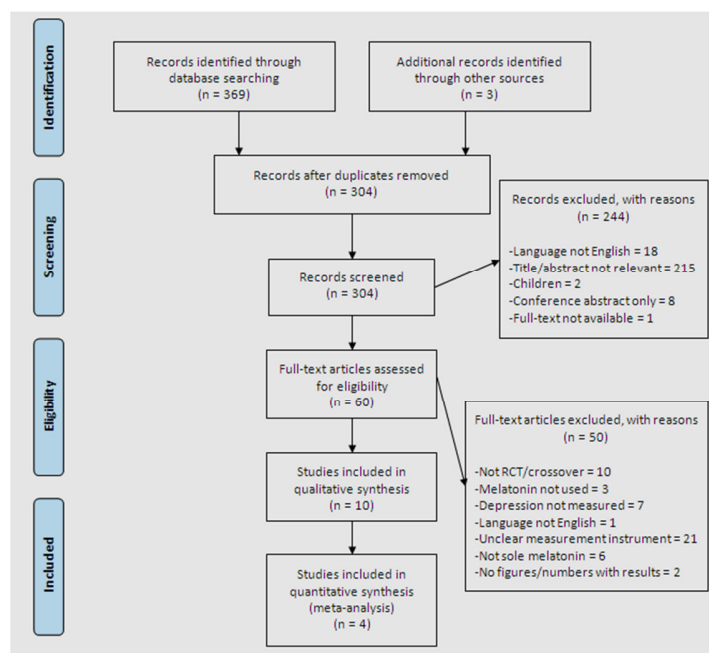
Strengths and limitations:
The overall strength of a systematic review lies in the systematic approach to summarizing and appraising the current literature relevant to a specific research question. A systematic review is regarded as the strongest form of medical evidence 1a [140], as it is better at assessing the strength of evidence, than single studies alone. In this systematic review we have examined quantitative evidence, including both RCTs and crossover trials. This in itself is a strength as we have summarized high levels of evidence, although a systematic review is by no means better than the studies included in it, hence we considered both the heterogeneity and assessed the quality of the included studies.

We used the Cochrane risk-of-bias tool which is one of the most widely used tools for this type of assessment [136]. Due to the crossover design of half of the included studies, we chose to add an assessment of risk of bias considering whether statistical consideration was given to the carry-over and/or period-effect in these trials. The overall risk of bias was intermediate with the main problem being that many of the studies had a high risk of attrition and reporting bias. The studies were quite heterogeneous with regard to patient group, dose and timing of administration; hence we concluded that further studies were warranted.

In the search process publication, language and selection bias are possible issues of concern. We agreed on the

databases that were the most suitable and comprehensive for our literature search, although one could always argue that other databases e.g. CINAHL and SCOPUS also should have been searched. Furthermore it could have been relevant to search for unpublished studies or contact key authors in the field, although it is unlikely that this would have changed our overall conclusion as unpublished studies more often have negative results [126]. A method of assessing the presence of publication bias is by using a funnel plot, although since we had less than ten studies in our meta-analyses, a funnel plot was not appropriate.

Language bias is worth mentioning as we did exclude 18 records and 1 full-text article due to non-English language, although we do not expect that this could have changed our overall results significantly, as it has been shown that excluding non-English trials generally has little effect on the overall estimate of effect [141].

Two authors independently assessed full-text articles for eligibility, extracted data and evaluated risk of bias. Discrepancies were settled by discussion but no comparative statistics on the degree of disagreement were performed. Cohen's kappa coefficient is a statistical measure of inter-rater agreement between two raters, which could have been used in this case. We believe selection bias to be at an absolute minimum as disagreements in the process of including studies were rare.

An obvious limitation of our systematic review was that we did not register or publish a formal protocol on beforehand. The main advantages of conducting a review according to a prespecified and registered protocol are that it ensures transparency, promotes collaboration, minimizes the risk of duplication and minimizes publication bias and the risk that some of the results retrospectively could influence the inclusion of other studies, the data synthesis or other changes in the review [142].

Our decision not to include studies using an unclear measurement of mood or depressive symptoms was a choice based on scientific reasoning, as the heterogeneity would have increased and the comparability between studies decreased. It could however be interesting to conduct a systematic review on melatonin's effect on mood in general, although the possibility of performing meta-analyses would probably be lost, unless it could be an option with a widely used instrument; i.e. VAS scale for mood.

