UNIVERSIDADE CATÓLICA DE SANTOS
DOUTORADO EM SAÚDE COLETIVA

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EFEITO DA MELATONINA EXÓGENA SOBRE O PADRÃO ALIMENTAR DE TRABALHADORAS NOTURNAS COM EXCESSO DE PESO

Santos
2022

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EFEITO DA MELATONINA EXÓGENA SOBRE O PADRÃO ALIMENTAR DE TRABALHADORAS NOTURNAS COM EXCESSO DE PESO

Tese apresentada ao Programa de Pós-Graduação em Saúde Coletiva da Universidade Católica de Santos para a obtenção do grau de Doutora em Saúde Coletiva.

Área de concentração: Política, Condição Social e Saúde

Orientadora: Prof.ª Dr.ª Elaine Cristina Marqueze

SANTOS

2022

[Dados Internacionais de Catalogação]
Departamento de Bibliotecas da Universidade Católica de Santos
Viviane Santos da Silva - CRB-8/6746

N778e Nogueira, Luciana Fidalgo Ramos
Efeito da melatonina exógena sobre o padrão alimentar de trabalhadoras noturnas com excesso de peso / Luciana Fidalgo Ramos Nogueira ; orientadora Elaine Cristina Marqueze. -- 2022.
242 f.

Tese (doutorado) - Universidade Católica de Santos, Programa de Pós-Graduação stricto sensu em Saúde Coletiva, 2022.
Inclui bibliografia.

1. Melatonina. 2. Consumo Alimentar. 3. Dessincronização Circadiana. 4. Trabalho noturno. I. Marqueze, Elaine Cristina. II. Título.

CDU: Ed. 1997 -- 614(043.2)

AGRADECIMENTOS

O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), sob o código de financiamento nº 88887.356981/2019-00.

RESUMO

Introdução: A melatonina é um hormônio pineal essencial para a organização temporal interna dos ritmos circadianos e do metabolismo energético. Os ritmos biológicos estão intimamente ligados ao comportamento alimentar, no entanto, o papel da suplementação de melatonina exógena sobre a ingestão alimentar ainda não foi esclarecido. **Objetivos:** 1) Sintetizar os resultados de ensaios pré-clínicos e ensaios clínicos controlados, randomizados, duplo-cegos sobre os efeitos da suplementação de melatonina exógena no consumo alimentar e hormônios reguladores do apetite; 2) Determinar o intervalo entre a última refeição realizada e a hora de dormir, bem como sua relação com parâmetros de sono diurno e noturno, bem como a adequação nutricional desta refeição; e 3) Avaliar os efeitos da suplementação de melatonina exógena nos aspectos quali-quantitativos e na distribuição temporal da ingestão alimentar de trabalhadoras noturnas com excesso de peso, segundo o desalinhamento circadiano e cronotipo. **Métodos:** Para responder ao primeiro objetivo, uma revisão sistemática de ensaios pré-clínicos e clínicos foi realizada. Para os objetivos 2 e 3, um ensaio clínico randomizado, duplo-cego, crossover, controlado por placebo foi conduzido com 27 profissionais de enfermagem do sexo feminino, com excesso de peso, que trabalhavam em turnos noturnos fixos (idade: 37,1 [EP 0,6] anos, IMC: 29,8 [EP 0,4] kg/m², tempo total de exposição ao trabalho noturno: 9.1 [EP 0,7] anos). As participantes foram aleatoriamente alocadas para o grupo intervenção (3 mg de melatonina de liberação rápida por 12 semanas) e controle (12 semanas de placebo). Os aspectos qualitativos (nível de processamento), quantitativos (ingestão calórica total e percentuais de macronutrientes) e temporais (horários das refeições) da alimentação foram obtidos por diários alimentares no baseline e após cada fase de intervenção. **Resultados:** A influência da melatonina exógena sobre a ingestão alimentar apresenta resultados heterogêneos na literatura, e o número de ensaios clínicos é limitado. No presente estudo, nenhuma alteração significativa no consumo calórico, distribuição de macronutrientes, tipos de alimentos consumidos e horários de alimentação foi observada após a suplementação de melatonina nem do placebo. Diferentes padrões de desalinhamento circadiano e cronotipo não interferiram nos resultados. **Considerações finais:** Aspectos quali-quantitativos e distribuição temporal da dieta de trabalhadoras noturnas com excesso de peso não sofreram modificação após a suplementação de melatonina. Assim como verificado pela em estudos prévios, estes resultados sugerem que os efeitos metabólicos da melatonina podem ocorrer independentemente da ingestão alimentar.

Palavras-chave: Melatonina; Consumo Alimentar; Dessincronização Circadiana; Trabalho Noturno.

ABSTRACT

Background: Melatonin is a pineal hormone that plays an important role as an endogenous synchronizer of circadian rhythms and energy metabolism. As this circadian component has been closely related to eating behavior, an important question would be whether melatonin supplementation could influence food intake. However, this topic has not been addressed by literature yet, especially in individuals with overweight, chronic circadian misalignment, and frequent nutritional problems, such as shift workers. **Aims:** 1) To synthetize the results from clinical and preclinical studies on the effects of exogenous melatonin on food intake and appetite-regulating hormones; 2) To determine the interval between the last meal and bedtime and its relationship with diurnal and nocturnal sleep parameters, as well as the adequacy of this meal; and 3) To evaluate the effects of melatonin supplementation on the quality-quantitative aspects and temporal distribution of food intake in female overweight night workers, according to circadian misalignment and chronotype. **Methods:** To address the first aim, a systematic review of intervention studies was carried out. To address aims 2 and 3, a randomized, double-blind, placebo-controlled, cross-over clinical trial was conducted with 27 female overweight nursing professionals who worked permanent night shifts (age= 37.1 (SE ±0.6) years, BMI= 29.8 (SE ±0.4) kg/m², lifetime exposure to night work= 9.1 (SE ±0.7) years). Protocol was implemented under real-life conditions for 24 weeks, in two randomly allocated conditions (12 weeks of melatonin supplementation and 12 weeks of placebo). The quantitative aspects of food intake (total energy intake (TEI) and the proportion of calories from macronutrients) and meal timing were assessed by food diaries. **Results:** Findings on the influence of exogenous melatonin on food intake are heterogeneous and clinical trials are limited. In our trial, no significant modifications in total energy intake, macronutrient distribution, types of foods consumed and meal timing were observed after melatonin or placebo supplementation. Different patterns of circadian misalignment and chronotype did not interfere with these results. **Final considerations:** The quality-quantitative aspects of the diet of female overweight night workers remained unchanged after melatonin supplementation. Circadian misalignment and chronotype did not interfere in these results. In parallel with previous studies on the topic, these results suggest that the metabolic effects of melatonin may occur independently of food intake.

Keywords: Melatonin; Eating Behavior; Circadian Dysregulation; Night Shift Work.

SUMÁRIO

1. APRESENTAÇÃO DO ESTUDO

O presente estudo integra a pesquisa de pós-doutorado da minha orientadora, Dr.ª Elaine Marqueze, intitulada “Efeito da melatonina no sono e no metabolismo de trabalhadoras noturnas com excesso de peso”. Esta, por sua vez, faz parte do projeto temático “Melatonina e regulação do metabolismo energético: estudos básicos, clínicos e epidemiológicos”, coordenado pelo Prof. Dr. José Cipolla-Neto.

A coleta de dados para esta pesquisa, que foi realizada com enfermeiras e técnicas de enfermagem de trabalhavam no turno noturno de um grande hospital privado na cidade de São Paulo, SP, se iniciou enquanto eu cursava o último ano do mestrado. Participar do projeto desde a fase inicial, conhecer o campo, manter contato com as participantes e acompanhá-las durante sete meses foi uma experiência profissional e pessoalmente enriquecedora.

Durante o doutorado, em paralelo ao desenvolvimento desta tese, como membro do Grupo de Pesquisa em Cronobiologia e Sono, coordenado pela Dr.ª Elaine, tive a oportunidade de participar em diversas atividades que contribuíram para que eu me aprofundasse no meu tema de pesquisa. Em 2019 e 2021, apresentei alguns resultados da minha pesquisa em duas edições do Congresso Brasileiro do Sono (Apêndices 1 e 2, respectivamente), sendo o pôster apresentado na edição de 2019 premiado com o segundo lugar de melhor trabalho na área clínica (Anexo 3). Contribuí também para a elaboração dos resumos de outras pesquisas inseridas no mesmo projeto guarda-chuva (Apêndices 4 a 11), sendo um pôster premiado com a nota máxima na edição de 2021 do mesmo evento (Apêndice 12).

Contribuí, ainda, como coautora dos seguintes artigos científicos já publicados:

- *Eating habits, sleep and a proxy for circadian disruption are correlated with dyslipidemia in overweight night workers*, publicado na *Nutrition* em novembro de 2020 (Anexo 1). O artigo original foi elaborado a partir da iniciação científica da aluna Ananda Garrido, do curso de Enfermagem, e tinha como objetivo avaliar a relação entre cronorruptura circadiana, hábitos alimentares, características de sono e parâmetros lipídicos. A partir dos resultados, verificou-se que uma curta duração do sono noturno e um alto jetlag social foram fatores de risco para dislipidemia, enquanto cronotipo

vespertino e maior tempo de intervalo entre a última refeição do dia e o início do sono foram fatores de proteção contra a dislipidemia;

- *Exogenous melatonin decreases circadian misalignment and body weight among early types*, publicado na *Journal of Pineal Research* em junho de 2021 (Anexo 2). O artigo original foi elaborado a partir da referida pesquisa de pós-doutorado da Dr.ª Elaine, que tinha como objetivo avaliar os efeitos da melatonina exógena no desalinhamento circadiano e peso corporal das trabalhadoras noturnas de acordo com o cronotipo. A partir dos resultados, verificou-se que a suplementação intermitente de melatonina reduziu o desalinhamento circadiano em cerca de 20% em todos os cronotipos. Entre as participantes vespertinas e com maior desalinhamento circadiano, houve também redução do índice de massa corporal e circunferências de cintura e quadril.

Há também o artigo original elaborado a partir da iniciação científica da aluna Gabriella Habib, do curso de Nutrição, que foi submetido à *Sleep Epidemiology* em janeiro de 2022 e atualmente encontra-se na fase de revisão por pares (Anexo 3). Intitulado *A putative association between food intake, meal timing and sleep parameters among overweight nursing professionals working night shifts*, tinha como objetivo avaliar a relação entre ingestão de macronutrientes, horários das refeições e parâmetros subjetivos do sono das trabalhadoras noturnas, tanto em dias de trabalho quanto de folga. Os resultados mostraram uma associação entre curta duração do sono e maior ingestão de proteína de origem animal nos dias de folga.

Por fim, há dois artigos científicos que neste momento encontram-se em fase de elaboração:

- *Can preventive melatonin supplementation improve cardiometabolic parameters of overweight night workers?* (Anexo 4), elaborado a partir do projeto de iniciação científica da aluna Patrícia Santana, do curso de Farmácia. Tinha como objetivo avaliar o efeito da suplementação preventiva de melatonina sobre os parâmetros cardiovasculares das trabalhadoras noturnas com excesso de peso. A partir dos resultados, verificou-se que

houve uma interação significativa entre a suplementação de melatonina e a frequência cardíaca em repouso e nível de HDL-colesterol;

- *Efeito da melatonina no sono de trabalhadoras noturnas com excesso de peso* (Anexo 5), elaborado a partir da tese de doutorado em Saúde Coletiva da aluna Pollyanna Pellegrino. Com o objetivo de avaliar o efeito da suplementação de melatonina nos aspectos objetivos e subjetivos do sono das trabalhadoras de acordo com o cronotipo, os resultados mostraram que a suplementação de melatonina melhorou a percepção da qualidade, duração e tempo suficiente de sono para o descanso, além de ter diminuído os sintomas de insônia.

No que se refere especificamente a esta tese, ela foi elaborada de forma a apresentar seus resultados por meio dos três artigos científicos elaborados durante o doutorado. A introdução apresenta o papel da melatonina como principal sincronizador temporal interno, a expressão de ritmos circadianos pelo metabolismo energético, a relação entre composição e horários da alimentação e a dessincronização dos ritmos biológicos e o papel da suplementação de melatonina exógena neste contexto. Para responder aos objetivos propostos, cada artigo apresenta seus próprios métodos, resultados e discussão.

2. INTRODUÇÃO

A organização temporal interna permite a sincronização com o meio externo e envolve pelo menos três fatores: 1) social, representado pelo horário de referência (fuso horário) de uma determinada região; 2) solar, determinado pelos movimentos de rotação e translação do planeta, os quais por sua vez determinam a duração dos dias, noites e estações do ano; 3) biológico, que coordena todos os níveis da fisiologia humana, desde o metabolismo até o comportamento (ROENNEBERG et al., 2019). Até a era pré-industrial, o fator social tinha boa correspondência com os fatores solar e biológico, de modo que interações e eventos, como trabalho e ingestão de alimentos, forneciam boas pistas temporais (*zeitgebers*) para a organização temporal interna do ser humano. No entanto, nas sociedades industrializadas contemporâneas, o fator social perdeu a consistência: a maior parte do tempo é passada dentro de edifícios com pouca ou nenhuma iluminação natural durante o dia e artificialmente iluminados durante a noite. Neste contexto, a força dos *zeitgebers* foi enfraquecida numa velocidade muito maior do que a capacidade da evolução biológica é capaz de acompanhar (ROENNEBERG et al., 2019).

Apesar dos inúmeros benefícios alcançados pelo advento da luz elétrica, problemas fisiológicos devidos à exposição excessiva à iluminação artificial têm sido verificados (REITER et al., 2012). Como animal de hábitos diurnos, o ser humano está metabolicamente preparado para realizar suas atividades de vigília durante o dia e dormir à noite. Para atender às exigências da sociedade contemporânea, é cada vez mais frequente a organização do trabalho em sistemas de turnos (SOUZA et al., 2018). Estima-se que 20 a 30% das populações economicamente ativas da América do Norte, Canadá, Europa e Brasil estejam envolvidos em trabalhos em turnos que incluem trabalho noturno (ATKINSON et al., 2008; ÅKERSTEDT, WRIGHT, 2009; ALTERMAN et al., 2013; MORENO, 2004; STATISTICS CANADA, 2013). Entre trabalhadores noturnos fixos, menos de 3% apresentam ajuste circadiano completo (FOLKARD, 2008), o que evidencia que a grande maioria desses trabalhadores sofre com a dessincronização dos ritmos biológicos.

Em revisão a respeito dos efeitos deletérios dessa dessincronização, Rüger e Scheer (2009) afirmam que, em função da exposição à luz durante a noite, ocorre supressão aguda da produção de melatonina em trabalhadores noturnos, e se essa situação é mantida cronicamente, pode aumentar o risco de desenvolvimento de

doenças. A melatonina é um hormônio produzido pela glândula pineal que representa o mais importante sincronizador interno do organismo. Sua produção expressa um ritmo circadiano sincronizado pelo ciclo claro/escuro, ocorrendo exclusivamente na fase escura e sendo suprimida pela exposição à luz durante a noite (SAHIN; FIGUEIRO, 2013). Sua ação é essencial para a manutenção da organização temporal circadiana interna, incluindo a regulação do metabolismo para estoque e dispêndio energético (CIPOLLA-NETO et al., 2014).

A diminuição da produção de melatonina promove a dessincronização de processos metabólicos circadianos dos tecidos muscular, hepático e adiposo, levando à cronorruptura destes ritmos biológicos (CIPOLLA-NETO et al., 2014). Com a supressão da melatonina devido à iluminação artificial, portanto, trabalhadores noturnos estão especialmente propensos a sofrer cronorruptura circadiana, a qual, por sua vez, promove distúrbios endocrinometabólicos, de sono, sociotemporais e comportamentais (ARENDT; SKENE, 2005; REITER, 2009, TAN 2011, REITER et al., 2012, RAJARATNAM; HOWARD; GRUNSTEIN, 2013).

O comportamento alimentar também é regido por ritmos biológicos. Desde a década de 1980, estudos experimentais têm demonstrado que a melatonina está envolvida na regulação do apetite e ingestão alimentar em mamíferos (BARTNESS; WADE 1985, BUBENIK; PANG, 1994, BUBENIK 1996, HUETHER 1994). Atualmente, considera-se que a ingestão de alimentos, o número de refeições e os tipos de nutrientes podem tanto coordenar quanto ser coordenados pelos ritmos circadianos (MANOOGIAN; PANDA, 2016), portanto, o padrão alimentar de trabalhadores noturnos também tende a se modificar (FOLKARD, 2008). No entanto, não está plenamente esclarecido se as alterações no padrão alimentar e no metabolismo energético de trabalhadores noturnos ocorrem devido à cronorruptura circadiana e/ou ao estilo de vida não saudável (ATKINSON, 2008).]

Os processos de digestão e absorção de nutrientes são regulados por hormônios, como insulina, leptina e grelina, os quais expressam ritmos circadianos e agem como sinalizadores e influenciam osciladores periféricos (BAE et al., 2019). Uma vez que estes osciladores estão intimamente ligados ao ciclo vigília-sono, a dessincronização do ciclo alimentação-jejum pode impactar negativamente o metabolismo energético (HUTCHISON 2017, AMARAL et al., 2019, LEUNG et al., 2020).

Estudos genéticos em seres humanos têm associado a cronorruptura circadiana ao consumo alimentar excessivo e excesso de peso corporal (GARAULET 2010, GARAULET; MADRID 2010). Neste contexto, a alimentação de trabalhadores noturnos merece atenção especial. A ingestão de alimentos à noite pode ser um mascarador do ritmo circadiano e provocar arrastamento dos osciladores circadianos, exercendo papel fundamental na regulação do apetite (MANOOGIAN; PANDA, 2016). Portanto, a inversão do ciclo vigília-sono e dos horários das refeições, por si só, constitui um fator crítico neste contexto. Como ressaltam Souza e colaboradores (2018), até mesmo curtos períodos de dessincronização circadiana podem promover alterações metabólicas significativas em adultos saudáveis.

Estudos mostraram que a ingestão de alimentos por trabalhadores noturnos é determinada predominantemente pelas horas de trabalho, isto é, mais pelas oportunidades de comer que pela fome fisiológica (DELLA TORRE, 2020, COELHO et al., 2014). Com um sono de curta duração, aumenta-se o tempo disponível para comer e a fadiga, o que leva ao aumento da ingestão alimentar e à redução da taxa metabólica, resultando em excesso de peso. Não parece haver diferenças na ingestão calórica total de trabalhadores noturnos e diurnos, porém, verificou-se uma diferença na distribuição das refeições ao longo do período de 24 horas, na qual trabalhadores noturnos tendem a se alimentar de forma irregular durante o turno (FLANAGAN, 2020, BAE et al., 2019). A distribuição temporal da ingestão de nutrientes em horários fisiologicamente inadequados é um aspecto que tem despertado interesse, pois sabe-se que possui efeitos deletérios sobre o metabolismo energético e sobre a qualidade do sono (HUTCHISON et al., 2017, MANOOGIAN; PANDA, 2016).

Há evidências homogêneas de estudos básicos para sustentar a hipótese de que a suplementação de melatonina exógena pode prevenir e contribuir para reduzir os efeitos deletérios da dessincronização circadiana sobre o metabolismo e restaurar a homeostase (REITER, 2012, CIPOLLA-NETO et al., 2014). Porém, os ensaios clínicos são limitados e não está claro como a ingestão alimentar é influenciada pela melatonina, nem se há influência direta desta sobre o consumo alimentar de indivíduos dessincronizados e com excesso de peso (SHAJI; KULKARNI, 1998, WOLDEN-HANSON et al., 2000).

Dado o exposto, o presente estudo procura responder à seguinte pergunta primária: a suplementação de melatonina exógena influencia o padrão quali-quantitativo e a distribuição temporal da ingestão alimentar de trabalhadoras noturnas

com excesso de peso? Adicionalmente, duas perguntas secundárias se apresentam: 1) qual o estado da arte da influência da suplementação de melatonina exógena sobre a ingestão alimentar? e 2) qual o intervalo médio entre a última refeição e a hora de dormir e como é a qualidade nutricional desta refeição em trabalhadoras noturnas com excesso de peso?

3. HIPÓTESE

3.1 ARTIGO 1

- Estudos pré-clínicos e clínicos randomizados, duplo-cegos, controlados por placebo apresentam resultados homogêneos sobre os efeitos benéficos da suplementação de melatonina exógena no consumo alimentar e hormônios reguladores do apetite.

3.2 ARTIGO 2

- Quanto menor o intervalo entre a última refeição e o horário de dormir, e quanto mais inadequada nutricionalmente esta refeição, piores são os parâmetros de sono diurno e noturno de trabalhadoras noturnas com excesso de peso;

3.3 ARTIGO 3

- Considerando o potencial da melatonina em sincronizar os ritmos circadianos e o benefício desta sincronização para o comportamento alimentar, a suplementação de melatonina é capaz de melhorar o padrão quali-quantitativo e a distribuição temporal da ingestão alimentar de trabalhadoras noturnas com excesso de peso.

4. OBJETIVOS

4.1 ARTIGO 1

Sintetizar os resultados de ensaios pré-clínicos e ensaios clínicos controlados, randomizados, duplo-cegos sobre os efeitos da suplementação de melatonina exógena no consumo alimentar e hormônios reguladores do apetite;

4.2 ARTIGO 2

- Determinar o intervalo entre a última refeição realizada e a hora de dormir, bem como sua relação com parâmetros de sono diurno e noturno;
- Avaliar a associação entre a adequação nutricional da última refeição realizada e parâmetros objetivos do sono de trabalhadoras noturnas com excesso de peso;

4.3 ARTIGO 3

Avaliar os efeitos da suplementação de melatonina exógena nos aspectos quali-quantitativos e na distribuição temporal da ingestão alimentar de trabalhadoras noturnas com excesso de peso, segundo o desalinhamento circadiano e cronotipo.

5. MÉTODOS

A seguir, serão apresentados de forma sucinta os métodos dos três artigos que compõem a tese. Os métodos estão descritos detalhadamente na sessão Resultados, no corpo individual de carga artigo.

5.1 ARTIGO 1

Trata-se de uma revisão sistemática de estudos de intervenção. O desenho foi preparado de acordo com as diretrizes do *Preferred Reporting Items for Systematic Reviews and Meta-analyses* (PRISMA) (MOHER et al., 2015) e foi registrado no PROSPERO (número 42020175809). Por se tratar de uma revisão de literatura, nenhuma aprovação do comitê de ética foi requerida.

As bases virtuais de dados Medline, Scopus, Web of Science e Cochrane Library foram sistematicamente pesquisadas de janeiro de 2020 a fevereiro de 2021. Estudos potencialmente relevantes foram selecionados de forma independente por duas revisoras de acordo com os seguintes critérios de inclusão: 1) ser um ensaio clínico controlado randomizado ou pré-clínico publicado em um periódico revisado por pares; 2) ser pelo menos cego (para ensaios clínicos) com desenho paralelo ou *crossover*; 3) avaliar o efeito da suplementação de melatonina exógena, em qualquer dosagem e via de administração, em comparação ao placebo; 4) estudos experimentais em humanos saudáveis (exceto para Classificação Internacional de Doenças, 11ª Edição [CID-11] (OMS, 2018) código 5B81.0 - Obesidade por desequilíbrio energético), com idade entre 18 e 50 anos (para ensaios), ou espécies de mamíferos de ambos os sexos; e 5) ter o texto completo disponível em qualquer idioma e independentemente da data de publicação.

Estudos *in vitro* e observacionais (transversais, caso-controle, coorte e ecológicos) foram excluídos, bem como intervenções combinando a suplementação de melatonina com quaisquer outras substâncias. Também foram excluídos estudos que avaliaram mulheres menopausadas ou grávidas, indivíduos pinealectomizados, outras classes de animais que não mamíferos e vegetais.

Duas pesquisadoras realizaram de forma independente a seleção dos artigos por meio da leitura de seus títulos e resumos. Posteriormente, ambas leram os textos completos dos artigos que atenderam aos critérios de inclusão. Qualquer desacordo

sobre a elegibilidade dos artigos foi resolvido por consulta a um terceiro pesquisador. Dos 3.695 artigos identificados, dois ensaios clínicos e 13 pré-clínicos (n=15) preencheram os critérios de inclusão.

A ferramenta da *Cochrane Collaboration* para avaliar o risco de viés em estudos randomizados (HIGGINS et al., 2011) foi aplicada por duas revisoras independentes para avaliar a qualidade metodológica dos estudos realizados em humanos. O instrumento foi escolhido devido à estrutura mais semelhante ao que avalia ensaios pré-clínicos, de modo que ambos os desenhos pudessem ter o risco de avaliação de viés mais semelhante possível, mas considerando suas diferenças metodológicas. Os ensaios pré-clínicos, por sua vez, foram avaliados pelo *Systematic Reviews Center for Laboratory Animal Experimentation's Risk of Bias tool* (SYRCLE's RoB tool) (HOOIJMANS et al., 2014), que é baseado na ferramenta descrita acima e foi ajustado para aspectos de viés que desempenham um papel específico nos estudos de intervenção com animais.

5.2 ARTIGO 2

Trata-se de um artigo original com dados transversais referentes ao *baseline* do ensaio clínico (descrito no item a seguir). Um total de 30 profissionais de enfermagem (enfermeiras e técnicas de enfermagem) com excesso de peso que trabalhavam em turnos noturnos fixos de 12x36h (19:00 às 07:00) em um hospital de grande porte de São Paulo, SP, completou o monitoramento de actigrafia e diários alimentares. A coleta de dados de linha de base foi realizada de abril a novembro de 2018.

As variáveis dependentes foram dados objetivos de sono (valores médios) obtidos por meio de actigrafia (ActTrust, Condor Instruments®) durante 10 dias consecutivos. Optou-se por utilizar os parâmetros do sono obtidos pela actigrafia apenas nos dias de preenchimento dos diários alimentares, de forma a garantir que o episódio de sono analisado refletisse o consumo alimentar prévio no dia de trabalho e no dia de folga. Concomitantemente, as participantes foram orientadas a preencher registros de sono para complementar as informações e auxiliar na interpretação dos dados obtidos pela actigrafia. Os dados foram separados em sono diurno (após uma noite de trabalho) e sono noturno (a noite seguinte ao dia de folga que ocorreu a cada

15 dias). Os parâmetros avaliados foram duração do sono, latência do início do sono e despertar após o início do sono.

As variáveis independentes foram tempo e distribuição percentual de macronutrientes da última refeição antes de iniciar o sono. Assim, as participantes foram orientadas a preencher diários alimentares em um dia de trabalho e um dia de folga que considerassem típicos. Em ambos os casos, o período para registro dos dados alimentares foi das 19:00 às 19:00 do dia seguinte. Para determinar o intervalo entre a última refeição e o início do sono, os dados sobre o início do sono foram extraídos da actigrafia. Após o trabalho noturno, foi considerada para análise a última refeição realizada antes do sono diurno, enquanto no dia de folga foi considerada a última refeição antes do sono noturno.

A composição da última refeição foi avaliada utilizando as Recomendações Alimentares (RDA) estabelecidas pela Academia Nacional de Ciências [41] e adotadas pela Sociedade Brasileira de Alimentação e Nutrição [42]. A ingestão de macronutrientes na última refeição antes do episódio principal de sono foi classificada como adequada ou inadequada, sendo considerada inadequada quando abaixo do limite mínimo ou acima do limite máximo das faixas estabelecidas.