We acknowledge the fact that two studies in a meta-analysis is not a lot, however there was consensus that the uniformity of the measuring instrument and the prophylactic or therapeutic nature of the studies provided a solid base for comparing these studies quantitatively by using the mean difference. We chose not to use standardized mean difference as a summary statistic, although this could have given us the possibility of assessing the outcome of more of the studies by standardizing the results on the different psychometric scales to a uniform scale. We are aware of this limitation and would definitely try to do this in an updated version of the review, although we are convinced that this would not have changed the overall results either. We conducted both fixed- and random-effects meta-analyses. Random-effects are considered as more conservative with wider confidence intervals and since the difference between our results using fixed and random-effects were minimal, we chose to report the random-effects meta-analyses.

Conclusion:
The included studies were small and heterogeneous in respect of the dose and timing of melatonin administration and this systematic review highlighted the need for further studies. There was no clear evidence of a therapeutic or prophylactic effect of melatonin against depression or depressive symptoms, although some studies were positive.

DISCUSSION

PRINCIPAL FINDINGS
The conclusion of the case-control study (Study 1) included in this thesis was that POCD did not seem to be associated with the PER3$^{5/5}$ genotype one week after non-cardiac surgery.

The major positive findings in this thesis are derived from the RCT (Study 2). We found that melatonin had an effect on reducing the risk of developing depressive symptoms and could increase sleep efficiency perioperatively and total sleep time postoperatively in patients with breast cancer. However, we did not find an effect of melatonin on the subjective parameters of anxiety, sleep, general well-being, fatigue, pain or sleepiness; neither peri- nor postoperatively in patients with breast cancer. Postoperative cognitive function was not a prevalent problem in this patient group.

The systematic review (Study 3) included in this thesis did not find clear evidence of a prophylactic or treatment effect of melatonin for depression or depressive symptoms in adults, although further studies are warranted.

COMPARISON WITH PREVIOUS FINDINGS

The genetics of POCD
The etiology and risk factors of POCD are still under investigation and hereditary vulnerability has become a topic of recent investigations [40]. Many candidate genes have been investigated for their association with POCD after non-cardiac surgery. APOE is by far the most thoroughly investigated showing both positive [143-145] and negative results [111, 146, 147]. Polymorphisms in CYP2C19 or CYP2D6 genes have not shown any association with POCD after non-cardiac surgery [148]. Furthermore, the genes encoding complement component 3, complement factor H [149] and the promoter region of inducible nitric oxide synthase [150] have been shown to be associated with cognitive performance within 1 month or less after carotid endarterectomy, specifically.

The differences in the patient populations, types of surgery, the timing of the tests and divergent test batteries leading to various phenotypes of cognitive dysfunction makes comparisons between the genetic studies difficult. Most importantly, the scientific rationale for the association of cognitive dysfunction with a specific gene varies tremendously in the studies making it difficult to draw conclusions and as our study (Study 1) is the only study with the rationale of sleep deprivation leading to cognitive disturbances, direct comparisons to other studies are not obvious.

For future research, it is first of all necessary to reach consensus on the methods to identify POCD, otherwise it will hamper the genotype:phenotype correlation. The next step must be to identify possible genetic vulnerabilities by conducting family studies in twins and siblings, animal studies, sequencing of candidate regions, possibly from other conditions influencing cognitive function, and sequencing whole genomes in patients with outermost POCD phenotypes [40]. The final step is to test for association between these identified, suspect genes and POCD in large sample sizes.

Depressive symptoms and prophylaxis

Despite depression being a substantial problem with severe consequences for patients with breast cancer, the paucity of RCTs, open-label or head-to-head trials investigating treatment of depression in this population is evident [17, 151-153]. Interestingly, a high rate of dropouts, ranging up to as high as 55%, is a problem in many of the studies; two of the main reasons being a lack of effect or adverse effects [154-158].

Targeting prevention of the depressive symptoms rather than treatment once the symptoms arise is another option which is well-known in other populations, as antidepressants have shown to be able to prevent poststroke depression [159] and interferon-α-induced depression in patients with malignant melanoma [160] and hepatitis C [161]. The optimal timing and duration of treatment is yet to be known in these categories of patients and the potential benefits associated with the prevention of depression must be weighed against the risks associated with the use of antidepressant agents [162, 163], especially when giving them for another indication than treatment of depression.