O teste t pareado foi realizado para comparar a duração do sono, bem como a ingestão calórica total, porcentagem e tempo, para a última refeição após o turno da noite *versus* a última refeição no dia de folga. Para comparar a latência do início do sono e a média do despertar após o início do sono foi realizado o teste de Wilcoxon. A análise foi realizada por meio de regressões lineares simples e múltiplas (modelo linear geral), em que os parâmetros de sono foram variáveis dependentes e coeficientes β foram estimados para um intervalo de confiança de 95%. Os modelos foram construídos adotando como variáveis independentes o tempo e a composição da última refeição, todas ajustadas para idade, cronotipo e horário de trabalho noturno. Variáveis independentes com $p<0,20$ nos testes de hipóteses foram inseridas nos modelos múltiplos em ordem decrescente de significância estatística (técnica *backward stepwise*).

5.3 ARTIGO 3

Trata-se de um artigo original com dados do ensaio clínico fase 2, randomizado, *crossover*, duplo-cego e controlado por placebo. O protocolo foi implementado em

condições de vida real por 24 semanas. Todas as 27 profissionais de enfermagem trabalhavam em esquema 12x36h, conforme descrito no item anterior.

Os critérios de inclusão foram mulheres na faixa etária de 20 a 50 anos, com índice de massa corporal (IMC) ≥25 e <40 kg/m², trabalhando há pelo menos seis meses no atual turno noturno, que declarassem não ter intenção de seguir dietas restritas nem iniciar novas atividades físicas durante a participação no estudo. Os critérios de exclusão foram gestantes e lactantes; ter filhos menores de um ano; climatério ou menopausa; transtornos alimentares diagnosticados por um médico; ter um segundo emprego noturno; uso regular de medicamentos e/ou suplementos alimentares que influenciam o sono, o estado de alerta e o sistema de temporização circadiano; uso abusivo de drogas e álcool; história pregressa e/ou atual de distúrbios psiquiátricos, neurológicos, circadianos ou do sono diagnosticados por um médico; distúrbios metabólicos (exceto diabetes mellitus tipo 2 e dislipidemia tratados); doenças cardiovasculares (exceto hipertensão arterial sistêmica tratada); inflamação crônica e/ou infecção diagnosticada por um médico; anemia e/ou ter doado >400 mL de sangue nos últimos três meses anteriores ao estudo; cirurgia de grande porte nos seis meses anteriores ao estudo.

As participantes foram recrutadas de março de 2018 a junho de 2019. Elas foram alocadas aleatoriamente em dois grupos usando códigos gerados por computador. Comprimidos idênticos de 3 mg de melatonina de liberação rápida ou placebo (Aché Pharmaceutics®, São Paulo, Brasil) foram administrados por via oral por 12 semanas cada. As participantes foram orientadas a tomar um comprimido, uma hora antes de dormir, exclusivamente quando dormissem à noite, ou seja, nas noites entre turnos e folgas, e nunca durante o dia. Como a melatonina é completamente excretada na urina dentro de 24h após a administração [30], nenhum período de *washout* foi realizado entre as duas fases do estudo. O número médio de dias de administração de melatonina foi de 45 dias (EP 10,3 dias) e o uso de placebo foi de 44,3 dias (EP 8,2 dias).

Cada item nos diários alimentares revisados foi categorizado como alimentos não processados ou minimamente processados, processados ou ultraprocessados de acordo com a NOVA [33]. Quando não foi possível uma classificação direta, como preparações caseiras e alimentos contendo itens de diferentes grupos, além de ingredientes culinários, considerou-se a classificação do ingrediente principal. O

horário de cada refeição nos diários alimentares foi agrupado em quatro categorias: 19:00-00:59, 01:00-06:59, 07:00-12:59 e 13:00-18:59.

O desalinhamento circadiano foi estimado usando o desvio de fase composto (CPD) [34] do meio do sono baseado em actigrafia, que é calculado a partir dos dados de início e fim do episódio de sono. As participantes usaram actígrafos por 10 dias consecutivos, conforme descrito no item anterior. O cronotipo foi avaliado usando o ponto médio do sono em dias livres após os turnos noturnos, corrigido para *over-sleep* (MSFNsc), derivado do *Munich Chronotype Questionnaire for shift work* (MCTQshift) [35].

O peso corporal e a altura foram avaliados no *baseline*, e o peso corporal também foi avaliado nos últimos 10-15 dias da primeira e segunda fases do estudo. O índice de massa corporal (IMC) foi calculado como o peso corporal (kg) dividido pela altura ao quadrado (m^2). As necessidades energéticas das participantes foram calculadas individualmente pelas equações das necessidades energéticas estimadas (EER) [37].

Os dados descritivos foram apresentados como média ± erro padrão. O teste de Shapiro-Wilk foi usado para testar a normalidade dos dados. Equações de estimativas generalizadas (GEE) com teste post hoc de Bonferroni foram utilizadas para analisar o efeito da administração de melatonina e placebo nos aspectos quali-quantitativos e distribuição temporal da dieta. Sete modelos foram realizados para cada variável dependente (ingestão calórica total; porcentagens de calorias diárias retiradas de alimentos não processados ou minimamente processados, processados, ultraprocessados; carboidrato, proteína e gordura; e horário das refeições). As porcentagens de calorias para cada categoria de processamento foram obtidas: 1) calculando a ingestão média da jornada de trabalho e da folga registrada no *baseline* e durante as últimas quatro semanas de cada fase do estudo (semanas 12 e 24), e 2) calculando o consumo médio das 12 semanas de administração de melatonina e, em seguida, das 12 semanas de placebo. A decisão de utilizar os valores para o final de cada fase do estudo deve-se à ausência de diferenças significativas mês a mês na ingestão alimentar em modelos previamente testados.

Além disso, sete modelos foram realizados para analisar o efeito das intervenções nas mesmas variáveis dependentes citadas em associação com CPD e outros sete em associação com cronotipo. Tanto o CPD quanto o cronotipo foram tratados como variáveis contínuas. Todos os modelos foram ajustados para exposição

ao trabalho noturno ao longo da vida. A distribuição gama com log link foi escolhida considerando a menor quase verossimilhança sob o critério do modelo de independência (QIC). O nível de significância foi estabelecido em 5%.

As questões éticas relativas à pesquisa envolvendo seres humanos foram devidamente respeitadas. O projeto de pesquisa foi aprovado tanto pelo Comitê de Ética da Faculdade de Saúde Pública da Universidade de São Paulo (processo número 2.450.682) quanto pelo Comitê de Ética do hospital onde foi realizada (processo número 2.489.636). Todas as participantes deram consentimento escrito e informado para participar do estudo. O ensaio clínico foi registrado no Registro Brasileiro de Ensaios Clínicos - ReBEC (número RBR-6pncm9) e foi desenvolvido de acordo com os *Consolidated Standards of Reporting Trials* [38].

6. RESULTADOS

6.1 ARTIGO 1

CHRONOBIOLOGY INTERNATIONAL
https://doi.org/10.1080/07420528.2021.1918143

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Effects of melatonin supplementation on eating habits and appetite-regulating hormones: a systematic review of randomized controlled clinical and preclinical trials

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ABSTRACT

Melatonin is a hormone involved in appetite regulation and food intake. Circadian chronorrupture caused by its absence has been associated with excessive food consumption, and there is evidence that exogenous melatonin supplementation can restore homeostasis. Therefore, the aim of this systematic review was to synthesize evidence from randomized controlled clinical and preclinical trials that evaluated the effects of exogenous melatonin supplementation on eating habits and appetite-regulating hormones. The protocol was registered in PROSPERO (number 42020175809). Medline, Scopus, Web of Science and Cochrane Library were systematically searched from January 2020 to February 2021. Of 3.695 articles identified, 2 clinical and 13 preclinical trials (n = 15) met the inclusion criteria. The outcomes were total food intake, calories, macronutrients, cholesterol intake, leptin and ghrelin levels. Interventions ranged from 28 to 336 days and dose of melatonin varied between 0.2 µg/mL of drinking water and 10 mg/day. Clinical trials were conducted with healthy adults, and preclinical trials with rodents and dogs. Of the 15 articles, five assessed food intake and leptin, four assessed food intake only, five assessed leptin only, and one assessed leptin and ghrelin serum levels. The majority of the articles were judged as having low risk of bias. Although findings are heterogeneous and do not allow a robust conclusion, this review adds to the growing body of evidence suggesting that exogenous melatonin may be a potential therapeutic agent against endocrine-metabolic disorders. This reversal is not necessarily associated with changes in food consumption, signaling that melatonin's metabolic effects may occur independently of energy intake. Further studies, especially with humans, are needed provide more evidences for melatonin supplementation in clinical practice, as well as to understand its role on eating habits and appetite-regulating hormones.

ARTICLE HISTORY

Received 8 February 2021
Revised 19 March 2021
Accepted 12 April 2021

KEYWORDS

Melatonin; dietary supplements; feeding behavior; appetite regulation; hormones; leptin; ghrelin

INTRODUCTION

Melatonin is a hormone produced and secreted by the pineal gland that represents the most important internal synchronizer in the body. Its production expresses a circadian rhythm synchronized by the light-dark cycle, occurring exclusively during the dark phase and being suppressed by exposure to light at night (Sahin and Figueiro 2013).

The internal temporal organization that allows the individual to be synchronized

with the daily environmental cycles, such as the light-dark cycle, involves at least three factors: 1) the social factor, which represents the local time reference of a region or time zone; 2) the solar factor, determined by the movements of rotation and translation of the planet, which define the duration of the days, nights and seasons of the year; and 3) the biological factor, which coordinates all levels of human physiology, from metabolism to behavior (Roenneberg et al. 2019).

Until pre-industrial societies, the social clock has always shown good correspondence with the solar and biological clocks, so that social events and interactions, such as work activities and food intake, provided temporal clues (*zeitgebers*) for the internal organization of the human being. However, in contemporary industrialized societies the social clock has lost its consistency: most of the time is spent inside buildings protected from natural light and artificially lit during the night. The *zeitgebers*' strength has been significantly weakened at a much faster rate than the adaptive capacity of biological evolution is capable of keeping up with (Roenneberg et al. 2019).

The internal temporal organization for which melatonin is important includes the regulation of metabolism for storage and energy expenditure. The decrease in melatonin production promotes the desynchronization of the metabolic processes of muscle, liver and adipose tissue, leading to circadian chronorrupture (Cipolla-Neto et al. 2014). Thus, melatonin suppression can contribute to physiological changes that predispose obesity (Reiter et al. 2009; Tan et al. 2011) and, as Souza et al. (2019) point out, even short periods of circadian desynchronization can promote significant metabolic changes in healthy adults.

Despite the limited number of studies that have been carried out primarily with rodents, the homogeneity of the results suggests that melatonin has anti-obesogenic effects (Reiter et al. 2012). In experimental studies carried out with pinealectomized rats, it was found that the complete suppression of melatonin promoted metabolic desynchronization characterized by diabetogenic syndrome, which was reversed with the administration of exogenous melatonin (Nogueira et al. 2011; Shi et al. 2016).

Eating behavior is also controlled by biological rhythms. Food intake, number of meals and types of nutrients can both coordinate and be coordinated by circadian rhythms (Manoogian and Panda 2017). Since the 1980s, experimental studies have been demonstrating that melatonin is involved in appetite regulation and food intake in different mammal species (Bartness and Wade 1985; Bubenik and Pang 1994; Bubenik et al. 1996; Huether 1994).

Genetic studies carried out in humans, in turn, have associated circadian chronorrupture with excessive food

consumption and increased body mass (Garaulet et al. 2010; Garaulet and Madrid 2010). In individuals with sleep restriction, there was a reduction in leptin concentrations and an increase in ghrelin compared to individuals without sleep restriction, which results in high feeling of hunger, increased caloric intake and weight gain (Chin- Chance et al. 2010). Nevertheless, the results on the influence of melatonin in food consumption are still heterogeneous, since there are authors who found no effect or an increase in food consumption after exogenous melatonin supplementation (Shaji and Kulkarni 1998; Wolden-Hanson et al. 2000).

In summary, there is evidence to support that adequate supplementation of synthetic melatonin can prevent and/or contribute to reduce deleterious effects caused by metabolic disorders and restore homeostasis (Cipolla-Neto et al. 2014). However, it is not clear how appetite regulation and food intake are affected and how they participate in this process. Since systematic reviews are the gold standard for summarizing evidence in health care because of their rigorous methodological approach (Moher et al. 2015), the aim of the present systematic review is to synthesize the results of randomized controlled clinical and preclinical trials that evaluated the effects of exogenous melatonin supplementation on eating habits and appetite-regulating hormones.

MATERIALS AND METHODS

The design of this systematic review has been prepared according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement (Moher et al. 2015) and was registered in PROSPERO (number 42020175809). Because it is a literature review, no ethics committee approval was required.

Search strategy

The PICO (Population, Intervention, Control and Outcome) strategy was used to construct the research question (Table 1).

Online databases Medline, Scopus, Web of Science and Cochrane Library were systematically searched from January to July 2020 for identification of all the relevant controlled clinical and preclinical trials of melatonin supplementation in eating habits and appetite- regulating hormones. The key terms for searches were defined according to the Medical Subject Headings (MeSH) and are presented in Table 2.

The reference lists of all the included articles were manually investigated in order to find additional eligible studies.

Eligibility criteria

Potentially relevant studies were selected by two independent reviewers according to the following inclusion criteria: 1) being a randomized controlled clinical or preclinical trial published in a peer-reviewed

journal; 2) being at least single blind (for clinical trials) with either parallel or crossover design; 3) assessing the effect of exogenous melatonin,

Table 1. The PICO strategy for construction of the research question.

Criteria	Definition
Population	Animals (mammals only) or humans aged between 18 and 50 years
Intervention	Exogenous melatonin supplementation
Control	Placebo
Outcomes	Eating habits (consumption of calories and nutrients, meal patterns and/or time of food intake) and appetite-regulating hormones (leptin and/ or ghrelin levels)

Table 2. Search strategy for online databases.

Intervention	"melatonin supplementation" or "melatonin administration" or "melatonin intervention" or "melatonin intake"
Outcomes	"caloric intake" or "diet" " or "dietary factor" or "dietary intake" or "dietary pattern" or "eat" or "eating habit" or "eating habits" or "energy balance" or "energy expenditure" or "energy imbalance" or "energy intake " or "energy metabolism" or "feeding pattern" or "feeding phase" or "food habit" or "food intake" or "food pattern" or "food preference" or "food timing" or "meal pattern" or "meal timing" or "nutrition" or "nutritional intake" or "nutrient intake" or "nocturnal caloric intake" or "nocturnal eating" or "timing of meals" or "hunger" or "appetite" or "satiety response" or "leptin" or "ghrelin"
Limit	"Full-text available" and "Peer-reviewed articles"

at any dosage and route of administration, in comparison to placebo; and 4) experimental studies in healthy human (except for International Classification of Diseases, Eleventh Edition's [ICD-11] (World Health Organization 2018) code 5B81.0 – Obesity due to energy imbalance), aged between 18 and 50 years (for clinical trials), or mammal species of both sexes; and 5) having full text available in any language and regardless of the date of publication.

In vitro and observational studies (cross-sectional, case-control, cohort and ecological) were excluded, as well as interventions combining melatonin supplementation with any other substances. Studies that evaluated menopausal or pregnant women, pinealectomized individuals, classes of animals other than mammals and vegetables were also excluded.

Two researchers independently performed the selection of the articles by reading their titles and abstracts. Subsequently, both the reviewers read the full texts of the articles that met the inclusion criteria. Any disagreement on the eligibility of articles was resolved by consultation with a third researcher.

Reports of the number of included and excluded studies in the different stages of the current systematic review are presented afterward using PRISMA flow chart.

Data extraction

A standardized electronic abstraction form was designed by the researchers in order to extract data from the included articles. Data extraction was conducted independently by two reviewers and included the following variables: author, year of publication, country, design details, daily dose of melatonin supplementation, timing and route of administration, duration of the intervention, participants' species, sex and age, number of participants in both the intervention and control groups, tool used for assessing eating habits, methods for

assessing outcomes and main results found. Microsoft Office Excel 2010® was used to manage the selection of articles.

Quality assessment

The Cochrane Collaboration's tool for assessing risk of bias in randomized trials (Higgins et al. 2011) was applied by two independent reviewers to assess methodological quality of the studies performed in humans. This version of the tool was chosen due to the structure more similar to the tool that evaluates pre-clinical trials (described below), so that both types of trials could have the risk of bias assessment as similar as possible, but still considering methodological their differences. As outlined in The Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green 2008), the following sources of bias in clinical trials were considered: 1) Allocation sequence generation; 2) Allocation concealment; 3) Blinding of participants and personnel; 4) Blinding of outcome assessment; 5) Incomplete outcome data; 6) Selective outcome reporting; 7) Any other potential sources of bias. A third researcher was consulted in case of any discordance during the process of quality assessment.

Preclinical trials, in turn, were assessed by the Systematic Reviews Center for Laboratory Animal Experimentation's Risk of Bias tool (SYRCLE's RoB tool) (Hooijmans et al. 2014), which is based on Cochrane's RoB tool and has been adjusted for aspects of bias that play a specific role in animal intervention studies. As outlined by the developers, the following sources of bias in preclinical trials were considered: 1) Allocation sequence generation; 2) Allocation concealment; 3) Baseline characteristics; 4) Random housing; 5) Blinding of caregivers; 6) Random outcome assessment; 7) Blinding of assessors; 8) Incomplete outcome data; 9) Selective outcome reporting; 10) Any other potential sources of bias.

RESULTS

Search results

The search strategy identified 3.691 articles and four additional articles were identified through manual search of reference lists of included studies. After duplicate (n = 2.295) removal 1.400 articles were selected for title and abstract screening, from which 1.385 were excluded for not meeting the inclusion criteria. The remaining 15 articles were fully screened and met the inclusion criteria. Therefore, 15 studies were included in the present systematic review. Consensus between the two reviewers was reached for all included articles (Figure 1).

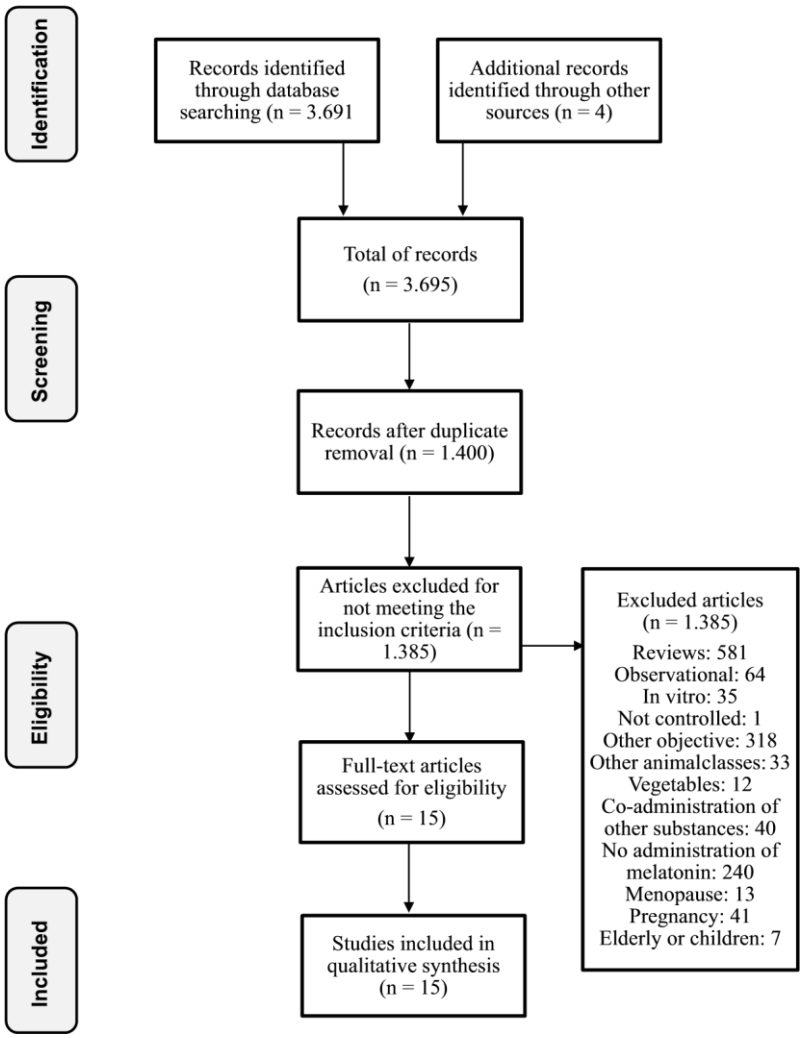

Figure 1. Search and selection process according to the PRISMA (Preferred reporting items for systematic reviews and meta analyses) statement.

Study characteristics

Two out of the 15 articles included consist in randomized- controlled clinical trials and 13 are preclinical trials. The clinical trials consisted in phase I (Szewczyk-Golec et al. 2017) and II (Bahrami et al. 2019) studies, performed mainly with female and male participants, respectively.

The participants of both studies were healthy and adults, with no statistical difference in mean age of intervention and control groups.

The preclinical trials were performed with male rats (Buonfiglio et al. 2018; Marková et al. 2004; Montano et al. 2010; Puchalski et al. 2003; Rasmussen et al. 1999, 2001; Ríos-Lugo et al. 2010, 2015; Terrón et al. 2013; Wolden-Hanson et al. 2000), mice (Farias et al. 2019a, 2019b) and dogs (Taheri et al. 2019). In this regard, it is important to highlight that C57BL/6 J mice strain used in both studies by Farias et al. has severely compromised synthesis of

melatonin, which can modify its circadian rhythmicity (Roseboom et al. 1998).

Mice were 8 weeks old at the beginning of the intervention, and dogs ranged from one to three years old. Rats' age ranged from 36 days to 10 months but was not available in all articles. Most articles were carried out in USA (Puchalski et al. 2003; Rasmussen et al. 1999, 2001; Wolden-Hanson et al. 2000), but also in Brazil (Buonfiglio et al. 2018; Farias et al. 2019a, 2019b), Spain (Montano et al. 2010; Ríos-Lugo et al. 2010; Terrón et al. 2013), Iran (Bahrami et al. 2019; Taheri et al. 2019), Argentine (Ríos-Lugo et al. 2015), Poland (Szewczyk-Golec et al. 2017) and Slovak Republic (Marková et al. 2004). The duration of interventions ranged from 28 to 336 days, and the dose of exogenous melatonin orally administered varied between 0.02 mg and 10 mg/day.

Table 3 presents the summary of the characteristics and main results of the included studies.

Table 3. Characteristics and main results of the included studies.

Author	Bahrami et al.	Szewczyk-Golec et al.	Buonfiglio et al.	Farias et al. (a)	Farias et al. (b)	Marková et al.	Montano et al.
Year	2019	2017	2018	2019	2019	2004	2010
Country	Iran	Poland	Brazil	Brazil	Brazil	Slovak Republic	Spain
Design details	Randomized double-blind controlled trial	Randomized triple-blind controlled trial	Preclinical trial	Preclinical trial	Preclinical trial	Preclinical trial	Preclinical trial
Number of participants	Intervention: 36 Control: 34	Intervention: 15 Control: 15	Intervention: 10 Control: 10	Intervention: 21 Control: 20	Intervention: 21 Control: 20	Control: 14 Intervention: 18	Control: 4 Intervention: 4
Species	Human	Human	Wistar rats	C57BL/6 mice	C57BL/6 mice	Sprague-Dawley rats	Wistar rats
Gender	Male (65.7%)	Male (33.3%)	Male	Male	Male	Male (43.7%)	Male
Age(mean ± SD)	Intervention: 42.5 years ± 9.8 years Control: 42.6 years ± 10.2 years	Intervention: 37.7 years ± 3.4 years Control: 36.3 years ± 4.2 years	NA*	8 weeks	8 weeks	36-37 days	NA
Daily dose and timing of melatonin administration	6 mg, 1h before bedtime	10 mg, 1h before bedtime	1 mg/kg BW†, during dark phase	1 mg/kg BW, during dark phase	1 mg/kg BW, during dark phase	0.004 mg/mL of drinking water, from 03:00 to 08:00 h	1 mg/kg BW, during dark phase
Estimated daily dose ingested	6 mg	10 mg	0.22 mg	0.02 mg	0.02 mg	Females: 0.11 mg Males: 0.16 mg ‡	0.17 - 0.19 mg
Route of administration§	Oral, received in tablets	Oral, received in cachets	Oral, received in drinking water	Oral, received in drinking water	Oral, received in drinking water	Oral, received in drinking water	Oral, received in drinking water
Duration of intervention	84 days	30 days	91 days	70 days	70 days	182 days	28 days
Outcomes of interest	Calories, proteins, carbohydrates, fats and cholesterol intake; serum leptin levels	Serum leptin levels	Food intake (grams/day); serum leptin levels	Food intake (grams/day)	Serum leptin levels	Food intake (g/day)	Meal frequency and food intake (grams/period)
Method for assessing outcomes	24h food recall, blood collection at baseline and end of protocol (ELISA//)	Blood collected at baseline and at the end of the protocol (ELISA)	Food weighing three times a week; blood collected at the end of protocol (multiplex assay)	Food weighing weekly	Blood collected at the end of protocol (ELISA)	Food weighing monthly	Food weighing daily
Main results	No difference in food intake and reduction in leptin levels after intervention	Leptin levels remained unchanged in both groups after intervention	Reduction in food intake by 9 and 13 weeks ($p<0.001$) and leptin after intervention ($p<0.05$)	No difference in food intake between groups after intervention	Reversal of the increase of leptin triggered by obesity ($p<0.05$)	No difference in average daily food intake after intervention	Lower total intake and higher meal frequency during scotophase after intervention ($p<0.05$)

Author	Puchalski et al.	Rasmussen et al.	Rasmussen et al.	Ríos-Lugo et al.	Ríos-Lugo et al.	Taheri et al.	Terrón et al.	Wolden-Hanson et al.
Year	2003	1999	2001	2015	2010	2019	2013	2000
Country	USA	USA	USA	Argentina	Spain	Iran	Spain	USA
Design details	Preclinical trial	Preclinical trial	Preclinical trial	Preclinical trial	Preclinical trial	Preclinical trial	Preclinical trial	Preclinical trial
Number of participants	Control: 14 Intervention: 14	Control: 14 Intervention: 10	Control: 12 young, 12 middle-age Intervention: 12 young, 12 middle-age	Control: 16 Intervention: 16	Control: 48 Intervention: 48	Control: 5 Intervention: 5	Control: 10 Intervention: 10	Control: 19 Intervention: 18
Species	Sprague-Dawley rats	Sprague-Dawley rats	Sprague-Dawley rats	Wistar rats	Wistar rats	Mixed breed dogs	Wistar rats	Sprague-Dawley rats
Gender	Male	Male	Male	Male	Male	Male	Male	Male

(*Continued*)

Table 3. (Continued).