A successful pharmacological strategy for prevention of depressive symptoms, aimed at individuals with an especially high risk and implementation during the initial, critical time period of breast cancer diagnosis, could limit the impact of depression, prevent a decrease in the quality of life, prevent non-compliance to oncological treatment and overall decrease the associated risk of morbidity and mortality. The scarcity of pharmacological trials in patients with breast cancer and cancer in general, where prevention of depression or depressive symptoms is clearly described as the main focus and where patients with depression are excluded, is even more evident than the scarcity of treatment trials.

Solely in patients with breast cancer, three RCTs which did not have depression as an eligibility criterion have been conducted; two positive and one negative study [164-166]. Razavi et al. [164] investigated the effect of alprazolam/placebo and a psychological support program in 57 patients and did not show any difference between the groups measured by the Montgomery and Asberg Depression Rating Scale and the Hospital Anxiety and Depression Scale. Roscoe et al. [165] investigated the effect of paroxetine hydrochloride/placebo in 94 patients and showed a significant reduction of depression scores measured by Center for Epidemiologic Studies-Depression and Profile of Mood States-Depression subscale. Navari et al. [166] investigated the effect of fluoxetine/placebo in 193 patients and showed a significant reduction in depressive symptoms on Brief Zung Self-Rating Depression Scale compared to placebo. However, the three studies are not entirely preventive. In one study patients had mean scores of mild depression at inclusion, although they did not have a DSM-III diagnosis as this was an exclusion criterion [164]. In another study, 28% of patients were significantly depressed at inclusion, although the use of psychotropic medications was an exclusion criterion [165]. In the last study, patients were eligible if they had some depressive symptoms on a 2-item depression screener, but were excluded if they were taking antidepressants [166]. Dropout rates in the three studies were 7% [166], 16% [164] and 34% [165] respectively, and there was no significant difference between the groups.

Two RCTs with the clear aim of preventing depression have been done in patients with head and neck cancer, investigating the prophylactic effect of citalopram and escitalopram, respectively [167, 168]. These studies excluded patients with a pre-existing depression and patients taking antidepressant medication. A trend towards a decrease in the incidence of depression was found in the pilot study in 36 patients [167] and a significant decrease in the risk of developing depression by 50% was found in the other study in 148 patients [168]. The dropout rates were 36% [167] and 16% [168] respectively, with no difference between the groups.

Three other RCTs have been conducted in a mix of cancer patients investigating the effects of sertraline, paroxetine, fluoxetine against placebo on depression [169-171]. Overall, these studies can be regarded as prophylactic as patients did not have a depression at inclusion, assessed by either a clinician-administered or a self-rating scale. Two of the studies [170, 171] showed a reduction in symptoms of depression and one study [169] was terminated prematurely due to no significant benefit of treatment. Dropout rates varied between 12-21% without any differences between the groups.

When considering a direct comparison with the MELODY trial (Paper 3), many aspects should be mentioned. Firstly, the MELODY trial was preventive as this was our main aim and baseline median MDI measurements were 6.5 and 7 in the melatonin and placebo groups, respectively, indicating no depression and very low total scores for depressive symptoms. Many of the aforementioned studies are not entirely prophylactic, as baseline depression scores vary, although exclusion of patients with depression or patients taking antidepressants was common for them all. Secondly, the advantage of using a relatively non-toxic drug, melatonin, compared with a benzodiazepine or SSRIs, which both have several known adverse effects [162, 163, 172]. Thirdly, the duration of treatment varies from about 8 weeks to 12 months. Fourthly, measuring instruments of depression/depressive symptoms with both self-rating and clinician-administered were used. Finally, dropout rates in all the aforementioned studies varied between 7-36% which is comparable with the dropout rate of 20% in the MELODY trial, although the dropouts varied significantly between the groups in our study with more placebo patients dropping out, which was not the case for any of the other studies. This again supports our hypothesis that melatonin has an effect on keeping participants in a trial. In many of the studies discussed above, adverse effects were a common reason for dropout in the treatment arm [165, 168], which was not the case in the MELODY trial as the only dropout in the melatonin group, withdrew due to not being able to cope with participation in the trial.