Author	Puchalski et al.	Rasmussen et al.	Rasmussen et al.	Ríos-Lugo et al.	Ríos-Lugo et al.	Taheri et al.	Terrón et al.	Wolden-Hanson et al.
Age (mean ± SD)	10 months	Control: 4 months Intervention: 10 months	3 months (young) and 10 months (middle-age) in each group	70 days	45 days	1-3 years	2-4 months	10 months
Daily dose and timing of melatonin administration	0.2 µg/mL of liquid diet, during the final 15 min of the light period	Exp. 1: 4 µg/mL of drinking water; Exp. 2: 0.4 µg/mL of drinking water, both free access	0.2 µg/mL of drinking water, free access	2,3 mg/kg BW, free access	25 µg/mL of drinking water, free access	3 mg/10 kg BW, NA	20 µg/mL of drinking water, free access	0.4 µg/mL of drinking water, free access
Estimated daily dose ingested	0.35-0.45 mg	Exp. 1: 1.6 - 2 mg Exp. 2: 0.16 - 0.2 mg	0.08 - 0.1 mg	0.53 - 0.6 mg	0.5 mg	6 mg	0.65 mg	0.14 - 0.19 mg
Route of administration §	Oral, received in liquid diet	Oral, received in drinking water	Oral, received in drinking water	Oral, received in drinking water	Oral, received in drinking water	Oral, received in gelatinous capsules	Oral, received in drinking water	Oral, received in drinking water
Duration of intervention	56 days	Exp. 1: 70 days; Exp. 2: 336 days	70 days	70 days	63 days	30 days	105 days	84 days
Outcomes of interest	Food intake (g/day); serum leptin levels	Serum leptin levels	Serum leptin levels	Serum leptin levels	Food intake (g/day); serum leptin levels	Serum leptin and ghrelin levels	Food intake (g/day)	Food intake (g/day), serum leptin levels
Method for assessing outcomes	Blood collected weekly (radioimmunoas-say kit)	Blood collected at the end of each protocol (radioimmunoassay kit)	Blood collected at the end of protocol (radioimmunoassay kit)	Blood collected at the end of protocol (multiplex assay)	Food weighing daily during the last 14 days; blood collected at the end of protocol (multiplex assay)	Blood collected weekly (ELISA)	Food weighing daily	Food weighing three times a week; blood collected at the end of protocol (radioimmunoassay kit)
Main results	No difference in daily food intake; decrease in fasting leptin after 3 weeks ($p<0.01$) and fed leptin after 8 weeks ($p<0.01$)	Leptin in middle-aged rats were restored to youthful levels after experiments 1 ($p<0.05$) and 2 ($p<0.01$)	Suppression by 51% in leptin levels in middle-aged aged rats after intervention ($p<0.001$)	Decrease in leptin levels after intervention ($p<0.05$)	No difference in daily food intake and disrupted daily pattern of leptin after intervention ($p<0.05$)	Decrease in leptin ($p<0.01$) at days 7, 14, 21 ($p<0,01$) and ghrelin ($p<0.01$) each sampling time	No difference in average daily food intake after intervention	No difference in daily food intake and decrease by 33% in nonfasted plasma leptin levels after intervention ($p<0.05$)

* Not available. † Body weight. ‡ No statistical significant difference between groups. § In all studies, except Bahrami et al. and Szewczyk-Golec et al. (clinical trials), melatonin was primarily diluted in a solution of drinking water with final ethanol concentration of 0.01%.//Enzyme-Linked Immunosorbent Assay.

Eating habits

Regarding the outcome, Buonfiglio et al. (2018), Farias et al. (2019a), Marková et al. (2004), Puchalski et al. (2003), Ríos-Lugo et al. (2010), Terrón et al. (2013) and Wolden-Hanson et al. (2000) assessed total food intake (g/animal/day). Montano et al. (2010), in turn, were the only authors that evaluated total food intake per period and meal frequency. Bahrami et al. (2019) assessed calories and macronutrients.

Buonfiglio et al. (2018) investigated how exogenous melatonin regulates energy intake and expenditure to promote a proper energy balance in rats, while Farias et al. (2019a) investigated the effects of melatonin supplementation on the prevention of obesity-associated complications in mice. To assess food intake, the authors used food weighing three times a week (Buonfiglio et al. 2018) and once a week (Farias et al. 2019a). Buonfiglio et al. (2018) found a reduction in food intake after nine weeks of intervention, and they attribute the result to melatonin's action on the hypothalamus, which modulates the expression of peptides related to food intake.

Additionally, the authors refer that food intake regulation by melatonin may occur *via* modulation of the hypothalamic suprachiasmatic nucleus (SCN), site of the master circadian clock that is sensitive to the chronobiotic effects of melatonin (Buonfiglio et al. 2018). On the other hand, Farias et al. (2019a) also did not find significant differences in food intake after the intervention but did not provide any hypothesis to explain this specific result.

Marková et al. 2004, who evaluated the effect of melatonin supplementation in food intake according to sex, found no difference in food intake comparing treated males and females with its respective control groups. However, the authors verified that both groups of males ate more than both groups of females and explain that the amount of food taken by the rats corresponds with their body weight. Thus, the results suggest that melatonin supplementation is able to influence energy metabolism without altering food consumption. Puchalski et al. (2003), Terrón et al. (2013), Ríos-Lugo et al. (2010) and Wolden-Hanson et al. (2000) also observed that exogenous melatonin promotes metabolic effects (presented below) without modifying food intake.

On the other hand, Montano et al. (2010), together with lower food intake, observed a higher meal frequency during scotophase (i.e., the dark phase) in treated rats compared with control group. The authors attribute the results to the influence of melatonin on leptin and ghrelin levels, which are involved in appetite control both in the long and short term.

Finally, the clinical trial conducted by Bahrami et al. (2019) used 24 h food recalls to assess calories, macronutrients and cholesterol intake. After melatonin supplementation, it was observed that the amount of food taken was significantly reduced. The potential mechanism presented to explain this result is the key role that leptin plays in regulating food intake, so the decrease in its serum concentration may be related to reduced ingestion.

Appetite-regulating hormones

Buonfiglio et al. (2018), Bahrami et al. (2019), Farias et al. (2019b), Puchalski et al. (2003), Rasmussen et al. (1999, 2001), Ríos-Lugo et al. (2010, 2015), Taheri et al. (2019), Wolden-Hanson et al. (2000) and Szewczyk- Golec et al. (2017) assessed appetite-regulating hormones. All the cited authors evaluated leptin concentration, but only Taheri et al. (2019) evaluated ghrelin levels. Buonfiglio et al. (2018), Bahrami et al. (2019), Farias et al. (2019b), Rasmussen et al. (1999, 2001), Ríos- Lugo et al. (2010, 2015) and Szewczyk-Golec et al. (2017) assessed appetite-regulating hormones at the baseline and at the end of the protocol by, while Puchalski et al. (2003) and Taheri et al. (2019) did weekly blood collections during the interventions. The outcomes were assessed using Enzyme-Linked Immunosorbent Assay (ELISA), multiplex assay or radioimmunoassay.

Regarding leptin, Buonfiglio et al. (2018), Farias et al. (2019b), Puchalski et al. (2003), Rasmussen et al. (1999, 2001), Ríos-Lugo et al. (2010, 2015), Taheri et al. (2019), Wolden-Hanson et al. (2000) and Szewczyk-Golec et al. (2017) observed a significant decrease in its serum levels after intervention. Ríos-Lugo et al. (2010), in turn, observed a disruption in the daily pattern of the hormone after intervention.

Buonfiglio et al. (2018) observe that while melatonin reduces food intake potentially because of its chronobiotic effects, a significant reduction in orexigenic signals causes an augment in molecules associated with anorexigenic signals, such as leptin. Farias et al. (2019b) evaluated melatonin's efficiency in delaying or blocking the damages caused by a high-fat diet in mice, as well as improving the inflammatory profile triggered by obesity. The authors also attribute the leptin reduction to changes at the molecular level, where genes encoding pro-inflammatory cytokines, such as leptin, have their expression reduced.

Puchalski et al. (2003) evaluated fed (rats were sacrificed 5 h after being fed a liquid diet) and fasting (rats were sacrificed >9 h after the liquid diet was removed from

the cage) plasma leptin levels and verified that eight weeks of melatonin supplementation suppressed fed leptin, while only three weeks suppressed fasting leptin levels. Considering that there were no effects on food intake, the authors suggest that melatonin effects are independent of effects on food intake.

Rasmussen et al. (1999) performed two experiments with different doses of melatonin to young and middle- aged rats and verified that both reduced leptin to youthful levels in the older group within 10 weeks of intervention. In another study, Rasmussen et al. (2001) administered an even lower dose and verified that melatonin was still able to reestablish leptin to youthful levels in middle-aged rats. However, parameters on young rats remained unchanged, suggesting an interesting aging-dependent effect of melatonin supplementation.

Ríos-Lugo et al. (2010) verified that exogenous melatonin disrupted the normal circadian rhythmicity of leptin by suppressing its night nadir in the plasma without significant effects of food intake but observed that the mechanism still remained to be defined. Afterward, Ríos-Lugo et al. (2015) evaluated the activity of melatonin on gene expression of some medio-basal hypothalamus signals involved in feeding behavior regulation, including leptin. The authors found that melatonin decreased leptin receptors and leptin blood levels in high-fat fed rats and suggest that the supplementation was able to restore homeostasis of feeding signals in the hypothalamus.

Taheri et al. (2019) assessed the effects of melatonin administration on metabolic hormones and verified a significant reduction in leptin and ghrelin levels after supplementation. The authors refer that melatonin has anorexigenic effect, and leptin and ghrelin levels consequently decrease. Despite claiming that melatonin may be useful in preventing and controlling obesity, no potential physiological mechanism is discussed.

Wolden-Hanson et al. (2000) observed a significant decrease in fed leptin levels after melatonin supplementation and provided a hypothesis to explain the dissociation of this result with changes in food consumption: since melatonin seems to increase sensitivity to leptin, even lower levels could facilitate energy expenditure in treated individuals.

Bahrami et al. (2019) assessed the effect of melatonin supplementation on metabolic syndrome components, as well as to measure leptin blood concentrations in adults of both sexes and found the same results by Wolden-Hanson et al. (2000).

However, as the latter authors suggest, extrapolating findings from rats to humans is biased, since previous studies have shown that melatonin supplementation may act differently in distinct photoperiodic species. As the authors observed, melatonin was able to restore plasma leptin to youthful level in middle-aged rats (nocturnal animals), but its effects in humans (diurnal animals) still remains unclear (Szewczyk-Golec et al. 2017; Wolden-Hanson et al. 2000).

Szewczyk-Golec et al. (2017), finally, estimated the effect of melatonin on oxidative stress and adipokine levels in obese patients on a calorie-restricted diet. Contrary to expectations, the authors found no difference in leptin levels after melatonin supplementation. To justify their finding, they reinforce that most animal model studies successfully reduced leptin levels with exogenous melatonin administration, while results from human studies on this subject are still inconsistent.

Quality assessment

Figure 2 presents the results of quality assessment. Among clinical trials (A), high risk of bias observed is due to non-blinding of assessors from knowledge of which intervention participants received until after processing and completion of data analysis.

Among preclinical trials (B), four out of five articles were judged as unclear and high risk of selection bias due to the lack information, respectively, on: 1) if any methods were used to adequately conceal the allocation sequence, and 2) description and comparison of any baseline characteristics in order to ensure that intervention and control groups were similar. Regarding performance bias, all the five articles were considered as unclear or high risk of bias due to lack of information on if any methods were used to: 1) ensure that animals' cages were randomly housed in the room, and 2) blind caregivers from knowing which intervention an animal received. Likewise, all the five articles presented unclear and risk of detection bias due to lack of information on if any methods were used to: 1) select animals at random for outcome assessment, and 2) blind assessors from knowledge of which intervention an animal received until after completion of data analysis.

Three studies, being one clinical and two preclinical trials, were judged as having high risk of bias due to other sources of bias. We considered the small sample size and low number of participants in the experimental groups of the studies as a

potential source of bias for the reliability of the statistical analysis performed, and, subsequently, for the results obtained.

A) Clinical trials

	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias
Bahrami et al, 2019	L	L	L	H	L	L	L
Szewczyk-Golec et al, 2017	L	L	L	L	L	L	H

B) Preclinical trials

	Random sequence generation	Allocation concealment	Baseline characteristics	Random housing	Blinding of caregivers	Random outcome assessment	Blinding of assessors	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Buonfiglio et al. 2018	L	U	H	U	U	U	H	L	L	L
Farias et al. 2019a	L	L	L	U	U	U	H	L	L	L
Farias et al. 2019b	L	U	L	U	U	U	H	L	L	L
Marková et al. 2004	L	L	L	U	U	U	H	L	L	L
Montano et al. 2010	L	L	L	L	U	U	H	L	L	H
Puchalski et al. 2003	L	L	L	L	U	L	H	L	L	L
Rasmussen et al. 1999	L	L	L	U	U	U	H	L	L	L
Rasmussen et al. 2001	L	L	L	U	U	U	H	L	L	L
Ríos-Lugo et al. 2010	L	L	L	U	U	U	H	L	L	L
Ríos-Lugo et al. 2015	L	U	L	U	U	U	H	L	L	L
Taheri et al. 2019	L	U	L	U	H	U	H	L	L	H
Terrón et al. 2013	L	L	L	U	U	U	H	L	L	L
Wolden-Hanson et al. 2000	L	L	L	L	U	L	H	L	L	L

Figure 2. Risk of bias assessments for studies. (a) Clinical trials, assessed by Cochrane's RoB tool; (b) Preclinical trials, assessed by SYRCLE's RoB tool. L indicates low risk of bias; H, high risk of bias; and U, unclear risk of bias.

DISCUSSION

This systematic review of 15 studies, including clinical and preclinical trials, found that the effect of exogenous melatonin on eating habits and appetite-regulating hormones are still controversial. Of the nine studies that evaluated food intake (Buonfiglio et al. 2018; Farias et al. 2019a; Bahrami et al. 2019), only two found a significant difference (Buonfiglio et al. 2018; Montano et al. 2010). The authors demonstrated that melatonin was able to reduce food intake from 54 to 28 days of supplementation, respectively. It is already established that melatonin has chronobiotic effects and controls the circadian activity of energy metabolism, including the feeding-fasting cycle (Cipolla-Neto et al. 2014). Hence, a possible mechanism to explain the anorexigenic effect of melatonin is that it acts directly on the hypothalamic suprachiasmatic nucleus (SCN) of mammals in order to modulate food intake (Buonfiglio et al. 2018).

The present review did not include studies performed with other species than mammals, but consistent reports can be found in literature. In zebrafish treated with melatonin for 35 days, it stimulated anorexigenic signals at the molecular level, such as genes encoding leptin, and inhibited orexigenic signals such as ghrelin (Montalbano et al. 2018). The same anorexigenic effect was observed in sea basses treated for 30 days with exogenous melatonin, which promoted a dose-response inhibition of food consumption from 9 to 34%. Furthermore, the authors verified changes in the pattern of macronutrient selection: carbohydrate intake decreased by 17 to 42%, showing a dose–response reduction as well. The amount of fat in the diet also decreased during the period in which the highest dose was administered (Rubio et al. 2004).

Evidence on the effect of melatonin supplementation on eating behavior, and food intake in general, are scant and incipient. The only clinical trial that met the inclusion criteria of the present review and evaluated total calories and composition of the diet (carbohydrate, protein, fat and cholesterol consumption) found no difference between intervention and control groups after 84 days of melatonin supplementation (6 mg/day) or placebo in healthy adult men (Bahrami et al. 2019). Likewise, the preclinical trial performed by Farias et al. (2019a) found no significant difference between groups of mice treated with melatonin or placebo for 70 days. The authors justify that it is possible that no results were found due to the low dosage administered (1 mg/kg BW [body weight]), which was chosen for being closer to the endogenous

physiological level. However, when evaluating the effect of exogenous melatonin on the expression of pro-inflammatory cytokines, the same authors found that the supplementation attenuated the inflammatory disorders triggered by the increase of leptin observed in obese mice (Farias et al. 2019b). In both studies, obesity was induced by high fat diet, and the authors highlight the potential of melatonin supplementation to prevent the deleterious effects caused by a hyperlipidic diet.

This finding is particularly interesting because the literature reports several situations that seem to lead to an increase in dietary fat intake in humans, such as increased fatigue, short sleep duration and night shift work (Heath et al. 2016; Kosmadopoulos et al. 2020; Shaw et al. 2019; St-Onge et al. 2011). The major concern in this topic is that, when checking which types of fats exceed the recommendations, most studies verify that saturated fats are those have the highest consumption (Morikawa et al. 2008). Though the relationship between dietary saturated fats and low-grade inflammation is not yet completely clear, international dietary guidelines recommend very limited amounts of saturated fat in the diet in order to reduce cardiovascular risk and metabolic disorders (<7% of total calories) (Jellinger et al. 2017; Rydén et al. 2013).

One of the criteria that define low-grade inflammation is the elevated circulating concentrations of pro- inflammatory cytokines (Calder et al. 2011). In this regard, leptin is a protein with a structure similar to cytokines and produced mainly in adipocytes in a circadian rhythm. In mammals, its action on the hypothalamus promotes a reduction in food intake and increases energy expenditure, in addition to regulating glucose and fat metabolism (Friedman and Halaas 1998). However, an increase of these cytokines, such as leptin, is linked with obesity-associated endocrine- metabolic disorders. On the other hand, obesity induced by a hyperlipidic diet might be associated with the desynchronization in biological rhythms of metabolic processes due to melatonin absence (Farias et al. 2019b).

A total of 11 out of 15 studies evaluated leptin concentrations. Of these, 10 found a reduction (Buonfiglio et al. 2018; Bahrami et al. 2019; Farias et al. 2019b; Puchalski et al. 2003; Rasmussen et al. 1999, 2001; Ríos- Lugo et al. 2010, 2015; Taheri et al. 2019; Wolden-Hanson et al. 2000) and one found no difference (Szewczyk-Golec et al. 2017) in leptin levels after melatonin supplementation. Bahrami et al. (2019) and Szewczyk-Golec et al. (2017), both clinical trials, equally administered melatonin 1 h before bedtime. Although the dosage administered by the former was

lower (6 vs. 10 mg/day), the duration of the intervention was longer (84 vs. 30 days) and the sample size was larger (36 vs. 15 participants in the intervention group), so that these two factors may have contributed to obtain a significant reduction in leptin levels. On the other hand, the preclinical trial performed by Taheri et al. (2019) found a significant reduction in leptin levels after seven days treating five mixed breed, male adult dogs with 3 mg/10 kg BW.

Moreover, it is noteworthy that Szewczyk-Golec et al. (2017) was the only study included in the present review conducted with a predominantly female sample. Since melatonin is locally synthesized by the ovary and its chronobiotic effects may affect ovarian function and reproduction (Tagliaferri et al. 2018), the fact that the authors did not take into consideration the phase of the menstrual cycle of the female participants may have affected the results.

Studies performed with rodents seem to obtain more consistent results than those performed in humans (Kitagawa et al. 2012; Wolden-Hanson et al. 2000). Evidence from animal model studies suggest that melatonin reduces leptin via lipogenesis suppression, that is, reducing leptin release from adipocytes into the circulation (Wolden-Hanson et al. 2000). It is well established that elevated levels of leptin induce leptin resistance and, consequently, dysregulation of appetite and food intake (Szewczyk-Golec et al. 2015). In humans, this scenario is associated not only with obesity *per se*, but also with its complications (Antuna-Puente et al. 2008).

An interesting result is found in Bahrami et al. (2019): the reduction in serum leptin levels after melatonin supplementation occurred without any changes in the consumption of total calories and percentages of macronutrient. According to Ríos-Lugo et al. (2015), a possible physiological explanation may be the increase in central orexigenic pathways in order to compensate increase of the satiety signals. This finding supports the hypothesis that alterations in food intake do not contribute for exogenous melatonin efficacy in mitigating the deleterious effects caused by inflammatory disorders, such as obesity (Reiter et al. 2012).

With regard more directly to satiety, only one included study evaluated serum levels of ghrelin (Taheri et al. 2019). The authors found reduction in ghrelin concentrations in all sampling times during the 30 days of protocol (blood analysis were performed weekly) in comparison with control group receiving placebo. Frequently less evaluated than leptin, ghrelin is a hormone predominantly produced by the Gr cells in the gastrointestinal tract, and for the context of the present review its

most important activity is the orexigenic, which stimulates food intake and increases adiposity (Nakazato et al. 2001). Therefore, the result found by Taheri et al. (2019) is consistent with the anorexigenic effect of melatonin previously described.

The present systematic review of clinical and preclinical trials is the first, to our knowledge, to summarize the effects of exogenous melatonin supplementation on eating habits and appetite-regulating hormones. Therefore, it allows to synthesize evidence from the gold standard in intervention-based studies in order to establish a reliable clinical application, and also highlights the existing gaps in the current knowledge on the subject. However, it has a few limitations. A very small number of studies met the inclusion criteria, which limits the ability of the present review to obtain a robust conclusion. Nevertheless, it is important to emphasize that we have chosen not to restrict the search according to the publication date due to the incipient knowledge of the therapeutic use of melatonin, especially in humans, and the small number of experimental studies on this topic (Cipolla-Neto and Amaral 2018). Also, the heterogeneity and reliability of the results found may be due to different factors, such as pharmaceutical forms (pills, cachets, gelatinous capsules, liquid solutions), dosage, duration of treatment, timing of administration, sample size and study protocols. Several risks of bias among the included preclinical trials remained unclear, which may also explain the inconsistence of the results. None of the included studies informed the pharmaceutical release forms of melatonin (slow, fast, mixed). Regarding the eating behavior, different methods used to evaluate food intake can also contribute to the inconsistency of the results between studies. Unfortunately, none of the included studies evaluated meal timing and eating duration, two emergent aspects of the diet that have been discussed as possible targets of intervention to counteract metabolic consequences of obesity and shift work (Gill and Panda 2015; Kosmadopoulos et al. 2020).

It is important to highlight that there is a large interindividual variation in absorption, metabolism and elimination of melatonin. This variation, among other factors, is related to age, clinical condition and presence of pathologies, which can influence the expected clinical efficacy. There is also an interindividual variability in the response of the circadian system to artificial light exposure at night, which induces higher or lower melatonin suppression according to the degree of sensitivity and light intensity (Phillips et al. 2019). Thus, dosage and pharmaceutical formulation must be individually considered (Cipolla-Neto and Amaral 2018). It is also important to

emphasize that the studies here reviewed include nocturnal and diurnal mammals, which may raise doubts about the obtained results. However, melatonin immediate and prospective effects are known to be the same in both species, the only difference being the phase of the daily cycle in which they occur (Cipolla- Neto and Amaral 2018). A significant advantage is that, though studies on pharmacokinetics are limited, exogenous melatonin is shown to be safe and lacks adverse effects in comparison to placebo (Cipolla-Neto and Amaral 2018). More studies are certainly needed to evaluate the effects of melatonin administration in humans, but the authors here reviewed point out that it may be an effective, low-cost hormone to treat and prevent obesity and its correlated conditions (Farias et al. 2019a; Bahrami et al. 2019; Farias et al. 2019b).

Findings about effects of exogenous melatonin supplementation on eating habits and appetite-regulating hormones are still heterogeneous and do not allow a robust conclusion. The present review adds to the growing body of evidence suggesting that exogenous melatonin may be a potential therapeutic agent against important issues brought up by the contemporary 24 h society, such as shorter sleep duration and exposure to artificial light at night due to shift work or long exposure to electronic devices. The included studies in the present review suggest that melatonin may be capable of preventing and reverting endocrine-metabolic alterations triggered by inflammatory disorders, especially obesity. This reversal does not seem to be necessarily associated with changes in total calories or macronutrient intake, signaling that melatonin's anorexigenic effect may occur independently of energy intake. However, it is important to reinforce that further studies, especially with humans, are needed to provide more evidence for adequate supplementation of exogenous melatonin in clinical practice. It is also important to highlight that specific groups at higher risk of metabolic disorders due to melatonin suppression, such as shift workers, have not been studied yet, and need to be considered in particular.

AUTHORSHIP

LFRN designed research, conducted research, analyzed data, wrote the paper and had primary responsibility for final content. ECM designed research, conducted research, analyzed data and had primary responsibility for final content.

FUNDING

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES under grant number [88887.356981/2019-00].

REFERENCES

Antuna-Puente B, Feve B, Fellahi S, Bastard JP. 2008. Adipokines: the missing link between insulin resistance and obesity. Diabetes Metab. 34(1):2–11. doi:10.1016/j. diabet.2007.09.004

Bahrami M, Cheraghpour M, Jafarirad S, Avalinejad P, Cheraghian B. 2019. The role of melatonin supplement in metabolic syndrome: a randomized double blind clinical trial. Food Sci Nutr. 49(5):965–977. doi:10.1108/NFS-01- 2019-0018

Bartness TJ, Wade GN. 1985. Body weight, food intake and energy regulation in exercising and melatonin-treated Siberian hamsters. Physiol Behav. 35(5):805–808. doi:10.1016/0031-9384(85)90415-9

Bubenik GA, Pang SF. 1994. The role of serotonin and melatonin in gastrointestinal physiology: ontogeny, regulation of food intake, and mutual serotonin-melatonin feedback. J Pineal Res. 16:91–99. doi:10.1111/j.1600-079x.1994. tb00088.x

Bubenik GA, Pang SF, Hacker RR, Smith PS. 1996. Melatonin concentrations in serum and tissues of porcine gastrointestinal tract and their relationship to the intake and passage of food. J Pineal Res. 21(4):251–256. doi:10.1111/j.1600- 079x.1996.tb00294.x

Buonfiglio D, Parthimos R, Dantas R, Silva RC, Gomes G, Andrade-Silva J, Ramos-Lobo A, Amaral FG, Matos R, Sinésio J Jr, et al. 2018. Melatonin absence leads to long-term leptin resistance and overweight in rats. Front Endocrinol. 9:122. doi:10.3389/fendo.2018.00122

Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, Esposito K, Jönsson LS, Kolb H, Lansink M, et al. 2011. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 106 (Suppl 3):5–78. doi:10.1017/S0007114511005460

Chin-Chance C, Polonsky KS, Schoeller D. 2010. Twenty-four hour leptin levels respond to cumulative short-term energy imbalance and predict subsequent intake. J Clin Endocrinol Metab. 85(8):2685–2691. doi:10.1210/jcem.85.8.6755

Cipolla-Neto J, Amaral FG. 2018. Melatonin as a hormone: new physiological and clinical insights. Endocr Rev. 39 (6):990–1028. doi:10.1210/er.2018-00084 Cipolla-Neto J, Amaral FG, Afeche SC, Tan DX, Reiter RJ. 2014. Melatonin, energy metabolism, and obesity: a review. J Pineal Res. 56(4):371–381. doi:10.1111/jpi.12137

Farias TSM, Cruz MM, Sá RCC, Severi I, Perugini J, Senzacqua M, Cerutti SM, Giordano A, Cinti S, Alonso-Vale MIC. 2019a. Melatonin supplementation decreases hypertrophic obesity and inflammation induced by high-fat diet in mice. Front Endocrinol. 10:750. doi:10.3389/fendo.2019.00750

Farias TSM, Paixão RI, Cruz MM, Paixão RI, Cruz MM, Sá RDCC, Simão JJ, Antraco VJ, Alonso-Vale MIC. 2019b. Melatonin supplementation attenuates the pro-inflammatory adipokines expression in visceral fat from obese mice induced by a high-fat diet. Cells. 8(9):1041. doi:10.3390/ cells8091041

Friedman JM, Halaas JL. 1998. Leptin and the regulation of body weight in mammals. Nature. 395:763–770. doi:10.1038/27376

Garaulet M, Lee YC, Shen J, Parnell LD, Arnett DK, Tsai MY, Lai CQ, Ordovas JM. 2010. Genetic variants in human CLOCK associate with energy intake and cytokine sleep factors in overweight subjects (GOLDN population). Eur J Human Gen. 18:364–369. doi:10.1038/ejhg.2009.176

Garaulet M, Madrid JA. 2010. Chronobiological aspects of nutrition, metabolic syndrome and obesity. Adv Drug Deliv Rev. 62(9–10):967–978. doi:10.1016/j.addr.2010.05.005

Gill S, Panda S. 2015. A smartphone app reveals erratic diurnal eating patterns in humans that can be modulated for health benefits. Cell Metab. 22(5):789–798. doi:10.1016/j. cmet.2015.09.005

Heath G, Coates A, Sargent C, Dorrian J. 2016. Sleep duration and chronic fatigue are differently associated with the dietary profile of shift workers. Nutrients. 8(12):771. doi:10.3390/nu8120771

Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JAC, et al. 2011. The Cochrane collaboration's tool for assessing risk of bias in randomized trials. BMJ. 343:d5928. doi:10.1136/bmj.d5928

Higgins JPT, Green S, editors. 2008. Cochrane handbook for systematic reviews of interventions. 1st ed. New Jersey: The Cochrane Collaboration and John Wiley & Sons.

Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. 2014. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 14(1):14–43. doi:10.1186/1471-2288-14-43

Huether G. 1994. Melatonin synthesis in the gastrointestinal tract and the impact of nutritional factors on circulating melatonin. Ann NY Acad Sci. 719:146–158. doi:10.1111/ j.1749-6632.1994.tb56826.x

Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, Grunberger GCK, Guerin CK, Bell DSH, Mechanick JI, et al. 2017. American association of clinical endocrinologists and American college of endocrinology guidelines for management of dyslipidemia and prevention of cardiovascular disease. Endocr Pract. 23
(Suppl 2):1–87. doi:10.4158/EP171764.APPGL

Kitagawa A, Ohta Y, Ohashi K. 2012. Melatonin improves metabolic syndrome induced by high fructose intake in rats. J Pineal Res. 52(4):403–413. doi:10.1111/j.1600-079X.2011.00955.x

Kosmadopoulos A, Kervezee L, Boudreau P, Gonzales-Aste F, Vujovic N, Scheer FAJL, Boivin DB. 2020. Effects of shift work on the eating behavior of police officers on patrol. Nutrients. 12(4):999. doi:10.3390/nu12040999

Manoogian ENC, Panda S. 2017. Circadian rhythms, time-restricted feeding, and healthy aging. Ageing Res Rev. 39:59–67. doi:10.1016/j.arr.2016.12.006

Marková M, Adámeková E, Bojková B, Kubatka P, Kassayová M, Ahlersová E, Ahlers I. 2004. Effect of low-dose chronic melatonin administration on metabolic and hormonal variables in young laboratory rats. Acta Vet BRNO. 73:445–453. doi:10.2754/avb200473040445

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA; PRISMA-P Group. 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 4(1). doi:10.1186/2046-4053-4-1.

Montalbano G, Mania M, Abbate F, Navarra M, Guerrera MC, Vega JA, Levanti M, Germanà A. 2018. Melatonin treatment suppresses appetite genes and improves adipose tissue plasticity in diet-induced obese zebrafish. Endocrine. 62 (2):381–393. doi:10.1007/s12020-018-1653-x

Montano ME, Molpeceres V, Mauriz JL, Garzo E, Cruz IBM, González P, Barrio JP. 2010. Effect of melatonin supplementation on food and water intake in streptozotocin-diabetic and non-diabetic male Wistar rats. Nutr Hosp. 25(6):931–938. doi:10.3305/nh.2010.25.6.4803

Morikawa Y, Miura K, Sasaki S, Yoshita K, Yoneyama S, Sakurai M, Ishizaki M, Kido T, Naruse Y, Suwazono Y, et al. 2008. Evaluation of the effects of shift work on nutrient intake: a cross-sectional study. J Occup Health. 50
(3):270–278. doi:10.1539/joh.l7116

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H,
Kangawa K, Matsukura S. 2001. A role for ghrelin in the central regulation of feeding. Nature. 409:194–198. doi:10.1038/ 35051587

Nogueira TC, Lellis-Santos C, Jesus DS, Taneda M, Rodrigues SC, Amaral FG, Lopes MAS, Cipolla-Neto J, Bordin S, Anhê GF. 2011. Absence of melatonin induces night-time hepatic insulin resistance and increased gluconeogenesis due to stimulation of nocturnal unfolded protein response. Endocrinology. 152 (4):1253–1263. doi:10.1210/en.2010-1088

Phillips AJK, Vidafar P, Burns AC, Mcglashan EM, Anderson C, Rajaratnam SMW, Lockley SW, Cain SW. 2019. High sensitivity and interindividual variability in the response of the human circadian system to evening light. PNAS. 116(24):12019–12024. doi:10.1073/pnas.1901824116

Puchalski SS, Green JN, Rasmussen DD. 2003. Melatonin effect on rat body weight regulation in response to high-fat diet at middle age. Endocrine. 21(2):163–168. doi:10.1385/endo:21:2:163

Rasmussen DD, Boldt BM, Wilkinson C, Yellon SM, Matsumoto AM. 1999. Daily melatonin administration at middle age suppresses male rate visceral fat, plasma leptin, and plasma insulin to youthful levels. Endocrinology. 140 (2):1009–1012. doi:10.1210/endo.140.2.6674

Rasmussen DD, Mitton DR, Larsen SA, Yellon SM. 2001. Aging-dependent changes in the effect of daily melatonin supplementation on rat metabolic and behavioral responses. J Pineal Res. 31(1):89–94. doi:10.1034/j.1600- 079x.2001.310113.x

Reiter RJ, Tan DX, Korkmaz A. 2009. The circadian melatonin rhythm and its modulation: possible impact on hypertension. J Hypertens.
27(Suppl. 6):17–20. doi:10.1097/01.hjh.0000358832.41181.bf

Reiter RJ, Tan DX, Korkmaz A, Ma S. 2012. Obesity and metabolic syndrome: association with chronodisruption, sleep deprivation, and melatonin suppression. Ann Med. 44(6):564–577. doi:10.3109/07853890.2011.586365

Ríos-Lugo MJ, Cano P, Jiménez-Ortega V, Fernández-Mateos MP, Scacchi PA, Cardinali DP, Esquifino AI. 2010. Melatonin effect on plasma adiponectin, leptin, insulin, glucose, triglycerides and cholesterol in normal and high fat-fed rats. J Pineal Res. 49(4):342–348. doi:10.1111/j.1600- 079x.2010.00798.x

Ríos-Lugo MJ, Jiménez-Ortega V, Cano-Barquilla P, Mateos PF, Spinedi EJ, Cardinali DP, Esquifino AI. 2015. Melatonin counteracts changes in hypothalamic gene expression of signals regulating feeding behavior in high-fat fed rats. Horm Mol Biol Clin Investig. 21 (3):175–183. doi:10.1515/hmbci-2014-0041

Roenneberg T, Pilz LK, Zerbini G, Winnebeck EC. 2019. Chronotype and social jetlag: a (Self-) critical review. Biology. 8:3. doi:10.3390/biology8030054

Roseboom PH, Namboodiri MA, Zimonjic DB, Popescu NC, Rodriguez IR, Gastel JA, Klein DC. 1998. Natural melatonin 'knockdown' in C57BL/6J mice: rare mechanism truncates serotonin N-acetyltransferase. Brain Res Mol Brain Res. 10 (63):189–197. doi:10.1016/s0169-328x(98)00273-3

Rubio VC, Sanchez-Vazquez FJ, Madrid JÁ. 2004. Oral administration of melatonin reduces food intake and modifies macronutrient selection in European sea bass (Dicentrarchus labrax, L.). J Pineal Res. 37(1):42–47. doi:10.1111/j.1600-079X.2004.00134.x

Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N, Deaton C, Escaned J, Hammes HP, Huikuri H, et al. 2013. The task force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J. 34(39):3035–3087. doi:10.1093/eurheartj/eht108

Sahin L, Figueiro MG. 2013. Alerting effects of short-wave-length (blue) and long-wave-length (red) lights in the after- noon. Physiol Behav. 116–117:1–7. doi:10.1016/j.physbeh.2013.03.014
Shaji AV, Kulkarni SK. 1998. Evidence of GABAergic modulation in melatonin-induced short-term memory deficits and food consumption. Methods Find Exp Clin Pharmacol. 20 (4):311–319. doi:10.1358/mf.1998.20.4.485685
Shaw E, Dorrian J, Coates AM, Leung GKW, Davis R, Rosbotham E, Warnock R, Huggins CE, Bonham MP. 2019. Temporal pattern of eating in night shift workers. Chronobiol Int. 36(12):1613–1625. doi:10.1080/07420528.2019.1660358
Shi SQ, Ansari TS, McGuinness OP, Wasserman DH, Johnson CH. 2016. Circadian disruption leads to insulin resistance and obesity. Curr Biol. 23(5):372–381. doi:10.1016/j.cub.2013.01.048
Souza RV, Sarmento RA, de Almeida JC, Canuto R. 2019. The effect of shift work on eating habits: a systematic review. Scand J Work Environ Health. 45(1):7–21. doi:10.5271/sjweh.3759
St-Onge MP, Roberts AL, Chen J, Kelleman M, O'Keeffe M, RoyChoudhury A, Jones PJH. 2011. Short sleep duration increases energy intakes but does not change energy expenditure in normal-weight individuals. Am J Clin Nutr. 94 (2):410–416. doi:10.3945/ajcn.111.013904
Szewczyk-Golec K, Rajewski P, Gackowski M, Mila- Kierzenkowska C, Wesołowski R, Sutkowy P, Pawłowska M, Woźniak A. 2017. Melatonin supplementation lowers oxidative stress and regulates adipokines in obese patients on a calorie-restricted diet. Oxid Med Cell Longev. 2017:8494107. doi:10.1155/2017/8494107.
Szewczyk-Golec K, Woźniak A, Reiter JR. 2015. Interrelationships of the chronobiotic, melatonin, with leptin and adiponectin: implications for obesity. J Pineal Res. 59(3):277–2791. doi:10.1111/ jpi.12257
Tagliaferri V, Romualdi D, Scarinci E, de Cicco S, di Florio C, Immediata V, Tropea A, Santarsiero CM, Lanzone A, Apa R. 2018. Melatonin treatment may be able to restore menstrual cyclicity in women with PCOS: a pilot study. Reprod Sci. 25(2):269–275. doi:10.1177/1933719117711262
Taheri P, Mogheiseh A, Tabrizi AS, Nazifi S, Salavati S, Koohi F. 2019. Changes in thyroid hormones, leptin, ghrelin and, galanin following oral melatonin administration in intact and castrated dogs: a preliminary study. BMC Vet Res. 145. doi:10.1186/s12917-019-1894-9
Tan DX, Manchester LC, Fuentes-Broto L, Paredes SD, Reiter RJ. 2011. Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. Obes Rev. 12(3):167–188. doi:10.1111/j.1467-789X.2010.00756.x
Terrón MP, Delgado-Adámez J, Pariente JA, Barriga C, Paredes SD, Rodríguez AB. 2013. Melatonin reduces body weight gain and increases nocturnal activity in male Wistar rats. Physiol Behav. 118:8–13. doi:10.1016/j. physbeh.2013.04.006
Wolden-Hanson T, Mitton DR, McCants RL, Yellon S, Wilkinson CW, Matsumoto A, Rasmussen D. 2000. Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. Endocrinology. 141(2):487–497. doi:10.1210/ endo.141.2.7311
World Health Organization. 2018. International classification of diseases for mortality and morbidity statistics, 11th revision. [accessed June 2020]. https://icd.who.int/browse11/l-m/en

6.2 ARTIGO 2

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Article

Timing and Composition of Last Meal before Bedtime Affect Sleep Parameters of Night Workers

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Citation: Nogueira, L.F.R.; Pellegrino, P.; Cipolla-Neto, J.; Moreno, C.R.C.; Marqueze, E.C. Timing and Composition of Last Meal before Bedtime Affect Sleep Parameters of Night Workers. *Clocks&Sleep* **2021**, *3*, 536–546. https://doi.org/10.3390/ clockssleep3040038

Received: 3 August 2021

Accepted: 9 October 2021

Published: 14 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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Abstract: Night workers tend to eat irregularly, both in terms of meal times and composition. The disruption in energy metabolism caused by inappropriate eating habits can negatively affect the sleep quality of these individuals. The objectives of this study were to determine the interval between the last meal and bedtime and its relationship with both diurnal and nocturnal sleep parameters, as well as to evaluate the association of the adequacy of this meal with sleep parameters. The analyses were carried out for a usual sleep routine on a workday and a day off. This cross-sectional study was part of a controlled, randomized, double-blind, crossover clinical trial. The sample comprised 30 female nursing professionals who worked permanent night shifts of 12 × 36 h. Timing and composition of the last meal were obtained from food diaries, and sleep parameters were collected via actigraphy. On multiple linear regression analysis, every hour decrease in the interval between the last meal and sleep onset there was an increase of 0.39 h on diurnal sleep duration. Regarding food intake, every 1 g of fat and 1 g of carbohydrate consumed was associated with an increase in diurnal sleep onset latency of 0.13 h and 0.02 h, respectively. These findings suggest that both timing and composition of the last meal before bedtime may be potential key factors for good diurnal and nocturnal sleep among night-shift workers.

Keywords: feeding behavior; nutrients; sleep; night-shift work; nursing personnel

1. Introduction

Heterogeneous findings regarding the influence of diet composition on the sleep of night workers may be associated with meal timing [1]. This issue is attracting growing research interest and is especially relevant in the context of dietary patterns of permanent night workers, a group subject to chronic circadian misalignment [2].

Most studies on the subject have failed to compare differences in patterns of daily calorie intake between night workers and permanent day workers. These groups differ in the distribution of meals over a 24-h period, where night workers tend to eat irregularly during their shifts, consuming carbohydrates and high-fat snacks [3,4]. However, the metabolism of carbohydrates and lipids is especially impaired during the night [5], where the intake of these nutrients at biologically inappropriate times has deleterious effects on the proper functioning of the metabolism and quality of sleep [6].

The processes of digestion and absorption of nutrients are regulated by hormones that express circadian rhythms, such as insulin, leptin, and ghrelin. Once secreted, these hormones act as metabolic signals for peripheral tissues and influence peripheral oscillators [4].

Clocks&Sleep **2021**, *3*, 536–546. https://doi.org/10.3390/clockssleep3040038 https://www.mdpi.com/journal/clockssleep

Since humans are diurnal animals, food intake at night adversely affects the homeostasis of energy metabolism [5,7]. As these oscillators are closely linked to the sleep-wake cycle, the desynchronization of the feeding-fasting cycle for central and peripheral oscillators can negatively affect sleep by disrupting energy metabolism [8].

Previous findings show that night workers tend to display poorer adherence to nutritional recommendations [9]. However, new evidence has suggested that controlling the timing of food intake represents a potential therapeutic approach in the context of preventing overweight and metabolic disorders [10,11]. Current trends indicate that this relationship, which is potentially mediated by sleep duration since childhood [12], is explained by the hypothesis that both sleep and metabolism share, and are modulated by, the same hypothalamic circuits [13].

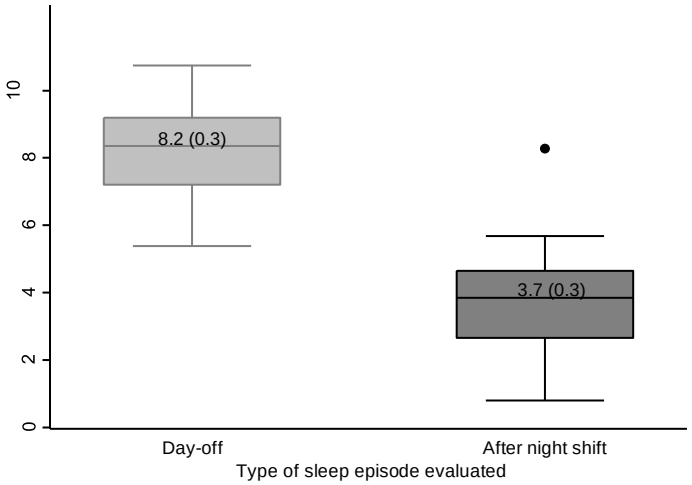
A review on nutritional deficits of nursing professionals revealed that the food intake of night workers is governed more by working hours, i.e., opportunities to eat during the shift than by hunger [14]. Torre et al. [11], in a recent evaluation of the eating habits of night workers, found that both the composition and timing of meals varied according to working hours, where the recovery day (after a night shift) proved the most affected. Also, a previous study has shown that meal content had a BMI-mediated effect on sleep, with obese workers the most affected [15].

In addition to nutrients, mealtimes can also affect sleep through circadian changes responsible for hormonal regulation of the central and metabolic nervous system [16]. When food consumption occurs close to bedtime, it leads to a shorter sleep duration and to a change in the time of sleep stages [17]. However, the time interval during which food interferes with sleep, and the nature of this interference, have yet to be elucidated [18]. Given the above, our hypothesis holds that the shorter the time interval between last meal and bedtime, and the more inadequate the content of this meal, the worse the sleep outcomes. Therefore, the objectives of this study were to determine the interval between last meal and bedtime, and its relationship to both diurnal and nocturnal sleep parameters, as well as to evaluate the association of the adequacy of this meal with sleep characteristics in overweight night shift workers.

2. Results

The diurnal sleep sample comprised a total of 30 participants and the nocturnal sleep sample comprised 22 participants (see Methods). The mean age of participants was 39.2 years (SE 1.0 years). Overall, participants were predominantly nurses (52.6%), married (63.2%), and had worked the night shift for more than five years (52.6%). Most participants were educated to postgraduate level (42.1%). The main reasons for choosing this work regimen were to reconcile work with home and childcare (44.7%) and to supplement income (21.1%). Most participants were non-smokers (89.5%) and consumed alcohol only on special occasions (65.8%). Mean body mass index (BMI) was 30 kg/m^2 (SE 0.5 kg/m^2).

Mean sleep duration was higher on the day-off (nocturnal sleep) than after the night shift (diurnal sleep). There was no significant difference for mean sleep onset latency (SOL) and mean wake-up after sleep onset (WASO) between the two groups (after night work and day-off) (Figure 1).



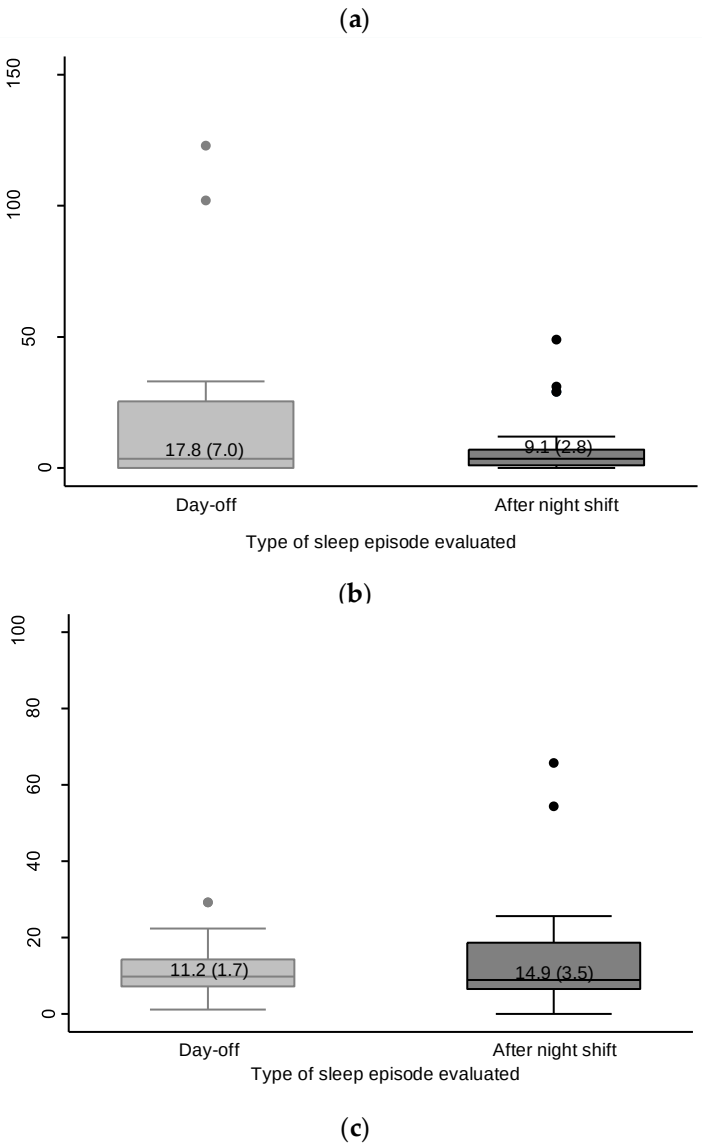
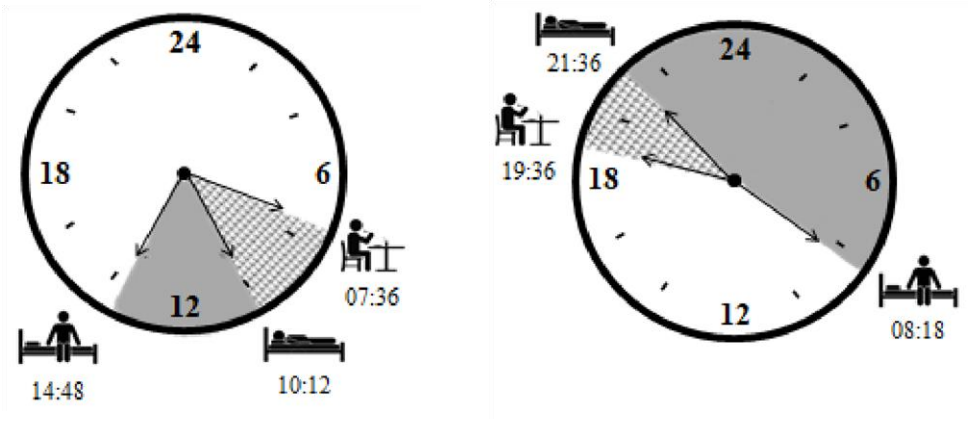


Figure 1. Sleep parameters obtained by actigraphy after night work (diurnal sleep, n = 22) and on day-off (nocturnal sleep, n = 22): (**a**) sleep duration (hours) (Paired t-test, $p < 0.01$) (**b**) sleep onset latency (minutes) (Wilcoxon $p = 0.87$) and (**c**) wake-up after sleep onset (%) (Wilcoxon $p = 1.00$). White lines represent means, gray boxes represent standard errors, and whiskers represent 95% confidence intervals.

The last meal after night work occurred 2.6 h (SE 0.3 h) before the participants initiated sleep. On the day-off, this interval was 2 h (SE 0.3 h). There were no significant differences between the intervals for the days evaluated (Paired t-test $p = 0.52$) (Figure 2).



(**a**) (**b**)

Figure 2. Mean times (clock time) of last meal before bedtime, of sleep onset and of getting up after: (**a**) night shift (*n* = 22); and (**b**) day-off (*n* = 22).

Results for mean daily intake of macronutrients on workdays and days-off reveal that both were in line with nutritional recommendations: workdays (fat: 31.1%, SE 1.3%; carbohydrate: 49.8%, SE 1.7%; protein: 18.3%, SE 1%); days-off (fat: 29.4%, SE 1.3%; carbohydrate: 51.5%, SE 1.8%, protein: 18%, SE 1.1%). Analysis of the composition of last meal before bedtime showed that mean percentages of macronutrients were adequate: workdays (fat: 26.4%, SE 2.5%; carbohydrate: 60.5%, SE 3.2%; protein: 14.9%, SE 1.8%); days-off (fat: 28.5%, SE 2.7%; carbohydrate: 58.5%, SE 3.7%; protein: 14.7%, SE 2.1%).

There was no statistically significant difference between workday and the day-off for the 24-h period and last meal before bedtime. Although these results suggest macronutrient intake of participants was adequate, most participants had inadequate fat and carbohydrate consumption, both for the 24 h recording period, and last meal before bedtime after the night shift and on the day-off (Figure 3).

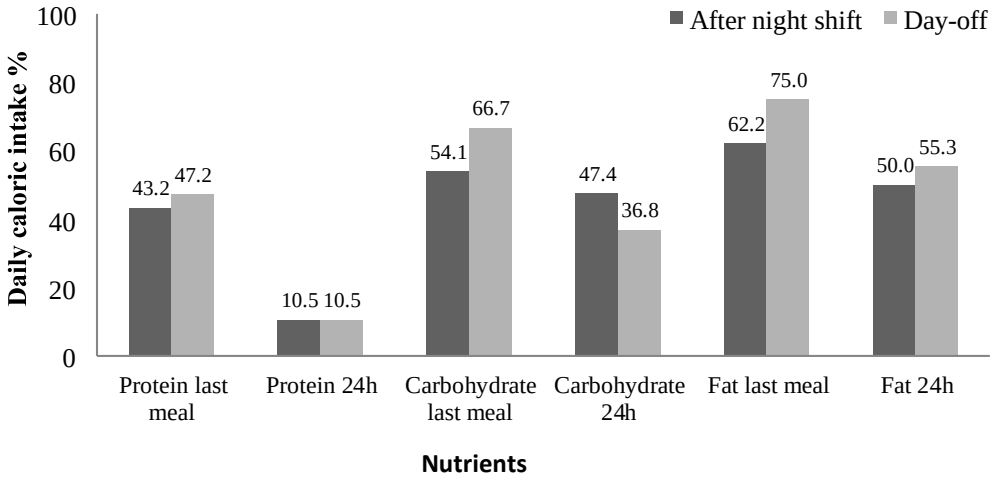


Figure 3. Percentage of participants with inadequate daily macronutrient intake for 24 h period and last meal before bed, after night shift (*n* = 30), and on day-off (*n* = 22). *t*-test, $p > 0.05$.

There was also no difference in total caloric intake after a night shift in comparison to day-off (1801 kcal vs. 1645.8 kcal, *t*-test $p = 0.38$). However, the proportion of calories provided by the last meal before the main episode of sleep was significantly higher on the day-off than after the night shift (25.5% vs. 15.2%, *t*-test $p = 0.01$).

2.1. Interval between Last Meal and Bedtime

On the multiple linear regression analysis of sleep duration on the workday, every hour decrease in interval between last meal and sleep onset was associated with an increase of 0.39 h on sleep duration (Table 1). No statistically significant results were found for the day-off.

Table 1. Sleep duration after night shift (diurnal sleep) (*n* = 28).

Variables	Crude β	95% CI	Multiple β Adj [&]	95% CI
Fat	−0.01	−0.02; 0.01	0.00	−0.01; 0.02
Carbohydrate	0.00	−0.01; 0.00	0.00	−0.01; 0.00
Protein	−0.01	−0.03; 0.00	−0.01	−0.02; 0.01
Interval between last meal and sleep onset	−0.32	−0.59; −0.05	−0.39	−0.63; −0.16

[1] Adjusted for age, chronotype and time (years) working nights.

On the multiple linear regression model of SOL on the day-off, no statistically significant results were found on the day-off. Nevertheless, on the crude linear regression for every one-hour increase in the interval between the last meal and sleep onset, there was an increase of 11.51 min in SOL (Table 2).

Table 2. Sleep onset latency (SOL) on day-off (nocturnal sleep) (*n* = 22).

Variables	Crude β	95% CI	Multiple β Adj [&]	95% CI
Fat	−0.10	−0.36; 0.16	0.09	−0.06; 0.24
Carbohydrate	−0.03	−0.16; 0.11	0.01	−0.10; 0.12
Protein	−0.18	−0.47; 0.10	0.11	−0.03; 0.25
The interval between last meal and sleep onset	11.51	3.25; 19.76	3.05	−6.41; 12.50

[&] Adjusted for age, chronotype, and time working nights.

2.2. Composition of Last Meal

On the multiple linear regression analysis of SOL on the workday, for every 1 g of fat and 1 g of carbohydrate consumed there was an increase of 0.13 min and 0.02 min (borderline significance) on SOL, respectively (Table [1]).

Table 3. Sleep onset latency after night shift (diurnal sleep) (*n* = 28).

Variables	Crude β	95% CI	Multiple β Adj [&]	95% CI
Fat	0.08	−0.07; 0.24	0.13	0.06; 0.20
Carbohydrate	0.06	0.01; 0.11	0.02	0.00; 0.05
Protein	0.03	−0.10; 0.15	0.02	−0.08; 0.12
Interval between last meal and sleep onset	−1.32	−2.99; 0.35	−0.44	−1.65; 0.77

[1] Adjusted for age, chronotype and time working nights.

No statistically significant results for WASO were found on the workday or day-off.

3. Discussion

This study found that a shorter interval between the last meal and sleep onset was associated with an increase in the duration of diurnal sleep of overweight night shift. Additionally, higher macronutrient (fat and carbohydrate) consumption during the last meal before sleep onset was associated with higher sleep onset latency (SOL) for diurnal sleep. Lastly, a longer interval between the last meal and sleep onset was associated with increased SOL for nocturnal sleep in the crude model.

In a discussion paper by Lowden et al. [9], the authors observed that both meal content and timing are important for the nutritional management of night shift workers. More recently, previous studies have shown that energy metabolism exhibits circadian rhythms and that nutrient intake during the night may disrupt these rhythms [19]. In parallel, it is well established that sleep quality is associated with several hormonal and metabolic changes, including macronutrient modulation [20]. Therefore, it is hypothesized that eating at night affects sleep more than eating the same foods during the day. In this context, the current recommendation is that night shift workers should limit food intake during the night [21]. However, there is little information available in the literature on the impact of this recommendation on daytime sleep after night shifts.

Diurnal sleep is one of the factors promoting misalignment of internal biological clocks with the external environment; when associated with insufficient sleep, this may lead to temporal changes in the rhythm of hormonal and enzymatic activities responsible for metabolic regulation [22]. Moreover, the timing of food intake is a strong zeitgeber for peripheral circadian clocks, as is dietary composition [23]. Thus, these factors give rise to a scenario in which the organism is less able to cope with food, that is, to digest and use it as an energetic substrate [24]. Taking these factors together, the relationship between sleep duration and diet composition may be bidirectional, since sleep debt is associated with a higher intake of both fat and carbohydrate and vice-versa [25,26].

In this regard, the association found in the present study refers to daytime sleep after night work, which proved very short in comparison to the 7–9 h recommended for the adult population [27]. It is known that the sleep of night workers during the day is less restful and shorter compared to night sleep [22]. Therefore, the short duration of diurnal sleep in the present study deserves attention, since sleeping less than needed is associated with cardiometabolic diseases [28] and the diet contributes to the development of the condition [29].

A possible mechanism to explain this dietary influence is that the distribution of macronutrients can affect sleep continuously, promoting changes in neuroendocrine signals related to energy metabolism [28,30]. However, these differences in sleep patterns caused by the last meal highlight, as observed in a systematic review by Souza et al. [31], that night work has a greater influence on the timing of consumption than on total daily intake. Supporting this theory, a recent meta-analysis concluded that night work does not alter energy intake [32].

According to Kogevinas et al. [33], eating 2 h or less before initiating sleep contributes to negative health outcomes. Corroborating these findings, the present study found negative effects on sleep due to inadequate intake of macronutrients less than 2 h before bedtime. Unlike the present investigation, the analysis performed by Kogevinas et al. [33] evaluated only episodes of nocturnal sleep and was not performed exclusively with night workers. In fact, to our knowledge, the present study is the first to evaluate the effects of the last meal, both before diurnal and nocturnal sleep, on night shift workers alone.

The shorter sleep duration when the meal is taken a long time after the diurnal sleep episode might be related to feeling hungry. As Lowden et al. suggested, eating breakfast before going to sleep in the morning is a strategy to prevent awakening due to hunger [9]. Regarding meal content, Jansen et al. [29] found an inverted U-type curve when assessing the association between sleep duration and diet adequacies in adults. Therefore, as demonstrated by the results of the present study, inadequate consumption of nutrients, either below or above recommended values, seems to negatively impact sleep. This result reported by Jansen et al. [29] is consistent with the findings of the present study since the highest percentage of inadequacies in macronutrient intake was observed at the last meal.

The present study has some limitations. Although the participants were instructed to record food intake on typical days, we cannot guarantee that the pattern of distribution of macronutrients observed truly reflects their usual eating behaviors in everyday life. In addition, the adequacy of macronutrient intake at the last meal was calculated based on a recommendation originally intended to assess food intake over a 24-h period. However, it is important to highlight that the decision to adopt this recommendation was due to the absence of nutritional recommendations that take into account the timing of food intake, reiterating the need to establish consensus on this topic [34]. In addition, the physical and cognitive demands of the night work performed before the diurnal sleep episode, but not before nocturnal sleep (after a day-off) may have been a key factor determining sleep quality and should therefore be controlled in future studies. The difference in the diurnal and nocturnal sleep samples, which occurred because the

day-off was not included in 10 days of data collected by actigraphy for 6 participants, may also represent a statistical bias for the results obtained. However, assessing sleep and dietary habits of night shift workers in real-life conditions, and evaluating the associations of meal timing and composition with both diurnal and nocturnal sleep, remain the main strengths of the study. Finally, the present study evaluated permanent night workers only, and hence results cannot be generalized to other types of shift workers [11,35,36].

In conclusion, the findings of this study suggest that both timing and the composition of the last meal before bedtime may be potential key factors for good diurnal and nocturnal sleep in night shift workers, and emphasize the need for nutritional guidelines for night shift workers.

4. Materials and Methods

4.1. Study Sample and Design

This cross-sectional study was part of a controlled, randomized, double-blind, crossover clinical trial in a large hospital situated in the city of São Paulo, São Paulo state, Brazil. Detailed information about the study design is provided elsewhere [37]. For the present study, involving nursing professionals (nurses and nursing technicians) who worked permanent night shifts of 12 × 36 h (12 h of night work, from 19:00 to 07:00 h, followed by 36 h of rest, with a day-off every 15 days), only the baseline data were analyzed. A total of 238 professionals were initially invited to participate in the study. Of this group, 152 did not meet the inclusion criteria and a further 40 refused to take part. Of the 46 subjects who met the criteria and agreed to participate in the study, 30 completed the actigraphy monitoring and food diaries at the study baseline.

To calculate the sample size a priori, the comparison test of three means and a significance level of 5% were used as a reference, with a sampling power of 80% for a sample size of 33 individuals. The final sample of the present study had a sampling power of 73% (G*Power®).

The inclusion criteria were: female gender; age 20–50 years; body mass index (BMI) ≥25 and <40 kg/m^2; working the current night shift for at least six months; agreeing not to follow diets restricted in calories or food groups and/or change eating habits, as well as not to engage in new physical activities while participating in the study.

The exclusion criteria were: pregnant or lactating women; having infants under one year of age; eating disorders diagnosed by a physician; climacteric or menopause; holding a second nighttime job; regular use of medications or dietary supplements that influence sleep, alertness, or the circadian timing system; abusive use of drugs and alcohol; past and/or current history of neurological, psychiatric, circadian or sleep disorders; metabolic problems (except treated type 2 diabetes mellitus and dyslipidemia); cardiovascular diseases (except treated systemic arterial hypertension), inflammation and/or chronic infections diagnosed by a physician; anemia or >400 mL of blood donated in the three months preceding the study; major surgery in the six months preceding the study.

4.2. Data Collection and Processing

Baseline data collection was carried out from April to November 2018. Sociodemographic characteristics, as well as aspects related to work and health, were obtained using a self-administered questionnaire. The dependent variables were objective sleep data (mean values) obtained via actigraphy (ActTrust, Condor Instruments®) over 10 consecutive days [38]. The night shift and the day-off were not included in the original 10 days of data collection from actigraphy for two and eight participants, respectively, then the study was conducted with a diurnal sleep sample of 28 participants and a nocturnal sleep sample of 22 participants. For the present study, it was decided to use the sleep parameters obtained by actigraphy only on the days that food diaries (described above) were filled out. This approach ensured the sleep episode analyzed reflected the prior food consumption on the workday and day-off.

Concomitantly, participants were instructed to fill in sleep logs to complement the information and assist interpretation of the data obtained by actigraphy. The data were separated into diurnal sleep (after a night of work) and nocturnal sleep (the night following the day-off which occurred every 15 days). The parameters evaluated were sleep duration (hours), sleep onset latency (SOL) (minutes), and wake-up after sleep onset (WASO) (minutes).

The independent variables were timing and percentage distribution of macronutrients from the last meal before initiating sleep. Therefore, the participants were instructed to fill out food diaries on a workday and a day-off they considered typical. In both cases, the period for recording the food data was from 19:00 to 19:00 the next day. Participants were previously instructed to provide as much information as possible on the food and drinks consumed, including brands, ingredients used in homemade preparations, and meal timing. Portion sizes were estimated using household measures and subsequently converted to mass units of measurement (g) and capacity (mL), according to

Pinheiro et al. [39]. The diaries were reviewed by a nutritionist to obtain additional clarifications, where necessary, and analyzed using the Nutrition Data System for Research software (NDSR, 2007).

Due to cultural differences between Brazilian and North American eating habits, the composition of typical preparations was manually added to the software database using the Brazilian Food Composition Table [40] and labels of industrialized products. To determine the interval between last meal and sleep onset, data on sleep onset was extracted from actigraphy. After night work, the last meal taken before diurnal sleep was considered for analysis, whereas on the day-off, the last meal before nocturnal sleep was considered.

The composition of the last meal was evaluated using the current Recommended Dietary Allowances (RDA) established by the National Academy of Sciences [41] and adopted by the Brazilian Society of Food and Nutrition [42]. The intake of macronutrients in the last meal before the main sleep episode was then classified as adequate or inadequate. The percentages of calories from each macronutrient classified as suitable were 25–35% for fats, 45–65% for carbohydrates, and 10–35% for proteins. Intakes were deemed inadequate when below the minimum limit or above the maximum limit of the aforementioned ranges. The decision to include both lower and higher-than-recommended intakes (i.e., 30 and 70% of carbohydrates) in the inadequate group was due to previous evidence on the association between sleep and diet in a representative sample of the North American adult population. Jansen et al. [29] observed that both lower and higher-than-recommended macronutrient intakes can negatively impact sleep outcomes. This decision was also based on a previous study on the same population analyzed in the present investigation [39]. The proportion of calories and macronutrients provided by the last meal in relation to daily intake was also calculated. Food diaries were filled in on a typical day of work and day-off, within the period of actigraphy monitoring.

4.3. Statistical Analysis

Data are expressed as mean and ± standard error (SE). Shapiro–Wilk's test was performed to assess data distribution. Paired t-test was performed to compare sleep duration, as well as total caloric intake, percentage, and timing, for the last meal after the night shift (participants left work at 07:00 h) versus last meal on the day-off (sleep was nocturnal, therefore last evening meal was considered). To compare sleep onset latency (SOL) and the mean of wake-up after sleep onset (WASO) we performed the Wilcoxon test.

Analysis was performed using univariate and multiple linear regressions (general linear model), on which sleep duration, SOL, and WASO (on a workday and day-off), were dependent variables and β coefficients estimated for a 95% confidence interval. The models were constructed adopting timing and composition of the last meal (protein, fat, and carbohydrate) as independent variables, all adjusted for age, chronotype, and time working nights. For sleep duration, we performed a linear regression (general linear model) with normal distribution, and for SOL and WASO we performed a linear regression (general linear model) with Gamma distribution. Independent variables with $p < 0.20$ on the hypothesis tests were input to the multiple models, in decreasing order of statistical significance (backward stepwise technique). Statistical analyses were performed using Statistica 7 (TIBCO Software Inc., Palo Alto, CA, USA).

4.4. Ethical Aspects

Ethical issues related to research involving humans have been duly respected and informed consent was drafted in accordance with Resolution 466/2012. The study was approved by the Ethics Committees of the School of Public Health of the University of São Paulo (permit no 2.450.682, 20 December 2017) and the participating hospital (permit no 2.489.636, 7 February 2018). The protocol of the clinical trial is registered on the World Health Organization's International Clinical Trials Registry Platform (UTN no U1111-12387395) and the Brazilian Registry of Clinical Trials (ReBEC–RBR-6pncm9).

Author Contributions: Conceptualization, L.F.R.N. and E.C.M.; methodology, L.F.R.N., C.R.C.M. and E.C.M.; formal analysis, L.F.R.N. and E.C.M.; data curation, L.F.R.N., P.P. and E.C.M.; writing— original draft preparation, L.F.R.N., P.P., C.R.C.M. and E.C.M.; writing— review and editing, L.F.R.N., P.P., J.C.-N., C.R.C.M. and E.C.M.; supervision, E.C.M.; project administration, E.C.M.; funding acquisition, J.C.-N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES, under grant numbers 88887.356981/2019-00 and 88887.150178/2017-00", the Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP, under grant numbers 2014/50457-0, 2018/09704-4 and 2019/03624-1, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico–CNPq, under grant number 150781/2017-2.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the School of Public Health of the University of São Paulo (permit 2.450.682 and 20 December 2017), and the participating hospital (permit 2.489.636).

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy concerns.

Acknowledgments: Adriana de Sousa Duarte and Ananda Laís Felix Garrido, undergraduate Nursing students at the Catholic University of Santos, for helping with data collection.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Kosmadopoulos, A.; Kervezee, L.; Boudreau, P.; Gonzales-Aste, F.; Vujovic, N.; Scheer, F.A.J.L.; Boivin, D.B. Effects of shift work on the eating behavior of police officers on patrol. *Nutrients* **2020**, *12*, 999. [CrossRef] [PubMed]
2. Folkard, S. Do permanent night workers show circadian adjustment? A review based on the endogenous melatonin rhythm. *Chronobiol. Int.* **2008**, *25*, 215–224. [CrossRef] [PubMed]
3. Flanagan, A.; Lowson, E.; Arber, S.; Griffin, B.A.; Skene, D.J. Dietary patterns of nurses on rotational shifts are marked by redistribution of energy into the nightshift. *Nutrients* **2020**, *12*, 1053. [CrossRef] [PubMed]
4. Bae, S.; Fang, M.Z.; Rustgi, V.; Zarbl, H.; Androulakis, I. At the interface of lifestyle, behavior, and circadian rhythms: Metabolic implications. *Front. Nutr.* **2019**, *6*, 132. [CrossRef]
5. Hutchison, A.T.; Wittert, G.A.; Heilbronn, L.K. Matching meals to body clocks—Impact on weight and glucose metabolism. *Nutrients* **2017**, *9*, 222. [CrossRef]
6. Manoogian, E.N.C.; Panda, S. Circadian rhythms, time-restricted feeding, and healthy aging. *Age Res. Rev.* **2016**, *39*, 59–67. [CrossRef]
7. Leung, G.K.W.; Huggins, C.E.; Ware, R.S.; Bonham, M.P. Time of day difference in postprandial glucose and insulin responses: Systematic review and meta-analysis of acute postprandial studies. *Chronobiol. Int.* **2020**, *37*, 311–326. [CrossRef]
8. Amaral, F.G.; Andrade-Silva, J.; Kuwabara, W.M.T.; Cipolla-Neto, J. New insights into the function of melatonin and its role in metabolic disturbances. *Expert Rev. Endocrinol. Met.* **2019**, *14*, 293–300. [CrossRef]
9. Samhat, Z.; Attieh, R.; Sacre, Y. Relationship between night shift work, eating habits and BMI among nurses in Lebanon. *BMC Nurs.* **2020**, *19*, 25. [CrossRef]
10. Singh, R.B.; Cornelissen, G.; Mojto, V.; Fatima, G.; Wichansawakun, S.; Singh, M.; Kartikey, K.; Sharma, J.P.; Torshin, V.I.; Chibisov, S.; et al. Effects of circadian restricted feeding on parameters of metabolic syndrome among healthy subjects. *Chronobiol. Int.* **2019**, *37*, 395–402. [CrossRef]
11. Della Torre, S.B.; Wild, P.; Dorribo, V.; Danuser, B.; Amati, F. Energy, nutrient and food intakes of male shift workers vary according to the schedule type but not the number of nights worked. *Nutrients* **2020**, *12*, 919. [CrossRef] [PubMed]
12. Kaar, J.L.; Schmiege, S.J.; Vadiveloo, M.; Simon, S.L.; Tovar, A. Sleep duration mediates the relationship between health behavior pattern and obesity. *Sleep Health* **2018**, *4*, 442–447. [CrossRef] [PubMed]
13. Herrera, C.G.; Ponomarenko, A.; Korotkova, T.; Burdakov, D.; Adamantidis, A. Sleep & Metabolism: The multitasking ability of lateral hypothalamic inhibitory circuitries. *Front. Neuroendocrinol.* **2016**, *44*, 27–34. [CrossRef] [PubMed]
14. Coelho, M.P.; Pinto, O.O.; Mota, M.C.; Crispim, C.A. Nutritional damages and disturbances in the sleep pattern of nursing workers. *Rev. Bras. Enferm.* **2014**, *67*, 832–842. [CrossRef] [PubMed]
15. Nehme, P.; Marqueze, E.C.; Ulhôa, M.; Moulatlet, E.; Codarin, M.A.; Moreno, C.R. Effects of a carbohydrate-enriched night meal on sleepiness and sleep duration in night workers: A double-blind intervention. *Chronobiol. Int.* **2014**, *31*, 453–460. [CrossRef]
16. Peuhkuri, K.; Sihvola, N.; Korpela, R. Diet promotes sleep duration and quality. *Nutr. Res.* **2012**, *32*, 309–319. [CrossRef]
17. Bandín, C.; Scheer, F.A.; Luque, A.J.; Ávila-Gandía, V.; Zamora, S.; Madrid, J.A.; Gómez-Abellán, P.; Garaulet, M. Meal timing affects glucose tolerance, substrate oxidation and circadian-related variables: A randomized, crossover trial. *Int. J. Obes.* **2015**, *39*, 828–833. [CrossRef]
18. Frank, S.; Gonzalez, K.; Lee-Ang, L.; Young, M.C.; Tamez, M.; Mattel, J. Diet and sleep physiology: Public Health and clinical implications. *Front. Neurol.* **2017**, *8*, 393. [CrossRef]
19. Phoi, Y.Y.; Keogh, J.B. Dietary Interventions for Night Shift Workers: A Literature Review. *Nutrients* **2019**, *11*, 2276. [CrossRef]
20. Lindseth, G.; Lindseth, P.; Thompson, M. Nutritional effects on sleep. *West J. Nurs. Res.* **2011**, *35*, 497–513. [CrossRef]
21. Gupta, C.C.; Dorrian, J.; Grant, C.L.; Pajcin, M.; Coates, A.M.; Kennaway, D.J.; Wittert, G.A.; Banks, S. It's not just what you eat but when: The impact of eating a meal during simulated shift work on driving performance. *Chronobiol. Int.* **2016**, *34*, 66–77. [CrossRef] [PubMed]

22. Moreno, C.R.C.; Marqueze, E.C.; Sargent, C.; Wright, K.P., Jr.; Ferguson, S.A.; Tucker, P. Working Time Society consensus statements: Evidence-based effects of shift work on physical and mental health. *Ind. Health* **2019**, *57*, 139–157. [CrossRef] [PubMed]
23. Patel, S.A.; Velingkaar, N.; Makwana, K.; Chaudhari, A.; Kondratov, R. Calorie restriction regulates circadian clock gene expression through BMAL1 dependent and independent mechanisms. *Sci. Rep.* **2016**, *6*, 25970. [CrossRef]
24. Johnston, J.D.; Ordovás, J.M.; Scheer, F.A.; Turek, F.W. Circadian rhythms, metabolism, and chrononutrition in rodents and humans. *Adv. Nut.* **2016**, *7*, 399–406. [CrossRef]
25. Grandner, M.A.; Jackson, N.; Gerstner, J.R.; Knutson, K.L. Dietary nutrients associated with short and long sleep duration. Data from a nationally representative sample. *Appetite* **2013**, *64*, 71–80. [CrossRef]
26. Kant, A.K.; Graubard, B.I. Association of self-reported sleep duration with eating behaviors of American adults: NHANES 2005–2010. *Am. J. Clin. Nut.* **2014**, *100*, 938–947. [CrossRef]
27. Hirshkowitz, M.; Whiton, K.; Albert, S.M.; Alessi, C.; Bruni, O.; DonCarlos, L.; Hillard, A. National Sleep Foundation's sleep time duration recommendation: Methodology and result summary. *Sleep Health* **2015**, *1*, 40–43. [CrossRef]
28. St-Onge, M.P.; Mikic, A.; Pietrolungo, C.E. Effects of diet on sleep quality. *Adv. Nut.* **2016**, *7*, 938–949. [CrossRef]
29. Jansen, E.C.; Prather, A.; Leung, C.W. Associations between sleep duration and dietary quality: Results from a nationallyrepresentative survey of US adults. *Appetite* **2020**, *153*, 104748. [CrossRef] [PubMed]
30. Melaku, Y.A.; Reynolds, A.C.; Gill, T.K.; Appleton, S.; Adams, R. Association between macronutrient intake and excessive daytime sleepiness: An iso-caloric substitution analysis from the North West Adelaide Health Study. *Nutrients* **2019**, *11*, 2374. [CrossRef] [PubMed]
31. Souza, R.V.; Sarmento, R.A.; de Almeida, J.C.; Canuto, R. The effect of shift work on eating habits: A systematic review. *Scand. J. Work Environ. Health* **2019**, *45*, 7–21. [CrossRef]
32. Bonham, M.P.; Bonnell, E.K.; Huggins, C.E. Energy intake of shift workers compared to fixed day workers: A systematic review and meta-analysis. *Chronobiol. Int.* **2016**, *33*, 1086–1100. [CrossRef]
33. Kogevinas, M.; Espinosa, A.; Castelló, A.; Gómez-Acebo, I.; Guevara, M.; Martin, V.; Romaguera, D. Effect of mistimed eating patterns on breast and prostate cancer risk (MCC-Spain Study). *Int. J. Cancer* **2018**, *143*, 2380–2389. [CrossRef]
34. Dashti, H.S.; Gómez-Abellán, P.; Qian, J.; Esteban, A.; Morales, E.; Scheer, F.A.J.L.; Garaulet, M. Late eating is associated with cardiometabolic risk traits, obesogenic behaviors, and impaired weight loss. *Am. J. Clin. Nut.* **2020**, *113*, nqaa264. [CrossRef] [PubMed]
35. Heath, G.; Dorrian, J.; Coates, A. Associations between shift type, sleep, mood, and diet in a group of shift working nurses. *Scand. J. Work Environ. Health* **2019**, *45*, 402–412. [CrossRef]
36. Lopez-Minguez, J.; Gómez-Abellán, P.; Garaulet, M. Timing of breakfast, lunch, and dinner. Effects on obesity and metabolic risk. *Nutrients* **2019**, *11*, 2624. [CrossRef] [PubMed]
37. Garrido, A.L.F.; Duarte, A.S.; Santana, P.T.; Rodrigues, G.H.; Pellegrino, P.; Nogueira, L.F.R.; Marqueze, E.C. Eating habits, sleep and a proxy for circadian disruption are correlated with dyslipidemia in overweight night workers. *Nutrition* **2020**, *83*, 111084. [CrossRef]
38. Littner, M.; Kushida, C.A.; Anderson, W.M.; Bailey, D.; Berry, R.B.; Davila, D.G.; Johnson, S.F. Practice Parameters for the Role of Actigraphy in the Study of Sleep and Circadian Rhythms: An Update for 2002. *Sleep* **2003**, *26*, 337–341. [CrossRef]
39. Pinheiro, A.B.V.; Lacerda, E.M.A.; Benzecry, E.H.; Gomes, M.C.S.; Costa, V.M. *Tabela Para Avaliação de Consumo Alimentar em Medidas Caseiras*, 4th ed.; Atheneu: São Paulo, Brazil, 2000.
40. NEPA-UNICAMP. *Tabela Brasileira de Composição de Alimentos—TACO*, 4th ed.; NEPA-UNICAMP: Campinas, Brazil, 2011.
41. Otten, J.J.; Hellwig, J.P.; Meyers, L.D. *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*; The National Academies Press: Washington, DC, USA, 2006.
42. Padovani, R.M.; Amaya-Farfán, J.; Colugnati, F.A.B.; Domene, S.M.A. Dietary reference intakes: Application of tables in nutritional studies. *Rev. Nut.* **2006**, *19*, 741–760. [CrossRef]

6.3 ARTIGO 3

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ARTICLE

The Effect of Exogenous Melatonin on Eating Habits of Female Night Workers with Excessive Weight

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Citation: Nogueira, L.F.R.; Crispim, C.A.; Cipolla-Neto, J.; de Castro Moreno, C.R.; Marqueze, E.C. The Effect of Exogenous Melatonin on Eating Habits of Female Night Workers with Excessive Weight. *Nutrients* **2022**, *14*, 3420. https://doi.org/10.3390/nu14163420

Academic Editor: Michael Wirth

Received: 20 July 2022

Accepted: 16 August 2022

Published: 19 August 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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Abstract: Background and Aims: Melatonin is a pineal hormone that plays an important role as an endogenous synchronizer of circadian rhythms and energy metabolism. As this circadian component has been closely related to eating behavior, an important question on this topic would be whether melatonin administration could influence eating habits. However, this topic has been rarely studied in the literature in individuals with excessive weight and chronic circadian misalignment, such as shift workers. Therefore, the present study aims to evaluate the effects of exogenous melatonin administration on the quali/quantitative aspects and temporal distribution of food intake in female night workers with excessive weight (overweight and obesity). An additional aim is to evaluate the association of the referred outcomes with circadian misalignment and chronotype. Methods: A randomized, double-blind, placebo-controlled, crossover clinical trial was conducted with 27 female nursing professionals with excessive weight who worked permanent night shifts. The protocol was implemented under real-life conditions for 24 weeks, in two randomly allocated conditions (12 weeks of melatonin and 12 weeks of placebo). The quali/quantitative aspects of food intake (NOVA classification, total energy intake and the proportion of calories from macronutrients) and meal timing were assessed using food diaries. Timing for every meal recorded in the diaries was assessed to evaluate the temporal distribution of food intake. Generalized estimating equations were performed for each dependent variable. Results: No significant modifications in total energy intake, macronutrient distribution, types of foods consumed, and meal timing were observed after melatonin administration. Different levels of circadian misalignment and chronotype did not interfere with these results. Conclusion: Eating habits of female night workers with excessive weight remained unchanged after melatonin administration, and no association of these results with circadian misalignment and chronotype was found. These results suggest that the metabolic effects of melatonin may occur independently of food intake.

Keywords: melatonin; dietary supplements; eating behavior; circadian dysregulation; night work

Nutrients **2022**, *14*, 3420. https://doi.org/10.3390/nu14163420 https://www.mdpi.com/journal/nutrients

1. INTRODUCTION

The modern world, including the growing percentage of workers involved in night shift work, challenges circadian and energy homeostasis by promoting food intake during the rest phase [1,2]. The desynchronization between the suprachiasmatic nucleus and the temporal clues of the peripheral tissues in shift workers has been associated with a higher perceived appetite for calorically dense food, later meal timing, and more snacks before sleep, independent of sleep duration [3,4]. These poorer eating habits have been linked with a higher prevalence of metabolic diseases in shift workers [5,6]. It is estimated that 20 to 30% of the economically active populations in North America, Canada, Europe, and Brazil are involved in shift work that includes night shifts [7–11].

Night work is considered the most extreme situation of circadian misalignment [12]. Previous findings suggest that the reduction in total daily energy expenditure during night shifts [13], as well as lower energy expenditure in response to dinner [14], may increase weight gain and the risk of obesity and adverse metabolic outcomes [13]. In addition, it has been suggested that evening-type individuals, for example, are used to eating at unfavorable eating times [15], showing high energy consumption late at night [16]. The same temporal distribution of food intake is observed in shift workers [17].

Food intake has been shown to be an important synchronizer for peripheral clocks [18] and, therefore, a modifiable temporal cue for the circadian system that can be influenced by innumerous factors [19]. One of these factors might be the plasma concentration of melatonin, a hormone produced by the pineal gland that plays an important role as an endogenous synchronizer of the circadian rhythms and energy metabolism, including energy intake and expenditure [20,21].