POCD in patients with breast cancer

In general cognitive disturbances are a common problem in cancer patients before, during and after treatment [173]. It has been shown that even before the start of any adjuvant therapy, 35% of women with breast cancer exhibit objectively measured cognitive impairment [22], highlighting that the cancer itself or the surgery may be possible contributing factors. Moreover, the high, sustained levels of psychological stress experienced by patients after diagnosis of cancer could also lead to cognitive changes [21, 174] and these changes could also be a contributing factor or a symptom of depression [17].

When specifically considering the type of cognitive disturbances arising after surgery, POCD, we found a surprisingly low incidence in the MELODY study (Paper 4) when compared to a study in a similar age-group of patients after major abdominal or orthopedic surgery [33] and another study after major non-cardiac surgery in patients ranging from 18 years and up [35]. The first study found an incidence of POCD of 19.2% after one week and 6.2% after 3 months [33]. The other study found,

specifically in middle-aged patients (40-59 years), an incidence of POCD of 30.4% after one week and 5.6% after 3 months [35]. Both studies also used the ISPOCD test battery, although the timing of the first test was about one week later and the second test two weeks earlier in the MELODY trial, which could influence detection of POCD, especially at the first test.

When taking into consideration the possible risk factors for the development of POCD of advanced age, duration of anesthesia and type of surgery, several possible explanations for our findings must be considered. With regard to age, the comparability of the MELODY trial (Paper 4) with these two aforementioned studies is obvious with the age of patients ranging from approximately 40-60 years. The duration of surgery in the MELODY trial did not differ much from the two other studies either. The type and severity of the surgery could have a small part in the explanation, as it can be discussed whether lumpectomy and mastectomy are major surgery, even though the study by Monk et al. [35] did include some minimally invasive surgery. In the MELODY trial approximately 50% of the patients in both the melatonin and placebo group stayed one night postoperatively at the hospital, whereas the rest were discharged on the day of surgery. This is a major difference with respect to the two other studies as the inclusion criteria were an expected stay of two [35] and four [33] days after surgery, respectively. Finally, an overall explanation could be that much focus has been on improving anesthetic regimens and pain treatment and shortening length of hospital stay since these studies were conducted in 1998-2002.

Melatonin and its effect on sleep
In accordance with a previous review, we also found that melatonin increased total sleep time and sleep efficiency [175]. We also found the known effect on sleep latency when calculating our sleep diary data in median (IQR) and using a Mann Whitney test between the two groups; however, this effect was not present when using the bootstrapping method.

With regard to subjective sleep quality it has also previously been reviewed that melatonin significantly improves sleep quality in patients with primary sleep disorders [176]. The sleep quality data extracted in the meta-analysis of this review [176] included sleep quality assessed by various different rating scales. In comparison, we did not find any significant differences in subjective sleep quality assessed by VAS between the groups. One explanation for this could be that the VAS was too crude a measure to detect any minor differences. Yet another explanation could be that there simply was no difference, as the patients in both groups displayed normal sleep on all sleep diary parameters. In this case, the findings suggest that a possible effect of melatonin on sleep and sleep quality was not the basis for the difference in development of depressive symptoms.

Cytokine sickness and inflammation
Inflammation, whether resulting from cancer or its treatment, poses a risk for the development of pain, fatigue, cognitive impairment, anxiety and depression [57, 177, 178]. It could be proposed that the difference in dropouts in the MELODY study could be related to an overall well-being due to an attenuation of all of the symptoms mentioned above, even though we were not able to detect this in all parameters with our measuring instruments. The physiological/immunological explanation for this could be the effect of melatonin on the proinflammatory cytokines and the "sickness behavior", although this is theoretical and measure-

ments of IL-1, IL-6 and TNF-α would be needed to confirm any direct causality.

In addition, a possible link between depressive and insomnia symptoms in cancer patients could also be the process of inflammation [179]. This process has found to be both generated and enhanced by the sleep disturbances, the depression and the cancer itself, as well as by the adjuvant treatment with chemotherapy or radiation [179]. As far as these theories of sick cytokines and inflammation are concerned, it is once again highlighted why it is necessary to investigate a possible common etiology and a possible common treatment of the symptom clusters experienced by many cancer patients.