Until now, only three studies have evaluated the effects of exogenous melatonin on night workers in real-life conditions [22–24], but did not evaluate eating habits. These studies assessed the effect of melatonin on (1) circadian misalignment: administration prior to daytime sleep attenuated interference from circadian alerting process, demonstrating both phase-shifting and sleep-promoting actions [22]; (2) sleep and alertness: administration reduced sleep problems and increased alertness during working hours [23]; and (3) body weight: administration reduced body weight, BMI, and waist and hip circumferences [24].

Additionally, it is important to highlight that the participants of this study had excessive weight (overweight and obesity), and that body mass seems to affect daily rhythmicity and concentrations of circulating metabolites that express circadian rhythms [25]. In addition, it has been previously established that circadian rhythms and metabolism are closely linked, and that meal timing plays a role in synchronizing peripheral circadian rhythms in humans, which is particularly relevant for shift workers [26]. The choice of focusing on females is due to the higher prevalence of excessive weight in women [27], and because nursing is a female-dominated occupation [28].

Therefore, the present study aims to evaluate the effects of melatonin administration on the quali/quantitative aspects and temporal distribution of food intake. An additional aim is to evaluate the association of the referred with circadian misalignment and chronotype. The question we expect to answer is: Does melatonin administration influence food choices, the quali/quantitative aspects and temporal distribution of food intake among female night workers with excessive weight? Given the potential of melatonin to regulate energy metabolism and to synchronize circadian rhythms including energy metabolism, we hypothesize that melatonin administration will be able to improve eating habits.

2. MATERIALS AND METHODS

2.1. Design and Sampling

A randomized, double-blind, placebo-controlled, crossover clinical trial was conducted with female nursing professionals (nursing technicians and nurses) with excessive weight who worked permanent night shifts in a large private hospital in São Paulo, SP, Brazil. The protocol was implemented under real-life conditions for 24 weeks. All nurses worked in a 12 × 36 scheme (12 h on night shifts, from 19:00 to 7:00 h, followed by 36 h off) and had one day off every 15 days.

The sample was calculated considering a test of difference of means of repeated measures (within–between interaction), the effect size of 0.30, alpha error of 5%, two groups (intervention and CPD), and two measures (melatonin and placebo), in which the sample of 27 people represented a power of 85% (G×Power). Detailed information on the study sample is published elsewhere [24].

The inclusion criteria were women aged 20 to 50 years, with body mass index (BMI) ≥25 and <40 kg/m^2, working for at least six months in the current night shift system, who declared having no intention of following any restricted diets and starting new physical activities while participating in the study. The exclusion criteria were pregnant or lactating women; having children under one years old; climacteric or menopause; eating disorders diagnosed by a physician; having a second night's work; regular use of medications or dietary supplements that influence sleep, alertness, and the circadian timing system; abusive use of drugs and alcohol; past and/or current history of psychiatric, neurological, circadian or sleep disorders diagnosed by a physician; metabolic disorders (except treated type 2 diabetes mellitus and dyslipidemia); cardiovascular diseases (except treated systemic arterial hypertension); chronic inflammation and/or infection diagnosed by a physician; anemia and/or having donated >400 mL of blood in the last three months preceding the study; major surgery in the six months preceding the study.

2.2. Study Protocol

Participants were recruited from March 2018 to June 2019. They were randomly allocated into two groups using codes generated by a computer. Since it is a crossover trial, in the first phase, one group received melatonin and the other group received a placebo; then, in the second phase, the participants switched groups. Identical tablets of 3 mg of fast-release melatonin or placebo (Aché Pharmaceutics®, Sao Paulo, Brazil) were orally administered for 12 weeks each. Participants were instructed to take one tablet, one hour before bedtime, exclusively when they slept at night, that is, in the nights between shifts and days off, and never during the day. It is important to highlight that fast-release melatonin was chosen due to its higher effectiveness in circadian synchronization in comparison to other types [29]. Since melatonin is completely excreted in urine within 24 h after administration [30], no washout period was performed between the two phases of the study. The average number of days of melatonin administration was 45 days (EP 10.3 days) and the use of placebo was 44.3 days (EP 8.2 days). More information about the study protocol is provided elsewhere [24], and Figure S1 presents the study flow chart.

2.3. Study Variables

2.3.1. Outcomes

• *Quantitative aspect of the diet: Total energy intake and macronutrient distribution*

Food intake was assessed by food diaries, which participants were previously instructed to fill in on typical workdays and days off (from 19:00 to 19:00 h). They completed these diaries two days per month while participating in the study, therefore totaling seven months (baseline plus 24 weeks of intervention) and 14 days recorded (two days per month, being one workday and one day off).

The participants were also given instructions to provide as detailed information as possible about food and drinks consumed, including brand names, ingredients used in homemade recipes, and meal timing. Portion sizes were estimated using household measures and subsequently converted to mass units of measurement (g) and capacity (mL), according to Pinheiro et al. [31]. Due to cultural differences between Brazilian and North American eating habits, the composition of typical Brazilian preparations was manually added to the software database using the Brazilian Food Composition Table [32] and labels of industrialized products.

The diaries were reviewed by a nutritionist to obtain additional clarifications, when necessary, and analyzed using the Nutrition Data System for Research software (NDSR, 2007). The total energy intake (TEI) and the proportion of calories from each macronutrient (carbohydrate, protein, and fat) in relation to TEI were calculated.

• *Qualitative aspect of the diet: Food classification according to processing*

Every item in the reviewed food diaries was categorized as unprocessed or minimally processed, processed, or ultra-processed foods according to the NOVA classification [33]. NOVA classifies food into four groups based on the nature, extent, and purpose of the industrial processing: (1) unprocessed or minimally

processed foods, such as rice, beans, frozen meat, and milk; (2) processed culinary ingredients, such as vegetable oils and table sugar; (3) processed foods, such as vegetables in brine and cheeses; and (4) ultra-processed foods, such as carbonated soft drinks, meat products, and instant noodles. A detailed description of the NOVA classification can be found in [33].

All the foods consumed were coded as a number corresponding to a food group (n = 5.549) according to NOVA. When a direct classification was not possible, such as homemade preparations and foods containing items from different groups, as well as culinary ingredients, the classification of the main ingredient was considered [33].

- *Temporal distribution of food intake*

Timing for every meal in the food diaries was grouped into four categories: 19:00–00:59 h, 01:00–06:59 h, 07:00–12:59 h and 13:00–18:59 h.

2.3.2. Independent Variables

- *Composite Phase Deviations*

The circadian misalignment was estimated using the composite phase deviation (CPD) [34] of actigraphy-based mid-sleep, which is calculated from the sleep onset and offset data. Participants wore actigraphs for 10 consecutive days (ActTrust and Basic Motionlogger Actigraph, Condor Instruments®, Sao Paulo, Brazil) and filled in sleep activity diaries to validate the recorded data. The CPD was calculated according to the following equation:

$$|CPD_i| = \left|\frac{x_i}{y_i}\right| = \sqrt{x_i^2 + y_i^2}$$

in which CPD_i = composite phase deviation on day i; x_i = distance of mid-sleep on day i to chronotype (MSFNsc)*; y_i = distance of mid-sleep on day i to previous day i − 1. *MSFNsc = chronotype (mid-sleep on free days after night shifts, corrected for over-sleep).

- *Chronotype*

Chronotype was assessed using the mid-point of sleep on free days after night shifts, corrected for over-sleep (MSFNsc) derived from the Munich Chronotype Questionnaire for shift work (MCTQshift) [35].

2.3.3. Descriptive Variables

The participants went through a two-week baseline period in which a self-administered questionnaire was completed. Sociodemographic (age, marital status, education) and work characteristics (current position, reason to work at night, lifetime exposure to night work, second job) and health behaviors (alcohol intake, smoking, physical activity) were evaluated.

Body weight and height were assessed at baseline, and body weight was also assessed in the last 10–15 days of the first and second phases of the study. Both measurements were performed according to the standardization method by Lohman et al. [36]. Body weight was assessed to the nearest 0.1 kg using a calibrated digital balance. Height was assessed using a wall-mounted portable stadiometer to the nearest 0.1 cm. Body mass index (BMI) was calculated as the body weight (kg) divided by the height squared (m^2).

The energy requirements of the participants were individually calculated by the equations of estimated energy requirements (EER) [37].

2.4. Statistical Analysis

Descriptive data are shown as mean ± standard error (SE). Shapiro–Wilk's test was used to test data normality. Generalized estimating equations (GEE) with the Bonferroni post hoc test were used to analyze the effect of melatonin and placebo administration on quali/quantitative aspects and temporal distribution of the diet. Seven models were performed for each dependent variable (TEI; percentages of daily calories taken from unprocessed or minimally processed, processed, ultra-processed foods; carbohydrate, protein, and fat; and meal timing).

The percentages of calories for each category of processing were obtained: (1) calculating the average intake of the workday and the day-off registered at the baseline and during the last four weeks of each phase of the study (weeks 12 and 24), and (2) calculating the average consumption from the 12 weeks of melatonin administration, and then from the 12 weeks of placebo. The decision to use the values for the end of each phase of the study is due to the absence of significant month-to-month differences in food intake in previously tested models.

Additionally, seven models were performed to analyze the effect of the interventions on the same dependent variables aforementioned in association with CPD, and another seven in association with chronotype. Both CPD and chronotype were treated as continuous variables. All models were adjusted for lifetime night work exposure. Gamma distribution with log link was chosen considering the smaller quasi-likelihood under the independence model criterion (QIC). Statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). The significance level was set at 5%.

2.5. Ethical Aspects

Ethical issues regarding research involving human beings have been duly respected. The research project was approved both by the Ethics Committee of the School of Public Health of the University of São Paulo (process number 2.450.682) and the Ethics Committee of the hospital where it was conducted (process number 2.489.636). All participants gave written and informed consent to participate in the study. The clinical trial was registered at the Brazilian Registry of Clinical Trials-ReBEC (number RBR-6pncm9) and was developed according to the Consolidated Standards of Reporting Trials [38].

3. RESULTS

Sociodemographics, work characteristics, health behaviors, BMI, CPD, and chronotype are shown in Table 1. All the participants were female, and the majority were nursing technicians, married, with a major degree. The main reason mentioned by the participants to work at night was to reconcile work with home and/or children's care, and the majority did not have a second job. None were smokers, and most consumed alcohol only on special occasions.

Table 1. Participants' characteristics at baseline (*n* = 27).

Variables	*n* (%) or Mean ± SE
Age (years)	37.1 ± 0.6
Marital status (married)	17.0 (63.0)
Current position (nursing technician)	14.0 (51.9)
Education level (complete or incomplete graduation)	16.0 (59.2)
Lifetime exposure to night work (years)	9.1 ± 0.7
The main reason to work at night (reconcile work with home and/or children's care)	11.0 (40.7)
Second job (yes)	2.0 (7.4)
Smoking (no)	27.0 (100.0)
Alcohol intake (only on special occasions)	17.0 (63.0)
Physical activity (none)	17.0 (63.0)
BMI (kg/m^2)	29.8 ± 0.4
CPD (hours)	2.9 ± 0.2
Chronotype (hours)	3.3 ± 0.2

BMI: body mass index; CPD: composite phase deviations.

The analysis of the effect of melatonin and placebo administration showed no significant results. No associations between the quali/quantitative aspects of the diet and CPD or chronotype were found as well (Table 2).

Table 2. Effect of melatonin and placebo administration on the quali/quantitative aspects of the diet.

Variables	Mean ± SE		Effects (*p*)	Goodness of Fit ***
% TEI *	Melatonin	Placebo	Intervention **	
Qualitative aspect				
Unprocessed or minimally processed foods	49.2 ± 3.2	52.6 ± 3.5	0.17	12.1
Processed foods	15.3 ± 1.9	13.1 ± 1.8	0.43	23.5
Ultra-processed foods	34.7 ± 2.4	34.8 ± 3.2	0.98	15.2
Quantitative aspect				
Carbohydrate	46 ± 9.7	44.6 ± 9.0	0.89	10.4
Protein	20.7 ± 10.2	21.3 ± 10.0	0.67	13.9
Fat	32.1 ± 7.9	33.1 ± 8.0	0.80	13.6

* Total energy intake. ** Means of the effect of melatonin and placebo administration, respectively, in association with CPD: unprocessed or minimally processed foods: 49.9 ± 9.1, 50.1 ± 9.8, $p = 0.27$; processed foods: 14.3 ± 5.0, 14.1 ± 5.2, $p = 0.33$; ultra-processed foods: 34.8 ± 4.9, 34.9 ± 8.1, $p = 0.95$; carbohydrate: 46.5 ± 10.9, 44.6 ± 10.5, $p = 0.95$; protein: 20.9 ± 12.2, 20.4 ± 10.7, $p = 0.60$; fat: 31.8 ± 10.7, 34.4 ± 11.8, $p = 0.56$. Means of the effect of melatonin and placebo administration, respectively, in association with chronotype: unprocessed or minimally processed foods: 49.1 ± 9.3, 52.6 ± 9.8, $p = 0.22$; processed foods: 15.4 ± 6.4, 13 ± 4.4, $p = 0.62$; ultra-processed foods: 34.6 ± 7, 34.9 ± 7.9, $p = 0.14$; carbohydrate: 46 ± 3.9, 44.7 ± 5, $p = 0.56$; protein: 20.7 ± 3.6, 21.3 ± 4.2, $p = 0.48$; fat: 32.1 ± 3.6, 33 ± 2.7, $p = 0.81$. *** Quasi-likelihood under independence model criterion (QIC) of interaction. All models are adjusted by lifetime exposure to night work (years).

Figure 1 presents the estimated energy requirements and total caloric intake after melatonin supplementation and placebo.

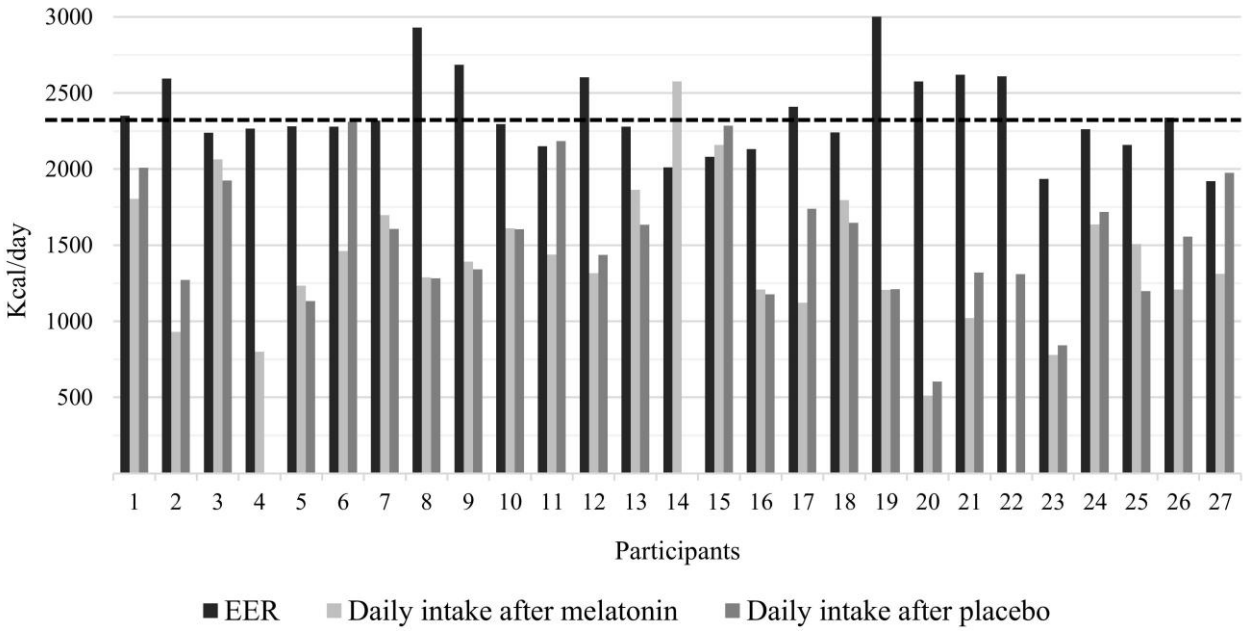

Figure 1. Estimated energy requirements and total caloric intake after melatonin and placebo administration. EER: estimated energy requirement. The dashed line represents the mean EER (kcal/day) of the participants. No food intake was recorded in the diaries after placebo administration for participants 4 and 14, and after melatonin administration for participant 22.

The mean EER of the participants at baseline was 2357.3 kcal/day. The mean TEI after melatonin administration was 1421.1 (SE = 88.3) kcal/day, and the mean TEI after placebo administration was 1532.9 (SE = 83.8) kcal/day (GEE $p = 0.15$). Additionally, no statistically significant differences in TEI between the intervention phases in association with CPD (melatonin: 1469.0 ± 262.4 kcal/day, placebo: 1482.7 ± 183.0 kcal/day, GEE $p = 0.94$) nor chronotype (melatonin: 1418.6 ± 174.5 kcal/day, placebo: 1531.8 ± 138 kcal/day, GEE $p = 0.99$) were observed.

In regard to the temporal distribution of food intake, no differences in meal timing between the intervention phases were observed (Figure 2). No associations between the temporal distribution of food intake and CPD or chronotype were found as well, as shown in Table 2.

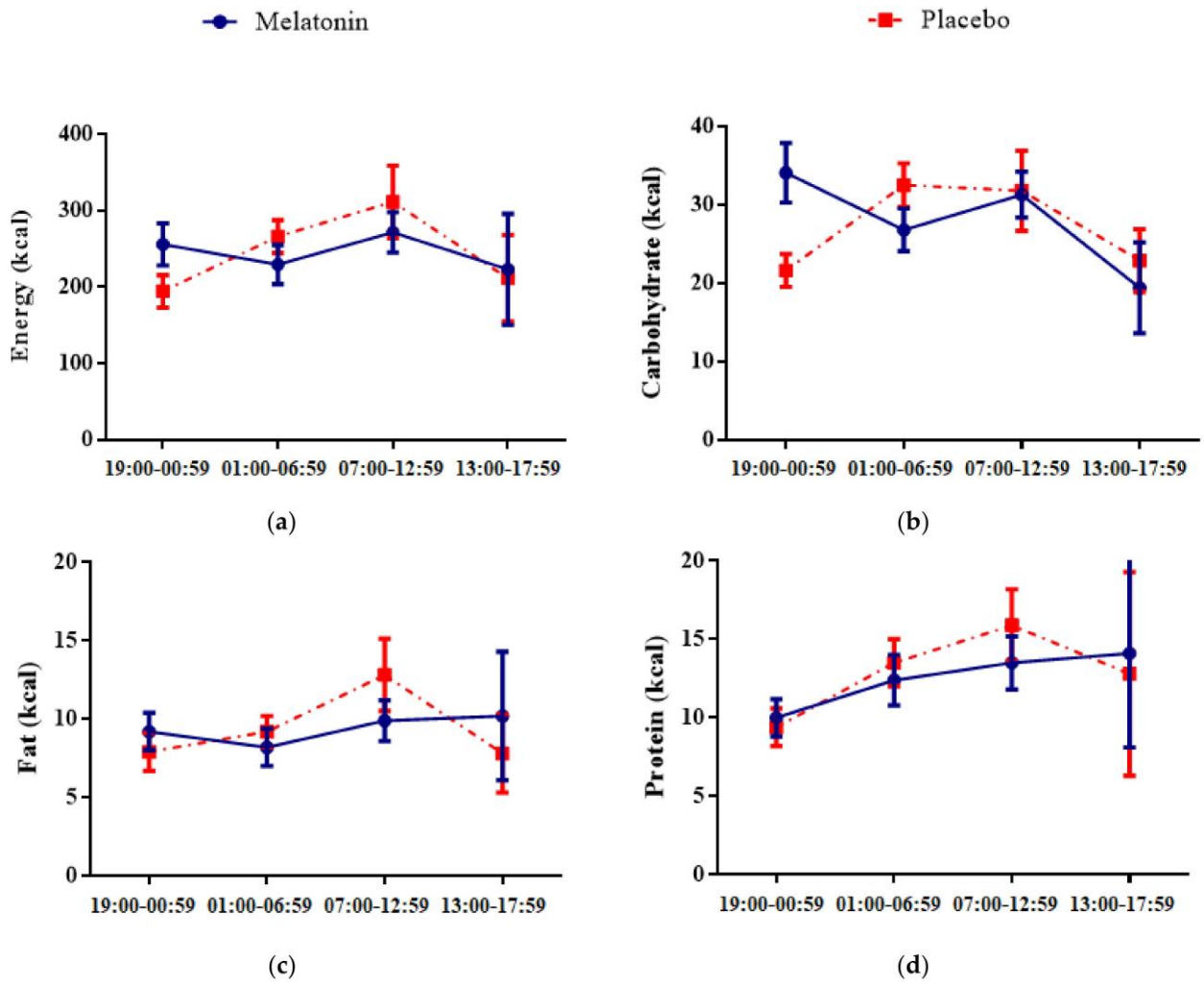

Figure 2. Temporal distribution of food intake after melatonin and placebo administration. Vertical lines represent standard error of mean. (**a**) Energy, (**b**) Carbohydrate, (**c**) Fat, and (**d**) Protein.

4. DISCUSSION

The present study showed that total energy intake, macronutrients distribution, types of foods consumed, and meal timing remained unchanged after melatonin administration in female night workers with excessive weight. No associations between these outcomes and circadian misalignment or chronotype were found either. These results contradict our initial hypothesis that melatonin administration would be able to improve eating habits.

4.1. No Effects of Melatonin on Energy, Quali/Quantitative Intake and Meal Timing

Little is known about the effect of melatonin administration on food intake [39]. In the present study, we found that melatonin administration had no effect on quali/quantitative aspects of the diet in female night workers with excessive weight. One of the hypotheses to justify our findings was recently emphasized by a systematic review that evaluated the effects of melatonin supplementation on eating habits and appetite-regulating hormones [39]. This review stated that melatonin's effects on energy metabolism can occur independently of food intake. Furthermore, the only clinical trial included in the review found no difference in

food intake after 84 days of melatonin supplementation (6 mg/day) in healthy men [40]. Although our results in the present study confirm these findings from the literature so far, the need for more randomized clinical trials to confirm these findings is evident.

Previous studies have reported that hunger increases and satiety decreases after a night shift, and that food preferences and appetite are altered to high-calorie density foods [41,42], that is, ultra-processed products. These foods contributed ~35% of TEI in the present study, and no changes were observed after the intervention. This amount is notably higher than the 22.7% observed in a cross-sectional analysis from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) cohort [43]. The higher intake might be related to chronic fatigue and sleep disturbances frequently experienced by shift workers, which promotes hedonic control of food intake and decreases the motivation to prepare meals, facilitating the choice of ready-to-eat, less healthy options [44].

The implication of modern industrial processing on health is overall underrated, but the quality of the energy consumed deserves particular attention in specific groups at higher risk of metabolic disorders due to melatonin suppression, such as night shift workers. The ELSA-Brasil cohort consumed ~65% of TEI from unprocessed or minimally processed foods [42], while the participants of the present study consumed ~50%. Even though melatonin supplementation did not change the contribution of this type of food to TEI, factors influencing eating behavior still need to be better understood. According to Gupta et al. [45], another interplay that still needs to be better understood is the role of obesity on food choices. Evidence suggests that consuming unprocessed or minimally processed foods regularly has a protective effect against metabolic disorders [46]. In this context, the low intake of this type of foods by specific groups, such as shift workers, deserves attention in future studies concerning diet.

Excessive weight is primarily caused by a positive energy balance, that is, a state in which energy intake is higher than its expenditure [47]. However, the female night workers with excessive weight participating in our study consumed an average of 900 kcal/day less than their EER. Previous studies have shown that insufficient sleep resulted in increased energy intake, while adequate sleep decreased energy intake [48,49]. Our hypothesis on this topic is that energy intake would be higher than energy requirements due to circadian misalignment, and that melatonin administration might be able to mitigate the adverse metabolic effects of night work [50,51]. However, this hypothesis was not confirmed either and more studies are needed.

4.2. No Effects of Melatonin Supplementation on Food Intake in Association with Circadian *MISALIGNMENT OR CHRONOTYPE*

Our findings in the present study showed melatonin administration had no effect on quali/quantitative aspects of the diet in female night workers with excessive weight, independent of their chronotype or level of circadian misalignment. A recent clinical trial published by our research group showed that 12 weeks of melatonin administration reduced circadian misalignment by 21% in the same cohort of female excessive night workers from the present study [24]. These results from our previous study led us to believe that greater changes in eating behavior would be more relevant in people who reduced their level of circadian misalignment, but this did not occur in the present study. This reinforces the aforementioned information that melatonin's effects on energy metabolism, which has been postulated in the literature [20], can occur independently of food intake [52].

Regarding an interplay between melatonin administration, chronotype, and food intake, we hypothesized that morning individuals would benefit the most from the intervention since this chronotype has been associated with high circadian misalignment [24].

However, this hypothesis was not confirmed. Moreover, our previous study [24] showed that the administration reduced the body weight of the early chronotypes—the ones that suffer all the adverse effects of night work the most [8]. In this context, it is reasonable to assume that these individuals would respond differently to the intervention in comparison to the vespertine ones, but this hypothesis was not confirmed either. More randomized clinical trials are needed to confirm our findings.

4.3. Limitations and Strengths

The present study has some limitations. The same dosage of melatonin was administered to all participants, but melatonin's metabolism has interindividual variations. The response to artificial light exposure at night also varies, leading to lower or higher melatonin suppression according to individual sensitivity and light intensity [53]. Hence, pharmaceutical formulation and dosage of exogenous melatonin could be individually considered [54]. Another possible limitation is that, although the participants were instructed to record food intake on typical days, it is not possible to guarantee that the records truly reflect their dietary behaviors in everyday life. It is also important to mention that the participants had the option of a nutritionist-planned dinner provided by the hospital. In addition, all units had a pantry in which they could store food brought in from outside the hospital, as well as have meals. This particular reality can influence the results obtained with regard to eating habits. Lastly, assessments of appetite-regulating hormones, i.e., leptin and ghrelin, might contribute toward understanding the lack of effect of melatonin administration on meal timing. However, these assessments were not performed in this study.

On the other hand, there are some strengths to be highlighted. A significant advantage of the present clinical trial is that, even though studies on pharmacokinetics in humans are limited, exogenous melatonin has shown to be safe and lacks adverse effects in comparison to placebo [54]. Additionally, fewer studies have employed within-subject comparisons and collected data regarding workdays and days off [42,55,56]. Lastly, assessing the dietary habits of female night workers with excessive weight in real-life conditions is one of the main strengths of the study. The qualitative aspect of the diet, especially, has been generally neglected in experimental studies [33] and adds important knowledge on the eating behaviors of this specific population.

5. CONCLUSIONS

The present study showed that the eating habits of female night workers with excessive weight remained unchanged after 12 weeks of intermittent exogenous melatonin administration. Circadian misalignment and chronotype did not interfere with these results. These results suggest that the metabolic effects of melatonin may occur independently of total energy intake, macronutrient distribution, food processing level, and temporal distribution of food intake.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14163420/s1, Figure S1: Study flow chart.

Author Contributions: L.F.R.N.: Conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft, writing—review and editing. C.A.C.: Writing— review and editing. J.C.-N.: Funding acquisition, project administration, resources, writing—review and editing. C.R.d.C.M.: Conceptualization, methodology, writing—review and editing. E.C.M.: Conceptualization, data curation, investigation, methodology, project administration, resources, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CAPES, grant number 88887.356981/2019-00; FAPESP, grant number 2014/50457-0; and CNPq, grant number 150781/2017-2.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the School of Public Health of the University of São Paulo (protocol 2.450.682, 20 December 2017) and the Ethics Committee of the Oswaldo Cruz German Hospital (protocol 2.489.636, 7 February 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors. The data are not publicly available due to privacy and ethical reasons.