METHODOLOGICAL CONSIDERATIONS
Industry sponsorship and research funding can influence doctors in the decision-making process and create an industry bias that cannot be explained by the standard risk of bias assessment [180, 181]. With regard to the MELODY trial, a major strength is that the study is not fully industry-sponsored. The only form of funding received was the melatonin and placebo tablets from Pharma Nord who had no influence on the design and conduct of the study, collection, analysis and interpretation of data; preparation, review or approval of the manuscripts; or decision to submit the manuscripts for publication. Besides that, the study received no other financial support from the industry. We therefore perceive our study as being academic and not financially sponsored by the industry as grants were given from the University of Copenhagen and several private foundations and we do not believe this could have had any influence on our positive findings. This funding status is seen as a strength, adding to the validity of our results, as it has previously been shown that significantly more trials funded by for-profit organizations found conclusions recommending the experimental drug than in trials funded by non-profit organizations or a mixture of both types of funding [182], as the MELODY trial is. This could reflect some sort of bias in studies fully sponsored by for-profit organizations. Finally, a recent Cochrane Review has also shown that sponsorship of drug studies by the manufacturing company leads to more favorable results and conclusions than sponsorship by other sources [181].

This thesis includes both a systematic review with a 1a level of evidence, an RCT with a 1b- level of evidence with the "-" denoting wide confidence intervals in the RCT and finally, a case-control study with a 3b level of evidence [140]. In addition, prior to enrolling the first patient in the RCT, the study was registered on www.clinicaltrials.gov and a protocol article was published in an open-access journal shortly after trial commencement. The case-control study was also registered on www.clinicaltrials.gov before cases and controls were matched. Registration and publication of the protocol article both help to avoid reporting bias and possibly also publication bias. Furthermore, registration of a clinical trial also prevents unnecessary duplication of research and informs patients, the public and the ethics review boards of planned and ongoing trials [127]. Having published a protocol on beforehand prespecifies the methods of the randomized trial and restricts the likelihood of posthoc changes to the trial methods and hereby selective reporting [129].

The MELODY study (Paper 3 and 4) was reported according to the CONSORT guidelines [129] and the systematic review (Paper 5) according to the PRISMA guidelines [139]; both to ensure the transparent and complete reporting of an RCT and a systematic review with meta-analyses, respectively and also to

avoid selective reporting. As the SPIRIT guidelines [128] were not yet developed at the time Paper 2 was written and published, it does not strictly adhere to these guidelines.

FUTURE PERSPECTIVES

Not only the process of conducting the studies in this thesis but also the results, prompt for further research in this field; important research questions remain to be answered and important studies need to be completed:

> 1. A fully powered, multi-center version of the MELODY trial
> 2. Head-to-head trials with other anti-depressants in prevention studies
> 3. Dose-response studies of melatonin in depressive symptoms/depression
> 4. Melatonin's effect on the symptom clusters of breast cancer- and cancer patients in general – possibly mediated by an effect on cytokines
> 5. Melatonin's role as an anti-oxidant and anti-inflammatory agent in the prevention or amelioration of neurocognitive changes (cancer-related and treatment-related cognitive dysfunction) in breast cancer- and cancer patients in general
> 6. Hypothesis-generating studies on the genetic heritability of POCD

Ad 1: Due to the MELODY trial being underpowered and due to the possible broad implications of the results in other patient populations, the primary outcome of prophylaxis/prevention of depressive symptoms requires further investigation. With regard to the population, the effect should be investigated in patients with cancer in general, but also in other groups of patients where post-diagnosis/illness depression is a common problem, i.e. patients undergoing coronary artery bypass graft surgery, patients with acute myocardial infarction, patients with a stroke or patients with a chronic illness such as multiple sclerosis, diabetes or rheumatic arthritis.
Moreover, the exclusion criteria should be thoroughly evaluated to be able to enhance the external validity. Further investigations should be made into interactions of melatonin with SSRI, antithrombotic drug therapy, MAO inhibitors and calcium channel blockers to conclude whether exclusion is necessary or possibly just needing an adjustment in drug dose. Candidates for inclusion should be screened with Mini-Mental State Examination (MMSE) to detect large cognitive difficulties and then include patients up to 85-90 years of age. Patients with previously treated depression or bipolar disorder could be included and the results could be stratified as to whether melatonin has a prophylactic effect on patients who have never experienced psychiatric disorders and those who have. Patients with a previous cancer diagnosis could also be included and the results stratified accordingly. By this overall broader inclusion, a larger sample could be obtained and thereby also the possibility of stratifying the results by subgroup analyses. Consequently, an association between PER3 genotypes and depressive symptoms, cognitive function and sleep disturbances could be investigated in a larger sample.
When considering the measuring instrument, both a self-rating and a clinician-administered scale should be considered. Likewise, a higher dose of melatonin and a longer

treatment and longer follow-up period should be considered. To be comparable to other doses of melatonin analogs [183] and to the human equivalent dose calculated from the animal doses in mg/kg(http://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf) doses in the range of 50-100mg/day are suggested. As the prophylactic effect of antidepressants in poststroke depression seems to more effective when continued over a period of at least 12 months [159], a longer period of treatment should be evaluated. Finally, quality of life, compliance of adjuvant treatment, morbidity and mortality should also be measured with a longer follow-up period.

Ad 2: Melatonin should be compared head-to-head with the SSRIs used in previous prophylactic studies to compare efficacy and adverse effects.

Ad 3: As this indication of melatonin is novel, dose-response studies need to be conducted to determine the minimum effective dose and the maximum tolerated dose; hereby revealing the therapeutic window for this indication.

Ad 4: The characteristics of cytokine-induced sickness behavior in animal models have much in common with some of the symptoms of cancer patients such as pain, gastrointestinal symptoms, fatigue, cognitive impairments, anxiety and depression [57]. A combination of animal and human research suggests that these cancer-related symptoms may involve the actions of proinflammatory cytokines, such as IL-1, TNF-α and IL-6 [57]. Therapeutic interventions that target cytokines and have anti-inflammatory effects, such as melatonin [72], can possibly be a promising candidate for suppressing and protecting against the development of cancer-related symptoms and the so-called symptom clusters. This of course implies the measurement of cytokine levels in the course of treatment to be able to correlate these with any possible changes in symptoms.

Ad 5: It has been proposed that among other factors, upregulated peripheral cytokine levels and peripheral oxidative stress may cause chemotherapy-related cognitive changes [174]. Correspondingly, surgery and radiation therapy may also damage peripheral tissue and activate inflammatory pathways [174]. On the basis of these proposed contributing mechanisms to the development of cognitive disturbances in the course of a cancer diagnosis, it could be interesting to investigate the effect of melatonin with its known anti-inflammatory and anti-oxidant effects [72].

Ad 6: Candidate genes should be considered from the available literature on other conditions known to negatively influence cognition and these genotypes could then be the basis of hypothesis-generating studies with regard to the genetic heritability of POCD. Ultimately, this could lead to the option of recommending preoperative genotypic assessment in order to individualize treatment.

CONCLUSION

With the objectives of this thesis in mind and on the basis of the conducted studies it can be concluded that:

> The PER3$^{5/5}$ genotype of the PER3 gene does not seem to be associated with POCD at one week after non-cardiac surgery

- In women with breast cancer, 6 mg oral melatonin has an effect on reducing the risk of developing depressive symptoms and can increase sleep efficiency perioperatively and total sleep time postoperatively
- In women with breast cancer 6 mg oral melatonin does not have an effect on the subjective parameters of anxiety, sleep, general well-being, fatigue, pain, and sleepiness
- Postoperative cognitive dysfunction was not detected in a significant proportion of patients with breast cancer 2 or 12 weeks after surgery
- No serious adverse effects were found in breast cancer patients who received 6 mg melatonin treatment for three months
- Treatment with melatonin positively influences the ability to complete trial participation compared to placebo
- The quantity, size and quality of trials investigating the prophylactic or therapeutic effect of melatonin in depression/depressive symptoms is not high and there is no clear evidence of an effect; both positive and negative studies are present
- Further research is needed on the prophylactic and therapeutic effect of melatonin in depression, depressive symptoms, cognitive disturbances and symptom clusters of cancer patients in general
- Further research is needed on the heritability of POCD

SUMMARY

Biological rhythms are essential for the regulation of many life processes. Disturbances of the circadian rhythm are known to affect human health, performance and well-being and the negative consequences are numerous and widespread. Cognitive dysfunction, fatigue, pain, sleep disturbances and mood disorders, such as anxiety and depression, are common problems arising around the time of surgery or in the course of a cancer diagnosis and subsequent treatment period. The importance of investigating prevention or treatment possibilities in these populations is significant due to the extent of the problems and the derived consequences on morbidity and mortality. Genetic predisposition to these problems is also an issue in focus.