Acknowledgments: The authors would like to thank the hospital staff and the funding agencies.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Buijs, R.M.; Kalsbeek, A. Hypothalamic integration of central and peripheral clocks. *Nat. Rev. Neurosci.* **2001**, *2*, 521–526. [CrossRef] [PubMed]
2. Albrecht, U. Timing to Perfection: The Biology of Central and Peripheral Circadian Clocks. *Neuron* **2012**, *74*, 246–260. [CrossRef] [PubMed]
3. Bodur, M.; Baspinar, B.; Özçelik, A.Ö. A cross-sectional evaluation of the relationship between social jetlag and diet quality. *Chronobiol. Int.* **2021**, *38*, 1557–1568. [CrossRef]
4. Rusu, A.; Ciobanu, D.; Vonica, C.L.; Bala, C.; Mocan, A.; Sima, D. Chronic disruption of circadian rhythm with mistimed sleep and appetite-an exploratory research. *Chronobiol. Int.* **2021**, *38*, 807–816. [CrossRef] [PubMed]
5. Dashti, H.S.; Merino, J.; Lane, J.M.; Song, Y.; Smith, C.E.; Tanaka, T.; McKeown, N.M.; Tucker, C.; Sun, D.; Bartz, T.M.; et al. Genome-wide association study of breakfast skipping links clock regulation with food timing. *Am. J. Clin. Nutr.* **2019**, *110*, 473–484. [CrossRef]
6. Richter, J.; Herzog, N.; Janka, S.; Baumann, T.; Kistenmacher, A.; Oltmanns, K.M. Twice as High Diet-Induced Thermogenesis after Breakfast vs Dinner on High-Calorie as Well as Low-Calorie Meals. *Int. J. Clin. Endocrinol. Metab.* **2020**, *105*, e211–e221. [CrossRef]
7. Atkinson, G.; Fullick, S.; Grindey, C.; Maclarem, D. Exercise, energy balance and the shift worker. *Sports Med.* **2008**, *38*, 671–685. [CrossRef]
8. Akerstedt, T.; Wright, K.P., Jr. Sleep loss and fatigue in shift work and shift work disorder. *Sleep Med. Clin.* **2009**, *4*, 257–271. [CrossRef]
9. Alterman, T.; Luckhaupt, S.E.; Dahlhamer, J.M.; Ward, B.W.; Calvert, G.M. Prevalence rates of work organization characteristics among workers in the U.S.: Data from the 2010 National Health Interview Survey. *Am. J. Ind. Med.* **2013**, *56*, 647–659. [CrossRef] 10. Fischer, F.M.; Moreno, C.R.C.; Rotenberg, L. *Trabalho em Turnos e Noturno na Sociedade 24 Horas*; Atheneu: São Paulo, Brazil, 2004.
11. Statistics Canada. Work-life balance of shift workers. *Perspectives* **2008**, *9*, 5–16.
12. Zerón-Rugerio, M.; Hernáez, Á.; Porras-Loaiza, A.P.; Cambras, T.; Izquierdo-Pulido, M. Eating Jet Lag: A Marker of the Variability in Meal Timing and Its Association with Body Mass Index. *Nutrients* **2019**, *11*, 2980. [CrossRef] [PubMed]
13. McHill, A.W.; Melanson, E.L.; Higgins, J.; Connick, E.; Moehlman, T.M.; Stothard, E.R.; Wright, K.P., Jr. Impact of circadian misalignment on energy metabolism during simulated nightshift work. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 17302–17307. [CrossRef]
14. Baron, K.G.; Reid, K.J.; Kern, A.S.; Zee, P.C. Role of sleep timing in caloric intake and BMI. *Obesity* **2011**, *19*, 1374–1381. [CrossRef] [PubMed]
15. Maukonen, M.; Kanerva, N.; Partonen, T.; Kronholm, E.; Tapanainen, H.; Kontto, J.; Männistö, S. Chronotype differences in timing of energy and macronutrient intakes: A population-based study in adults. *Obesity* **2017**, *25*, 608–615. [CrossRef] [PubMed]
16. Mazri, F.H.; Manaf, Z.A.; Shahar, S.; Ludin, A.F.M. The Association between Chronotype and Dietary Pattern among Adults: A Scoping Review. *Int. J. Environ. Res. Public Health* **2019**, *17*, 68. [CrossRef]
17. Hulsegge, G.; Picavet, H.S.J.; van der Beek, A.J.; Verschuren, W.M.M.; Twisk, J.W.; Proper, K.I. Shift work, chronotype and the risk of cardiometabolic risk factors. *Eur. J. Public Health* **2019**, *29*, 128–134. [CrossRef]
18. Vetter, C. Circadian disruption: What do we actually mean? *Eur. J. Neurosci.* **2020**, *51*, 531–550. [CrossRef]
19. Phoi, Y.Y.; Rogers, M.; Bonham, M.P.; Dorrian, J.; Coates, A.M. A scoping review of chronotype and temporal patterns of eating of adults: Tools used, findings, and future directions. *Nutr. Res. Rev.* **2021**, *35*, 112–135. [CrossRef]
20. Amaral, F.G.; Cipolla-Neto, J. A brief review about melatonin, a pineal hormone. *Arch. Endocrinol. Metab.* **2018**, *62*, 472–479. [CrossRef]
21. Garaulet, M.; Qian, J.; Florez, J.C.; Arendt, J.; Saxena, R.; Scheer, F.A.J.L. Melatonin Effects on Glucose Metabolism: Time to Unlock the Controversy. *Trends Endocrinol. Metab.* **2020**, *31*, 192–204. [CrossRef]
22. Sack, R.L.; Lewy, A.J. Melatonin as a chronobiotic: Treatment of circadian desynchrony in night workers and the blind. *J. Biol. Rhythm.* **1997**, *12*, 595–603. [CrossRef] [PubMed]
23. Folkard, S.; Arendt, J.; Clark, M. Can melatonin improve shift workers' tolerance of the night shift? Some preliminary findings. *Chronobiol. Int.* **1993**, *10*, 315–320. [CrossRef] [PubMed]
24. Marqueze, E.C.; Nogueira, L.F.R.; Vetter, C.; Skene, D.J.; Cipolla-Neto, J.; Moreno, C.R.C. Exogenous melatonin decreases circadian misalignment and body weight among early types. *J. Pineal Res.* **2021**, *71*, e12750. [CrossRef] [PubMed]
25. Isherwood, C.M.; van der Veen, D.R.; Johnston, J.D.; Skene, D.J. Twenty-four-hour rhythmicity of circulating metabolites: Effect of body mass and type 2 diabetes. *FASEB* **2017**, *31*, 5557–5567. [CrossRef]

26. Wehrens, S.M.T.; Christou, S.; Isherwood, C.; Middleton, B.; Gibbs, M.A.; Archer, S.N.; Skene, D.J.; Johnston, J.D. Meal Timing Regulates the Human Circadian System. *Curr. Biol.* **2017**, *27*, 1768–1775.e3. [CrossRef]
27. Silva, L.E.S.; Oliveira, M.M.; Stopa, S.R.; Gouvea, E.C.D.P.; Ferreira, K.R.D.; Santos, R.O.; Valença Neto, P.F.; Macário, E.M.; Sardinha, L.M.V. Temporal trend of overweight and obesity prevalence among Brazilian adults, according to sociodemographic characteristics, 2006–2019. *Epidemiol. Serv. Saude* **2021**, *30*, e2020294. [CrossRef]
28. ASchwiter, K.; Nentwich, J.; Keller, M. Male privilege revisited: How men in female-dominated occupations notice and actively reframe privilege. *Gender Work Organ.* **2021**, *28*, 2199–2215. [CrossRef]
29. Emens, J.S.; Burgess, H.J. Effect of Light and Melatonin and Other Melatonin Receptor Agonists on Human Circadian Physiology. *Sleep Med. Clin.* **2015**, *10*, 435–453. [CrossRef]
30. Ma, X.; Idle, J.R.; Krausz, K.W.; Gonzalez, F.J. Metabolism of melatonin by human cytochromes p450. *Drug Metab. Dispos.* **2005**, *33*, 489–494. [CrossRef]
31. Pinheiro, A.B.V.; Lacerda, E.M.A.; Benzecry, E.H.; Gomes, M.C.S.; Costa, V.M. *Tabela Para Avaliação de Consumo Alimentar em Medidas Caseiras*, 4th ed.; Atheneu: São Paulo, Brazil, 2000.
32. NEPA-UNICAMP. *Tabela Brasileira de Composição de Alimentos-TACO*, 4th ed.; NEPA-UNICAMP: Campinas, Brazil, 2011.
33. Monteiro, C.A.; Cannon, G.; Lawrence, M.; Louzada, M.L.C.; Machado, P.P. *Ultra-Processed Foods, Diet Quality, and Health Using the NOVA Classification System*; FAO: Rome, Italy, 2019.
34. Fischer, D.; Vetter, C.; Roenneberg, T. A novel method to visualize and quantify circadian misalignment. *Sci. Rep.* **2016**, *6*, 38601. [CrossRef]
35. Juda, M.; Vetter, C.; Roenneberg, T. The Munich ChronoType Questionnaire for shift-workers (MCTQshift). *J. Biol. Rhythm.* **2013**, *28*, 130–140. [CrossRef] [PubMed]
36. Lohman, T.G.; Roche, A.F.; Martorell, R. *Anthropometric Standardization Reference Manual*; Human Kinetics Books: Champaign, IL, USA, 1988.
37. Trumbo, P.; Schlicker, S.; Yates, A.A.; Poos, M.; Food and Nutrition Board of the Institute of Medicine; The National Academies. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J. Am. Diet. Assoc.* **2002**, *102*, 1621–1630. [CrossRef]
38. Schulz, K.F.; Altman, D.G.; Moher, D.; for the CONSORT Group. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomized trials. *PLoS Med.* **2010**, *7*, e1000251. [CrossRef] [PubMed]
39. Nogueira, L.F.R.; Marqueze, E.C. Effects of melatonin supplementation on eating habits and appetite-regulating hormones: A systematic review of randomized controlled clinical and preclinical trials. *Chronobiol. Int.* **2021**, *38*, 1089–1102. [CrossRef]
40. Bahrami, M.; Cheraghpour, M.; Jafarirad, S.; Avalinejad, P.; Cheraghian, B. The role of melatonin supplement in metabolic syndrome: A randomized double blind clinical trial. *Food Sci. Nutr.* **2019**, *49*, 965–977. [CrossRef]
41. Frank, S.; Christopher, J.M.; Steven, A.S. The internal circadian clock increases hunger and appetite in the evening independent of food intake and other behaviors. *Obesity* **2013**, *21*, 421–423.
42. Della Torre, S.B.; Wild, P.; Dorribo, V.; Danuser, B.; Amati, F. Energy, nutrient and food intakes of male shift workers vary according to the schedule type but not the number of nights worked. *Nutrients* **2020**, *12*, 919. [CrossRef]
43. Silva, F.M.; Giatti, L.; Figueiredo, R.C.; Molina, M.C.B.; Cardoso, L.O.; Duncan, B.B.; Barreto, S.M. Consumption of ultra-processed food and obesity: Cross sectional results from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) cohort (2008–2010). *Public Health Nutr.* **2018**, *21*, 2271–2279. [CrossRef]
44. Shechter, A.; O'Keeffe, M.; Roberts, A.L.; Zammit, G.K.; Choudhury, A.R.; St-Onge, M.P. Alterations in sleep architecture in response to experimental sleep curtailment are associated with signs of positive energy balance. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2012**, *303*, R883–R889. [CrossRef]
45. Gupta, C.C.; Coates, A.M.; Dorrian, J.; Banks, S. The factors influencing the eating behaviour of shiftworkers: What, when, where and why. *Ind. Health* **2019**, *57*, 419–453. [CrossRef]
46. World Health Organization. *Diet, Nutrition and the Prevention of Chronic Diseases. Report of a Joint WHO/FAO Expert Consultation*; WHO Technical Report Series, n. 916; World Health Organization: Geneva, Switzerland, 2003.
47. Webber, J. Energy balance in obesity. *Proc. Nutr. Soc.* **2003**, *62*, 539–543. [CrossRef] [PubMed]
48. Levine, J.A. Measurement of energy expenditure. *Public Health Nutr.* **2005**, *8*, 1123–1132. [CrossRef] [PubMed]
49. Markwald, R.R.; Melanson, E.L.; Smith, M.R.; Higgins, J.; Perreault, L.; Eckel, R.H.; Wright, K.P., Jr. Impact of insufficient sleep on total daily energy expenditure, food intake, and weight gain. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 5695–5700. [CrossRef]
50. Cipolla-Neto, J.; Amaral, F.G.; Afeche, S.C.; Tan, D.X.; Reiter, R.J. Melatonin, energy metabolism, and obesity: A review. *J. Pineal Res.* **2014**, *56*, 371–381. [CrossRef] [PubMed]
51. Flanagan, A.; Lowson, E.; Arber, S.; Griffin, B.A.; Skene, D.J. Dietary patterns of nurses on rotational shifts are marked by redistribution of energy into the nightshift. *Nutrients* **2020**, *12*, 1053. [CrossRef]

52. Amaral, F.G.D.; Andrade-Silva, J.; Kuwabara, W.M.T.; Cipolla-Neto, J. New insights into the function of melatonin and its role in metabolic disturbances. *Expert Rev. Endocrinol. Metab.* **2019**, *14*, 293–300. [CrossRef]
53. Phillips, A.J.K.; Vidafar, P.; Burns, A.C.; Mcglashan, E.M.; Anderson, C.; Rajaratnam, S.M.W.; Lockley, S.W.; Cain, S.W. High sensitivity and interindividual variability in the response of the human circadian system to evening light. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 12019–12024. [CrossRef]
54. Cipolla-Neto, J.; Amaral, F.G. Melatonin as a hormone: New physiological and clinical insights. *Endocr. Rev.* **2018**, *39*, 990–1028. [CrossRef]
55. Lowden, A.; Moreno, C.; Holmbäck, U.; Lennernäs, M.; Tucker, P. Eating and shift work-effects on habits, metabolism and performance. *Scand. J. Work Environ. Health* **2010**, *36*, 150–162. [CrossRef]
56. Wong, H.; Wong, M.C.; Wong, S.Y.; Lee, A. The association between shift duty and abnormal eating behavior among nurses working in a major hospital: A cross-sectional study. *Int. J. Nurs. Stud.* **2010**, *47*, 1021–1027. [CrossRef]

7. CONSIDERAÇÕES FINAIS

Os resultados sobre o efeito da melatonina exógena na ingestão alimentar e hormônios reguladores do apetite são heterogêneos e o número de estudos clínicos é limitado, não permitindo uma conclusão robusta sobre a suplementação deste hormônio. No presente ensaio clínico, não houve modificação no padrão alimentar de trabalhadoras noturnas com excesso de peso no que se refere à ingestão calórica total, distribuição de macronutrientes, nível de processamento dos alimentos nem horários de alimentação. Estes resultados vão ao encontro da literatura, que aponta que os efeitos da melatonina sobre o metabolismo energético podem ocorrer independentemente da ingestão alimentar.

8. PERSPECTIVAS FUTURAS

Conforme evidenciado ao longo desta tese, o conhecimento a respeito dos efeitos da melatonina exógena sobre diferentes aspectos relacionados à alimentação, perfil metabólico, peso e composição corporal são incipientes. Para permitir que os avanços científicos neste campo se traduzam em benefícios na prática clínica, com a prescrição de melatonina exógena para trabalhadores noturnos que vivenciam os efeitos crônicos da dessincronização circadiana, muitas lacunas ainda precisam ser preenchidas. Destaca-se a necessidade de estudos clínicos que administrem doses individualizadas, bem como avaliem a real biodisponibilidade da melatonina exógena. No que se refere ao perfil metabólico, em especial, a partir dos resultados obtidos no presente estudo e em outros já disponíveis na literatura, faz-se necessário esclarecer os efeitos da dessincronização circadiana crônica causada pelo trabalho noturno sobre as taxas metabólicas basal e total.

No momento da defesa desta tese (dezembro de 2022), outro ensaio clínico com administração de melatonina exógena está sendo conduzido sob coordenação da Prof.ª Dr.ª Cláudia Moreno (FSP-USP) e da Prof.ª Dr.ª Elaine Cristina Marqueze (UNISANTOS), intitulado “Menopausa em trabalhadoras noturnas: intervenção com melatonina exógena para a melhoria do sono e bem-estar”, e do qual também integro a equipe. Este estudo tem como objetivo administrar melatonina a profissionais de saúde expostas ao trabalho noturno a fim de reduzir efeitos da menopausa, particularmente sobre o sono, perfil hormonal do climatério e sintomas psíquicos. Neste estudo, estamos administrando doses individualizadas às participantes. O estudo integra o projeto temático “Melatonina, fisiologia e fisiopatologia, estudos básicos e clínicos: caracterização da síndrome hipomelatoninêmica e o papel da reposição terapêutica com melatonina”, coordenado pelo Prof. José Cipolla-Neto (FAPESP nº 2019/24327-5).

REFERÊNCIAS

ÅKERSTEDT, T. et al. Work organization and unintentional sleep: Results from the WOLF Study. J **Occup and Environ Med.**, v. 59, n. 9, p. 595-600, 2002.

ÅKERSTEDT, T.; WRIGHT, K.P. Sleep loss and fatigue in shift work and shift work disorder. **Sleep Med Clin.**, v. 4, n. 2, p. 257-271, 2009.

ALTERMAN, T. et al. Prevalence rates of work organization characteristics among workers in the US: data from the 2010 National Health Interview Survey. **Am J Ind Med**, v. 56, p. 647-659, 2013.

AMARAL, F.G.; ANDRADE-SILVA, J.; KUWABARA, W.M.T.; CIPOLLA-NETO, J. New insights into the function of melatonin and its role in metabolic disturbances. **Expert Rev. Endocrinol. Met.**, v. 14, p. 293-30, 02019.

ARENDT, J.; SKENE, D.J. Melatonin as a chronobiotic. **Sleep Med Rev.**, v. 9, p. 25-39, 2005.

ATKINSON, G. et al. Exercise, energy balance and the shift worker. **Sports Med.**, v. 38, n. 8, p. 671-685, 2008.

BAE, S.; FANG, M.Z.; RUSTGI, V. et al. At the interface of lifestyle, behavior, and circadian rhythms: Metabolic implications. **Front. Nutr.**, v. 6, p. 132, 2019.

CHIN-CHANCE, C.; POLONSKY, K.S.; SCHOELLER, D. Twenty-four-hour leptin levels respond to cumulative short-term energy imbalance and predict subsequent intake. **J Clin Endocrinol Metab.**, v. 85, p. 2685-2691, 2000.

CIPOLLA-NETO, J. et al. Melatonin, energy metabolism and obesity: a review. **J Pineal Res.**, v. 56, p. 371-381, 2014.

COELHO, M.P.; PINTO, O.O.; MOTA, M.C.; CRISPIM, C.A. Nutritional damages and disturbances in the sleep pattern of nursing workers. **Rev. Bras. Enferm.**, v. 67, p. 832-842, 2014.

COSTA, G. Saúde e trabalho em turnos e noturno. In: FISCHER, F.M.; MORENO, C.R.C.; ROTENBERG, L. **Trabalho em turnos e noturno na sociedade 24 horas.** São Paulo: Atheneu, 2004. p. 79-98.

CRISPIM, C.A. et al. Relationship between Food Intake and Sleep Pattern in Healthy Individuals. **J Clin Sleep Med.**, v. 17, n. 6, p. 659-664.

DELLA TORRE, S.B.; WILD, P.; DORRIBO, V. et al. Energy, nutrient and food intakes of male shift workers vary according to the schedule type but not the number of nights worked. **Nutrients**, v. 12, n. 919, 2020.

FLANAGAN, A.; LOWSON, E.; ARBER, S. et al. Dietary patterns of nurses on rotational shifts are marked by redistribution of energy into the nightshift. **Nutrients**, v. 12, n. 1053, 2020.

FOLKARD, S. Do permanent night workers show circadian adjustment? A review based on the endogenous melatonin rhythm. **Chronobiol Int.**, v. 25, n. 2, p. 215-224, 2008.

GARAULET, M. et al. Genetic variants in human CLOCK associate with energy Intake and cytokine Sleep factors in overweight subjects (GOLDN population). **Eur J Human Gen.**, v. 18, p. 364-369, 2010.

GARAULET, M.; MADRID, J.A. Chronobiological aspects of nutrition, metabolic syndrome and obesity. **Adv Drug Deliv Rev.**, v. 62, p. 967-978, 2010.

GORDIS, L. **Epidemiologia.** 4 ed. Rio de Janeiro: Revinter, 2010.

GRAHAM, I. et al. European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and

other societies on cardiovascular disease prevention in clinical practice. **Eur J Cardiovasc Prev Rehabl**, v. 14, suppl. 2, p. S1-113.

GRUNDY, S.M. et al. Implications of recent clinical trials for the National Cholesterol Education Program. Adult Treatment Panel III Guidelines. **J Am Coll Cardiol.**, n. 44, v. 3, p. 720-732, 2004.

GUILBERT, J.J. The world health report 2002: reducing risks, promoting healthy life. **Educ Health** (Abingdon), v. 16, n. 2, p. 230, 2003.

HINGORJO, M.R.; QURESHI, M.A.; MEHDI, A. Neck circumference as a useful marker of obesity: a comparison with body mass index and waist circumference. **J Park Med Assoc.**, v. 62, n. 1, p. 36-40, 2012.

HOLANDA, L.B.; BARROS FILHO, A.A. Applied methods in dietary assessment. **Rev Paul Pediatria**, v. 24, n. 1, p. 62-70, 2006.

HUTCHISON, A.T.; WITTERT, G.A.; HEILBRONN, L.K. Matching meals to body clocks - Impact on weight and glucose metabolism. **Nutrients**, v. 9, n. 222, 2017.

INSTITUTE OF MEDICINE. **Dietary Reference Intakes.** Estimated Average Requirements for Groups. Washington (DC): National Academy Press.

INTERNATIONAL DIABETES FEDERATION. **The IDF consensus worldwide definition of metabolic syndrome.** International Diabetes Federation, 2006. Available from: http://www.idf.org/webdata/docs/IDF_Meta_def_final.pdf. Acesso em 21 set. 2018.

JUDA, M.; VETTER, C.; ROENNEBERG, T. The Munich ChronoType Questionnaire for Shift-Workers ($MCQT^{Shift}$). **J Biol Rhythms.**, v. 28, n. 2, 2013.

LEUNG, G.K.W.; HUGGINS, C.E.; WARE, R.S. et al. Time of day difference in postprandial glucose and insulin responses: Systematic review and meta-analysis of acute postprandial studies. **Chronobiol. Int.**, v. 37, p. 311–326, 2020.

MA, X. et al. Metabolism of melatonin by human cytochromes p450. **Drug Metab Dispos**., v. 33. p. 489-494, 2005.

MANOOGIAN, E.N.C.; PANDA, S. Circadian rhythms, time-restricted feeding and healthy aging. **Ageing Res Rev.**, n. 39, p. 59-67, 2016.

MARQUEZE, E.C.; ULHÔA, M.A.; MORENO, C.R.C. Leisure-time physical activity does not fully explain the higher body mass index in irregular-shift workers. **Int Arch Occup Environ Health**, v. 87, p. 229-239, 2014.

MATSUDO, S. et al. Questionário Internacional de Atividade Física (IPAQ): estudo de validade e reprodutibilidade no Brasil. **Atividade Física & Saúde**, v. 6, n. 2, p. 5-18, 2001.

MORENO, C.R.C. Sono e estratégias relativas ao sono para lidar com os horários de trabalho. In: FISCHER, F.M.; MORENO, C.R.C.; ROTENBERG, L. **Trabalho em turnos e noturno na sociedade 24 horas.** São Paulo: Atheneu, 2004. p. 137-157.

NDSR SOFTWARE [internet]. Minneapolis: NCC - Nutrition Coordinating System. 2016. Disponível em: http://license.umn.edu/technologies/ndsr87072_nutrition-data-system-for-research-nutritional-analysis-software. Acesso em 16 set. 2018.

NEHME, P.; MARQUEZE, E.C.; ULHÔA, M. et al. Effects of a carbohydrate-enriched night meal on sleepiness and sleep duration in night workers: A double-blind intervention. **Chronobiol. Int.**, v. 31, p. 453-460, 2014.

NOGUEIRA, T.C. et al. Absence of melatonin induces night-time hepatic insulin resistance and increased gluconeogenesis due to stimulation of nocturnal unfolded protein response. **Endocrinology**, v, 152, p. 1253-1263, 2011.

PATE, R.R. et al. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports medicine. **JAMA**, v. 273, n. 5, p. 402-407, 1995.

RAJARATNAM, S.M.; HOWARD, M.E.; GRUNSTEIN, R.R. Sleep loss and circadian disruption in shift work: health burden and management. **Med J Aust.**, v. 21, n. 199(8), p. S11-15, out. 2013.

REITER, R.J. et al. Obesity and metabolic syndrome: association with chronodisruption, sleep deprivation and melatonin suppression. **Ann Med.**, v. 44, p. 564-577, 2012.

ROENNEBERG, T.; PILZ, L.K.; ZERBINI, G. et al. Chronotype and social jetlag: a (Self-) critical review. **Biology**, v. 8, p. 3, 2019.

RÜGER, M.; SCHEER, F.A. Effects of circadian disruption on the cardiometabolic system. **Rev Endocr Metab Disord.**, v. 10, n. 4, p. 245-260, dez. 2009.

SAHIN, L.; FIGUEIRO, M.G. Alerting effects of short-wave length (blue) and long-wave length (red) lights in the afternoon. **Physiol Behav.**, v. 117, p. 1-7, 2013.

SHAJI A.V.; KULKARNI, S.K. Evidence of GABAergic modulation in melatonin-induced short-term memory deficits and food consumption. **Methods Find Exp Clin Pharmacol.**, v. 20, n. 4, p. 311-319, 1998.

SHI, S.Q. et al. Circadian disruption leads to insulin resistance and obesity. **Curr Biol.**, v. 23, p. 372-381, 2013.

SOUZA, R.V. et al. The effect of shit work on eating habits: a systematic review. **Scand J Work Environ Health**, pii. 3759, ago. 2018.

STATISTICS CANADA. Work-life balance of shift workers. **Perspectives**, v. 9, n. 8, p. 5-16, 2008.

TABELA BRASILEIRA DE COMPOSIÇÃO DE ALIMENTOS. Núcleo de Estudos e Pesquisas em Alimentação - NEPA. Universidade Estadual de Campinas - UNICAMP. 4 ed. rev. e ampl. Campinas: NEPA-UNICAMP, 2011. 161 p.

WOLDEN-HANSON, T.; MITTON, D.R.; MCCANTS, R.L. et al.. Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. **Endocrinology**, v. 141, n. 2, p. 487-497, 2000.

WORLD HEALTH ORGANIZATION (WHO). **BMI classifications**, 2006. Disponível em: http://www.who.int/bmi/index.isp?introPage=intro3.html. Acesso em 16 set. 2018.

APÊNDICES

APÊNDICE 1 - Pôster apresentado no Congresso Brasileiro de Sono 2019

CONGRESSO BRASILEIRO DO SONO

2019 CONGRESSO BRASILEIRO DO SONO

04 a 07 de dezembro

FOZ DO IGUAÇU - PR

Certificamos que o trabalho intitulado **Reduction in calories and carbohydrates intake among overweight night workers after melatonin supplementation**, dos autores **Luciana Fidalgo Ramos Nogueira, Adriana Sousa Duarte, Ananda Laís Felix Garrido, Gabriella Habib Rodrigues, Patrícia Teixeira Santana, Pollyanna Pellegrino, José Cipolla-Neto, Claudia Roberta Castro Moreno, Elaine Cristina Marqueze** foi apresentado no "Congresso Brasileiro do Sono", realizado de 05 a 07 de Dezembro de 2019, em Foz do Iguaçu/PR, na forma de apresentação Pôster.