In this thesis we initially investigated whether the specific clock gene genotype $PER^{5/5}$ was associated with the development of postoperative cognitive dysfunction one week after non-cardiac surgery. We did not find any association, although this could have been due to the size of the study. Yet, if $PER3^{5/5}$ is associated with a higher incidence of postoperative cognitive dysfunction, the risk seems to be only modestly increased and by less than 10%.

Melatonin is a hormone with well-known chronobiotic and hypnotic effects. In addition, exogenous melatonin is also known to have anxiolytic, analgesic, antidepressant and positive cognitive effects. Based on the lack of studies investigating these effects of melatonin, we conducted the MELODY trial in which we investigated the effect of 6 mg oral melatonin on depressive symptoms, anxiety, sleep, cognitive function and fatigue in patients with breast cancer in a three month time period after surgery.

Melatonin had an effect on reducing the risk of developing depressive symptoms and also increased sleep efficiency perioperatively and total sleep time postoperatively. No effect was found on anxiety, sleep quality, sleepiness, general well-being or pain, however melatonin seemed to positively influence the ability to complete trial participation compared to placebo. Postoperative cognitive dysfunction was not a problem in this limited population. With regard to safety in our study, melatonin treatment for three months did not cause any serious adverse effects.

Finally, we systematically reviewed the literature on the prophylactic or therapeutic effect of melatonin for depression or depressive symptoms in adult patients and assessed the safety of melatonin in these studies. The quantity, size and quality of trials investigating this question were not high and there was no clear evidence of an effect, although some studies were positive.

In conclusion, further research is warranted with regard to the prophylactic effect and treatment effect of melatonin in depression, depressive symptoms, cognitive disturbances and symptom clusters of cancer patients in general. In addition, more hypothesis-generating studies with regard to the genetic heritability of POCD are needed.

ABBREVIATIONS

ANOVA: Analysis of variance
APOE: Apolipoprotein E
ASA: American Society of Anesthesiologists
AUC: Area under the curve
CONSORT: Consolidated Standards of Reporting Trials
DSM-III: The *Diagnostic and Statistical Manual* of Mental Disorders - III
DSM-IV: The *Diagnostic and Statistical Manual* of Mental Disorders - IV
HAM-D: The Hamilton Depression Scale
ICD-10: International Classification of Diseases – 10
IQR: Interquartile range
ISPOCD: International Study of Postoperative Cognitive Dysfunction
ITT: Intention to treat
KSS: Karolinska Sleepiness Scale
LOCF: Last observation carried forward
MAO: Monoamine oxidase
MDI: Major Depression Inventory
MELODY trial: The effect of MELatOnin on Depression, anxietY, cognitive function and sleep disturbances in patients with breast cancer
MMSE: Mini-Mental State Examination
PER3: PERIOD3
POCD: Postoperative cognitive dysfunction
PP: Per protocol
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT: Randomized controlled trial
SCAN: The Schedules for Clinical Assessment in Neuropsychiatry
SD: Standard deviation
SEM: Standard error of the mean
SPC: Summary of product characteristics
SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials
SSRI: Selective serotonin reuptake inhibitors
TSP: Total sleep period
VAS: Visual Analogue Scale
Zung-SDS: The Zung Self-Rating Depression Scale

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APPENDIX 1 – MAJOR DEPRESSION INVENTORY, SCORING KEY AND SCORING INSTRUCTION (REUSED WITH PERMISSION – WWW.CCMH.DK)

Psychiatric Research Unit
Psychiatric Centre North Zealand, Denmark

Major (ICD-10) Depression Inventory

The following questions ask about how you have been feeling over the last **two weeks**. Please put a tick in the box which is closest to how you have been feeling. A higher number signifies a higher degree of depression.

	How much of the time in the last two weeks...	All the time	Most of the time	Slightly more than half the time	Slightly less than half the time	Some of the time	At no time
1	Have you felt low in spirits or sad?	5	4	3	2	1	0
2	Have you lost interest in your daily activities?	5	4	3	2	1	0
3	Have you felt lacking in energy and strength?	5	4	3	2	1	0
4	Have you felt less self-confident?	5	4	3	2	1	0
5	Have you had a bad conscience or feelings of guilt?	5	4	3	2	1	0
6	Have you felt that life wasn't worth living?	5	4	3	2	1	0
7	Have you had difficulty in concentrating, e.g. when reading the newspaper or watching TV?	5	4	3	2	1	0
8a	Have you felt very restless?	5	4	3	2	1	0
8b	Have you felt subdued or slowed down?	5	4	3	2	1	0
9	Have you had trouble sleeping at night?	5	4	3	2	1	0
10a	Have you suffered from reduced appetite?	5	4	3	2	1	0
10b	Have you suffered from increased appetite?	5	4	3	2	1	0

Total score

Depression Inventory MDI: Scoring key

At the top the diagnostic demarcation line is indicated. The total score of the10 items is filled in below

| | | How much of the time... | All the time | Most of the time | **The diagnostic demarcation line** | | | |
					Slightly more than half the time	Slightly less than half the time	Some of the time	At no time
Core symptoms	1	Have you felt low in spirits or sad?	5	4	3	2	1	0
	2	Have you lost interest in your daily activities?	5	4	3	2	1	0
	3	Have you felt lacking in energy and strength?	5	4	3	2	1	0
Accompanying symptoms	4	Have you felt less self-confident?	5	4	3	2	1	0
	5	Have you had a bad conscience or feelings of guilt?	5	4	3	2	1	0
	6	Have you felt that life wasn't worth living?	5	4	3	2	1	0
	7	Have you had difficulty in concentrating, e.g. when reading the newspaper or watching TV?	5	4	3	2	1	0
Highest score	8a	Have you felt restless?	5	4	3	2	1	0
	8b	Have you felt subdued or slowed down?	5	4	3	2	1	0
	9	Have you had trouble sleeping at night?	5	4	3	2	1	0
Highest score	10a	Have you suffered from reduced appetite?	5	4	3	2	1	0
	10b	Have you suffered from increased appetite?	5	4	3	2	1	0

Total score (item 1 – 10)   ☐ ☐

Diagnosis: ICD-10 _____   DSM-IV_____

Major Depression Inventory (MDI): A depression questionnaire with a dual function
MDI: Scoring instructions

The questionnaire consists of the ten symptoms contained in the World Health Organization WHO's depression demarcation. WHO employs the last two weeks as the period of time in which to assess whether each symptom has been present for more than half the time. These symptoms are mainly subjective; therefore it is natural to ask the patient to complete the questionnaire, allowing the patient to tick each symptom. A higher number signifies a more constant presence of the symptom in question. Remember to fill in patient name and the date

The patient's completed questionnaire is scored using the scoring key. MDI (Major Depression Inventory) has a dual function, as it is scored both as an instrument of severity (A) similar to the Hamilton Depression Scale, and (B) as a diagnostic tool.

(A)   If MDI is used as a **rating scale** in the same way as the Hamilton scales, then the sum of the ten questions indicates the degree of depression. For items 8, 9 and 10, with two answer categories for each (a) and (b), the highest score is used. The theoretical score range is thus from 0 (no depression) to 50 (maximum depression).

Mild depression: MDI total score from 21 to 25
Moderate depression: MDI total score from 26 to 30
Severe depression: MDI total score of 31 or higher

(B)   MDI as a **diagnostic tool**: the vertical line (the diagnostic demarcation line) is used as indicated above. The three top symptoms which reflect the core symptoms of the WHO/ICD-10 diagnosis of depressions must have been present during the last two weeks for most of the time. The accompanying symptoms in the remaining seven MDI items must have been present during the last two weeks for more than half of the time.

The ICD-10 algorithm:

Mild depression: 2 core symptoms and 2 accompanying symptoms
Moderate depression: 2 core symptoms and 4 accompanying symptoms
Severe depression: 3 core symptoms and 5 accompanying symptoms.

MDI can also be employed when diagnosing DSM-IV major depression. According to DSM-IV only nine symptoms are used, as the DSM-IV item 4 is included in item 5. Thus the item with the highest score is used here.

The DSM-IV algorithm:

5 out of the 9 symptoms should be present. Of these one should be one of the two first items; according to DSM-IV these are core symptoms.