Apresentado em:

Luciano Ferreira Drager
Coordenador Científico

Andrea Frota Bacelar Rego
Presidente da ABS

Carolina Ferraz de Paula Soares
Presidente do Congresso

Fernanda Louise Martinho Haddad
Presidente da ABMS

Marco Antonio Machado
Presidente da ABROS

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APÊNDICE 2 - Pôster apresentado no Congresso Brasileiro de Sono 2021

APÊNDICE 3 - Premiação de pôster apresentado no Congresso Brasileiro de Sono 2019

APÊNDICE 4 - Coautoria em pôster apresentado no Congresso Brasileiro de Sono 2019

APÊNDICE 5 - Coautoria em pôster apresentado no Congresso Brasileiro de Sono 2019

CONGRESSO BRASILEIRO DO SONO

2019 CONGRESSO BRASILEIRO DO SONO

04 a 07 de dezembro

FOZ DO IGUAÇU - PR

Certificamos que o trabalho intitulado **Duration and quality of sleep associated with food consumption among overweight night workers**, dos autores **Gabriella Habib Rodrigues, Adriana Souza Duarte, Ananda Laís Felix Garrido, Patricia Teixeira Santana, Luciana Fidalgo Ramos Nogueira, Pollyanna Pellegrino, José Cipolla-Neto, Claudia Roberta Castro Moreno, Elaine Cristina Marqueze** foi apresentado no "Congresso Brasileiro do Sono", realizado de 05 a 07 de Dezembro de 2019, em Foz do Iguaçu/PR, na forma de apresentação Pôster.

Apresentado em:

Luciano Ferreira Drager
Coordenador Científico

Andrea Frota Bacelar Rego
Presidente da ABS

Carolina Ferraz de Paula Soares
Presidente do Congresso

Fernanda Louise Martinho Haddad
Presidente da ABMS

Marco Antonio Machado
Presidente da ABROS

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APÊNDICE 6 - Coautoria em pôster apresentado no Congresso Brasileiro de Sono 2019

APÊNDICE 7 - Coautoria em pôster apresentado no Congresso Brasileiro de Sono 2019

CONGRESSO BRASILEIRO DO SONO

2019 CONGRESSO BRASILEIRO DO SONO

04 a 07 de dezembro

FOZ DO IGUAÇU - PR

Certificamos que o trabalho intitulado **Relationship between social jet lag and diabetes markers in overweight night workers**, dos autores **Adriana Sousa Duarte, Ananda Lais Felix Garrido, Gabriella Habib Rodrigues, Patricia Texeira Santana, Luciana Fidalgo Ramos Nogueira, Pollyanna Pellegrino, José Cipolla-Neto, Claudia Roberta Castro Moreno, Elaine Cristina Marqueze** foi apresentado no "Congresso Brasileiro do Sono", realizado de 05 a 07 de Dezembro de 2019, em Foz do Iguaçu/PR, na forma de apresentação Pôster.

Apresentado em: 06/12/2019

Luciano Ferreira Drager
Coordenador Científico

Andrea Frota Bacelar Rego
Presidente da ABS

Carolina Ferraz de Paula Soares
Presidente do Congresso

Fernanda Louise Martinho Haddad
Presidente da ABMS

Marco Antonio Machado
Presidente da ABROS

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APÊNDICE 8 - Coautoria em pôster apresentado no Congresso Brasileiro de Sono 2021

APÊNDICE 9 - Coautoria em pôster apresentado no Congresso Brasileiro de Sono 2021

CONGRESSO BRASILEIRO DO SONO

Associação Brasileira do Sono

abms

ABROS

XVIII CONGRESSO BRASILEIRO DO SONO 2021

CERTIFICADO

Certificamos que o Trabalho Científico intitulado Eating habits, sleep, and a proxy for circadian disruption are correlated with dyslipidemia in overweight night workers

dos autores Ananda Lais Felix Garrido, Adriana Sousa Duarte, Patricia Teixeira Santana, Gabriella Habib Rodrigues, Pollyanna Pellegrino, Luciana Fidalgo Ramos Nogueira, José Cipolla-Neto, Claudia Roberta Castro Moreno, Elaine Cristina Marqueze

foi apresentado no Congresso Brasileiro do Sono, realizado no Centro de Convenções Frei Caneca,

12 a 15 de Dezembro de 2021, na forma de apresentação: Pôster.

São Paulo, 15 de dezembro de 2021

Dra. Andrea Bacelar
Presidente da ABS

Rowdley Robert Rossi
Presidente da ABROS

Dr. Pedro Rodrigues Genta
Presidente da ABMS

Dr. Luciano Ribeiro Pinto
Coordenador de Projeto da ABS

APÊNDICE 10 - Coautoria em pôster apresentado no Congresso Brasileiro de Sono 2021

APÊNDICE 11 - Coautoria em pôster apresentado no Congresso Brasileiro de Sono 2021

APÊNDICE 12 - Premiação em pôster apresentado no Congresso Brasileiro de Sono 2021

APÊNDICE 13 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

Código da participante		

I – DADOS DE IDENTIFICAÇÃO DO SUJEITO DA PESQUISA

1. Dados de Identificação
Iniciais do Nome: .....

Data de nascimento: ...../...../.....

II – DADOS SOBRE A PESQUISA

1. Título do Protocolo de Pesquisa: **Efeito da melatonina no sono e no metabolismo de trabalhadoras noturnas com excesso de peso.**

2. Pesquisador responsável: Elaine Cristina Marqueze
Documento de Identidade Nº : 3.415.216 Sexo: ( ) M (X) F

Cargo/Função: Pós-doutoranda pelo Departamento Saúde, ciclos de vida e sociedade da Faculdade de Saúde Pública/USP.

Professora Assistente do Programa de Mestrado e Doutorado em Saúde Coletiva da Universidade Católica de Santos/UniSantos.

3. Avaliação de Risco da Pesquisa
( ) Sem Risco (X) Risco Mínimo ( ) Risco Médio
( ) Risco Baixo ( ) Risco Maior

4. Duração da Pesquisa: 12 meses

III – REGISTRO DAS EXPLICAÇÕES DA PESQUISADORA AO SUJEITO DA PESQUISA, CONSIGNANDO:

Estamos convidando você a participar de uma pesquisa que será realizada aqui no hospital com as profissionais de enfermagem.

Esta pesquisa tem por objetivo principal avaliar os efeitos da melatonina sintética nas variáveis antropométricas (peso, circunferências) e também nos aspectos de sono (qualidade, duração, latência, eficiência), hormonais (leptina, grelina e insulina), fisiológicos e bioquímicos (pressão arterial, colesterol total, HDL, LDL, VLDL-colesterol, glicemia, triglicérides séricos) de trabalhadoras da área de enfermagem que trabalham em turnos noturnos fixos de 12x36 horas. A melatonina é um hormônio naturalmente produzido pelo organismo, sendo que o uso da melatonina sintética não altera a produção endógena.

A melatonina pode ser considerada um indutor de sono leve, porém, na dose e horário utilizados no estudo, não se esperam efeitos colaterais significativos. Estudos recentes têm demonstrado que a melatonina regula aspectos que influenciam o metabolismo energético, as lipidemias, o peso corporal e o sono; bem como, que o uso da melatonina não está associado a reações adversas ou toxicidade.

As profissionais de enfermagem, em especial as que trabalham no turno noturno, podem apresentar uma alta taxa de sobrepeso e obesidade, que podem estar associadas ao turno de trabalho, aos aspectos do sono, estilo de vida e entre outros. Desta forma, há necessidade de estudos com maior controle desses aspectos e como o uso regular e adequado da melatonina pode influenciá-los.

As informações produzidas por esta pesquisa poderão ser utilizadas para ajudar a elaboração de programas de saúde que visam melhorar a saúde, o sono e consequentemente a qualidade de vida destas profissionais.

Sua participação consiste em fazer uso da melatonina sintética, um hormônio naturalmente produzido pelo organismo. A presente pesquisa terá a duração total de seis meses, sendo que esse tempo será fracionado em duas etapas. Em uma etapa (três meses) você fará uso de melatonina e na outra (três meses), o uso de placebo, um comprimido que na aparência é igual à melatonina (sem glúten e sem lactose, e sem qualquer efeito sobre o organismo). A ordem de participação em cada uma dessas etapas será feita por sorteio, sendo que nem as participantes e nem as pesquisadoras saberão durante o processo de qual etapa está fazendo parte, para garantir a imparcialidade dos resultados.

O uso da melatonina ocorrerá somente nos seus dias de folga, ou seja, nos dias em que você realizar o sono durante a noite. Nos dias de trabalho noturno, a melatonina não será tomada. Será utilizado um diário de registro do total de dias de uso, para contabilização ao término da intervenção. Você tomará melatonina uma hora antes do seu horário desejado para dormir, sendo a dose de 3 mg. Também será necessário preencher um diário informando o horário que você tomou a melatonina, bem como os horários que dormiu e acordou.

Você terá que responder, se concordar, a um questionário que será entregue por um entrevistador de nossa equipe de pesquisa. Posteriormente, será coletado uma amostra de sangue para análise de colesterol total e frações, triglicérides, glicemia e hormônios reguladores do apetite. A quantidade de sangue a ser retirada não é muito grande e não implica em danos ao organismo. Cada coleta de sangue requer uma punção venosa, por isso o local pode ficar roxo, caso haja um pequeno trauma local. Uma das causas do “roxo” que pode aparecer no local da punção venosa é o uso de alguns remédios, como por exemplo, a aspirina. A punção venosa pode causar um certo incômodo. A quantidade de sangue a ser retirada não é muito grande, nada provocando no organismo. Será utilizado somente material descartável nesta coleta de sangue. Também será medida sua altura e peso, as circunferências abdominal e do quadril, assim como a pressão arterial (PA). Todas essas análises serão realizadas por pessoas especializadas, devidamente treinadas.

Os grupos serão divididos em dois subgrupos de acordo com IMC (25 a 29,9 Kg/m^2 e $\geq$ 30 kg/m^2), cada subgrupo será dividido em dois grupos, sendo um denominado ‘grupo intervenção’, que fará uso de melatonina e o outro ‘grupo controle’, que fará uso de placebo, por um período de três meses. Após esse período os grupos se invertem, ou seja, quem era grupo controle, nos próximos três meses será grupo intervenção e vice-versa. Vale ressaltar, que ao fim e ao início de cada etapa, os exames bioquímicos, as medidas antropométricas, a aferição de PA e aplicação dos questionários serão realizados novamente.

Será garantido o total sigilo das informações que você fornecer, assim como seu anonimato. Seu nome não será divulgado em nenhum momento da pesquisa, apenas os dados dos grupos serão utilizados para publicações em periódicos especializados.

A sua participação nesta pesquisa oferece riscos mínimos de desconforto emocional, como constrangimento durante a verificação das medidas antropométricas e em responder o questionário. A verificação das medidas antropométricas será realizada pela pesquisadora responsável do estudo, em uma sala fechada, em que somente você e a pesquisadora se farão presentes. No entanto, ressaltamos que você tem o direito de não responder a essas questões e pode parar de participar do estudo a qualquer momento, se assim quiser sem sofrer prejuízos por isso.

A hipótese do presente estudo é de que a administração de melatonina sintética poderá melhorar os aspectos metabólicos, bem como a qualidade de sono de trabalhadoras de turnos noturnos fixos que apresentam excesso de peso. Vale ressaltar que o uso de melatonina sintética não altera a produção do organismo, ou seja, quando você parar de tomar a melatonina seu copo continuará a produção desse hormônio como antes. O benefício de sua participação no estudo será conhecer a qualidade de seu sono e conseguir identificar possíveis problemas relativos a este tema.

Sua participação é voluntária e você pode interrompê-la a qualquer momento, mesmo depois de ter concordado em participar. Você tem liberdade para não responder a qualquer pergunta do questionário. A equipe de pesquisa somente voltará a contatá-la se for necessário completar informações fornecidas anteriormente e com sua autorização.

Se você tiver dúvidas sobre esse estudo, pode a qualquer momento, entrar em contato com a pesquisadora responsável, Elaine Cristina Marqueze, sito à Av. Dr. Arnaldo, 715, Cerqueira César – Departamento Saúde, ciclos de vida e sociedade – Faculdade de Saúde Pública/USP, 2º andar. Bairro: Cerqueira César. São Paulo – Telefone: (11) 98758-6384– e-mail: ecmarqueze@usp.br.

Em caso de dúvida ou denúncia sobre a ética você poderá entrar em contato com o Comitê de Ética em Pesquisa (CEP) da Faculdade de Saúde Pública da Universidade de São Paulo, sito à Av. Dr. Arnaldo, 715, Cerqueira César – CEP 01246-904, São Paulo, SP – Telefone: (11) 3061-7779 – e-mail: coep@fsp.usp.br.

Desejo ser contatado através de:
[ ] Correio eletrônico: _____
[ ] Telefone celular: _____
[ ] Telefone fixo: _____
[ ] Outros. Indicar o modo de contato: _____
[ ] Não desejo ser contatado para futuras pesquisas.

IV – ESCLARECIMENTOS DADOS PELO PESQUISADOR SOBRE GARANTIAS DO SUJEITO DA PESQUISA

1. A qualquer momento o participante dessa pesquisa poderá fazer perguntas sobre os riscos e o que será realizado na pesquisa;
2. A qualquer momento o participante da pesquisa poderá retirar seu consentimento e deixar de participar do estudo, sem nenhum prejuízo;

3. Os resultados de cada participante serão confidenciais, somente os pesquisadores envolvidos terão acesso aos resultados individuais. Caso o participante tenha interesse, poderá conhecer o resultado de suas avaliações individualmente;
4. Salvaguarda da confidencialidade, sigilo e privacidade;
5. A melatonina e o placebo serão fornecidos sem ônus às participantes;
6. Os resultados da pesquisa do coletivo serão apresentados em um encontro a ser marcado com todos os envolvidos no projeto ao término do mesmo.

V – CONTATOS

Nome: ELAINE CRISTINA MARQUEZE
Telefone: (11) 98758-6384
E-mail: ecmarqueze@usp.br
Endereço: Av. Dr. Arnaldo, 715 – Departamento Saúde, ciclos de vida e sociedade – Faculdade de Saúde Pública/USP, 2º andar. Bairro: Cerqueira César. São Paulo. CEP: 01246-904

VI – CONSENTIMENTO PÓS-ESCLARECIDO

Declaro que concordo em participar desse estudo. Recebi uma via deste termo de consentimento livre e esclarecido e me foi dada a oportunidade de ler e esclarecer as minhas dúvidas.

São Paulo, _____ de _____ de _____.

Nome do participante (letra de forma)	Pesquisadora Responsável
_____	Elaine Cristina Marqueze
Assinatura do sujeito de pesquisa	Assinatura da pesquisadora
_____	_____

APÊNDICE 7 - INSTRUMENTO DE COLETA DE DADOS

Universidade de São Paulo
Faculdade de Saúde Pública
Av. Dr. Arnaldo, 715 – CEP 01246-904 – São Paulo
coep@fsp.usp.br

PESQUISA: "EFEITO DA MELATONINA NO SONO E NO METABOLISMO DE TRABALHADORAS NOTURNAS COM EXCESSO DE PESO"

Código da participante		Código amostra de sangue	

QUESTÕES SOBRE DADOS SOCIODEMOGRÁFICOS

01. Data de nascimento: _____/_____/_____

02. Seu estado conjugal atual é:
 Solteira
 Casada / Vive com companheiro(a)
 Separada / Divorciada
 Viúva

03. Qual é o seu grau de escolaridade?
 Ensino Médio completo
 Faculdade incompleta ou cursando
 Faculdade completa
 Pós-Graduação incompleta ou cursando
 Pós-Graduação completa

04. Você já esteve grávida?
 Não
 Sim. Qual o número total de gestações? _____

05. Se sim, em alguma dessas gestações, você trabalhou à noite?
 Não
 Sim, até o primeiro trimestre, em _____ gestações
 Sim, até o segundo trimestre, em _____ gestações
 Sim, até o final da gravidez, em _____ gestações

06. Incluindo você, quantas pessoas moram na sua casa? _____ pessoa(s)

07. Incluindo você, quantas pessoas contribuem para a renda familiar? _____ pessoa(s)

08. Qual é aproximadamente sua renda familiar LÍQUIDA, isto é, a soma de rendimentos, já com descontos, de todas as pessoas que contribuem regularmente para as despesas de sua casa?
 Até R$ 900,00
 Entre R$ 901,00 e 1.800,00
 Entre R$ 1.801,00 e 2.700,00
 Entre R$ 2.701,00 e 3.600,00
 Entre R$ 3.601,00 e 4.500,00
 Entre R$ 4.501,00 e 5.400,00
 Entre R$ 5.401,00 e 7.200,00
 Entre R$ 7.201,00 e 9.000,00
 Mais de R$ 9.001,00
 Não sabe / Não quer responder

09. Informe o número de crianças que moram com você de acordo com a idade (PODE HAVER MAIS DE 1 OPÇÃO)
 Nenhuma criança
 Menor que 1 ano _____criança (s)
 De 1 a 5 anos _____criança (s)
 De 6 a 10 anos _____criança (s)
 De 11 a 14 anos _____criança (s)

QUESTÕES SOBRE O TRABALHO

10. Qual função você exerce nesse hospital?
 Enfermeira
 Técnica de enfermagem
 Auxiliar de enfermagem

11. Qual a sua unidade de trabalho nesse hospital atualmente? _____

12. Qual a sua carga horária semanal nesse hospital? _____ horas por semana

13. Há quanto tempo você trabalha nesse hospital? _____ ANOS _____ MESES

14. Há quanto tempo você trabalha nessa função atual nesse hospital?
_____ ANOS _____MESES

15. Há quanto tempo você trabalha no turno noturno atual nesse hospital?
_____ANOS _____ MESES

16. Qual o principal motivo que o levou a trabalhar à noite?
 Imposição do serviço
 Para conciliar com outro emprego
 Para conciliar com o estudo
 Para conciliar com o cuidado da casa e/ou filhos
 Porque gosta
 Para aumentar os rendimentos
 Outro _____
 Não sabe / Não lembra

17. Na maior parte das vezes, qual a primeira atividade (NÃO CONSIDERAR O BANHO) que você costuma fazer após sair do trabalho noturno nesse hospital?
□ Dorme assim que chega em casa
□ Descansa em casa (sem dormir)
□ Faz alguma atividade de lazer (ginástica, cinema, visita parentes, etc.)
□ Cuida da casa
□ Vai para outro emprego
□ Resolve algum assunto (pagamento, comprar coisas, etc.)
□ Faz uma refeição
□ Outros _____
□ Não sabe / não lembra

18. Você já trabalhou no turno noturno anteriormente (tanto nesse hospital, como em outro emprego)?
□ Não
□ Sim. Quanto tempo no total? _____ANOS _____ MESES

19. Quanto tempo você gasta para ir de casa até o trabalho (nesse hospital)?
_____ horas _____ minutos

20. Quanto tempo você gasta para voltar do trabalho (nesse hospital) para casa?
_____ horas _____ minutos

21. Nos últimos 12 meses ocorreu algum acidente durante o trabalho noturno nesse hospital?
□ Não (VÁ PARA QUESTÃO 24)
□ Sim

22. A que horas ocorreu o acidente? **(SÓ PARA QUEM SOFREU ACIDENTE)** (SE SOFREU VÁRIOS ACIDENTES FAVOR REFERIR-SE AO ÚLTIMO) _____:_____ horas

23. Devido a esse último acidente, você teve que ficar afastada do trabalho?
□ Não
□ Sim, _____ dias

24. Em média, quanto tempo você dedica às atividades domésticas e familiares (Considere atividades domésticas e familiares as atividades que envolvem a organização familiar, cuidado com os filhos ou crianças, como também cozinhar, lavar, passar, limpar a casa, fazer compras, etc)?
Nos dias de trabalho: _____ horas _____ minutos
Nos dias de folga: _____ horas _____ minutos

25. Além deste emprego, você possui outra atividade remunerada (PODE HAVER MAIS DE 1 OPÇÃO)?
□ Não (VÁ PARA QUESTÃO 31)
□ Sim, outra atividade não relacionada à assistência de enfermagem.
□ Sim, na assistência de enfermagem

26. Se você possui outra(s) atividade(s) remunerada(s), indique o número de locais que trabalha:
□ Em 1 local
□ Em 2 locais
□ Em 3 locais ou mais
□ Não quer responder

27. Qual sua carga horária de trabalho por semana nessa(s) outra(s) atividade(s) remunerada(s)?
_____ horas por semana

28. Há quanto tempo você trabalha em mais de um local? **(SÓ PARA QUEM POSSUI OUTRA ATIVIDADE REMUNERADA)** _____ ANOS _____ MESES

29. Quando você vem para o hospital, você vem direto de outro trabalho? **(SÓ PARA QUEM POSSUI OUTRA ATIVIDADE REMUNERADA)**
□ nunca □ raramente □ às vezes □ muitas vezes □ sempre

30. Quando você sai da empresa, você vai direto para outro trabalho? **(SÓ PARA QUEM POSSUI OUTRA ATIVIDADE REMUNERADA)**
□ nunca □ raramente □ às vezes □ muitas vezes □ sempre

31. No seu horário de "descanso" durante o plantão noturno nesse hospital, você diria que na maior parte das vezes:
□ Somente descansa (não consegue dormir)
□ Dorme. Se sim, quantas vezes por plantão? _____ vezes
Quanto tempo você dorme durante o plantão (tempo total)? ____h ____minutos
□ Não dorme, nem descansa
□ Não quer responder

32. Nos dias de trabalho noturno, qual horário habitualmente você costuma jantar?
_____:_____ horário

33. Além do jantar, você costuma comer outras coisas durante o plantão?
□ Não
□ Sim. Em média, quantas vezes durante o plantão? _____vezes

34. Suponha que a sua melhor capacidade para o trabalho tem um valor igual a 10 pontos. Assinale com X um número na escala de zero a dez, quantos pontos você daria para sua capacidade de trabalho atual.

0	1	2	3	4	5	6	7	8	9	10

Estou incapaz para o trabalho

Estou na minha melhor capacidade para o trabalho

35. Como você classificaria sua capacidade atual para o trabalho em relação às exigências físicas do seu trabalho? (por exemplo, fazer esforço físico com partes do corpo)
□ Muito boa
□ Boa
□ Moderada
□ Baixa
□ Muito baixa

36. Como você classificaria sua capacidade atual para o trabalho em relação às exigências mentais de seu trabalho? (por exemplo, interpretar fatos, resolver problemas, decidir a melhor forma de fazer)
□ Muito boa
□ Boa
□ Moderada
□ Baixa
□ Muito baixa

37. Sua lesão ou doença é um impedimento para seu trabalho atual? (você pode marcar mais de uma resposta nesta pergunta)
☐ não há impedimento / eu não tenho doenças
☐ eu sou capaz de fazer meu trabalho, mas ele me causa alguns sintomas
☐ algumas vezes preciso diminuir meu ritmo de trabalho ou mudar meus métodos de trabalho
☐ frequentemente preciso diminuir meu ritmo de trabalho ou mudar meus métodos de trabalho
☐ por causa de minha doença sinto-me capaz de trabalhar apenas em tempo parcial
 na minha opinião estou totalmente incapacitado para trabalhar

38. Quantos dias inteiros você esteve fora do trabalho devido a problema de saúde, consulta médica ou para fazer exame durante os últimos doze meses?
 Nenhum
 Até 9 dias
 De 10 a 24 dias
 De 25 a 99 dias
 De 100 a 365 dias

39. Considerando sua saúde, você acha que será capaz de daqui a 2 anos fazer seu trabalho atual?
☐ É improvável
☐ Não estou muito certo
☐ Bastante provável

40. Recentemente você tem conseguido apreciar suas atividades diárias?
☐ Sempre
☐ Quase sempre
☐ Às vezes
☐ Raramente
☐ Nunca

41. Recentemente você tem-se sentido ativo e alerta?
☐ Sempre
☐ Quase sempre
☐ Às vezes
☐ Raramente
☐ Nunca

42. Recentemente você tem se sentido cheio de esperança para o futuro?
☐ Continuamente
☐ Quase sempre
☐ Às vezes
☐ Raramente
☐ Nunca

43. Na sua opinião, quais das lesões por acidentes ou doenças, citadas abaixo, você possui atualmente. Marque também aquelas que foram confirmadas pelo médico que você possui atualmente.

		Em minha opinião	Diagnóstico médico
01	Lesão nas costas		
02	Lesão nos braços / mãos		
03	Lesão nas pernas / pés		
04	Lesão em outras partes do corpo. Onde? Que tipo de lesão? _____		
05	Doença da parte superior das costas ou região do pescoço, com dores frequentes.		
06	Doença na parte inferior das costas com dores frequentes		
07	Dor nas costas que se irradia para perna (ciática)		
08	Doença musculoesquelética afetando os membros (braços e pernas) com dores frequentes		
09	Artrite reumatoide		
10	Outra doença musculoesquelética. Qual? _____		
11	Hipertensão arterial (pressão alta)		
12	Doença coronariana, dor no peito durante exercício (angina pectoris)		
13	Infarto do miocárdio, trombose coronariana		
14	Insuficiência cardíaca		
15	Outra doença cardiovascular. Qual? _____		
16	Infecções repetidas do trato respiratório (incluindo sinusite aguda, amigdalite, bronquite aguda)		
17	Bronquite crônica		
18	Sinusite crônica		
19	Asma		
20	Enfisema		
21	Tuberculose pulmonar		
22	Outra doença respiratória. Qual? _____		
23	Distúrbio emocional severo (exemplo, depressão severa)		
24	Distúrbio emocional leve (exemplo, depressão leve, tensão, ansiedade, insônia)		
25	Problema ou diminuição da audição		
26	Doença ou lesão da visão (não assinale se apenas usa óculos e/ou lentes de contato de grau)		
27	Doença neurológica (acidente vascular cerebral ou “derrame”, neuralgia, enxaqueca, epilepsia)		
28	Outra doença neurológica ou dos órgãos dos sentidos. Qual? _____		
29	Pedras ou doenças da vesícula biliar		
30	Doença do pâncreas ou do fígado		
31	Úlcera gástrica ou duodenal		
32	Gastrite ou irritação duodenal		
33	Colite ou irritação do cólon		
34	Outra doença digestiva. Qual? _____		
35	Infecção das vias urinárias		
36	Doença dos rins		
37	Doença dos genitais e aparelho reprodutor (exemplo, problema nas trompas ou ovários, ou na próstata)		
38	Outra doença geniturinária. Qual? _____		
39	Alergia, eczema		
40	Outra erupção. Qual? _____		
41	Outra doença na pele. Qual? _____		
42	Tumor benigno		
43	Tumor maligno (câncer). Onde? _____		
44	Obesidade		
45	Diabetes		
46	Bócio ou outra doença da tireóide		
47	Outra doença endócrina ou metabólica. Qual? _____		
48	Anemia		
49	Outra doença do sangue. Qual? _____		
50	Defeito de nascimento. Qual? _____		
51	Outro problema ou doença. Qual? _____		

QUESTÕES SOBRE SAÚDE E ESTILO DE VIDA

44. Seu peso mudou no último ano?

☐ Não mudou

☐ Diminuiu. Quantos quilos? _____kg

☐ Aumentou. Quantos quilos? _____kg

☐ Não sei

45. Você se considera do tipo matutino (prefere acordar cedo e tem dificuldade de se manter acordado além do horário habitual de dormir) ou vespertino (prefere acordar mais tarde e dormir mais tarde)?

☐ Do tipo matutino
☐ Mais matutino que vespertino
☐ Indiferente
☐ Mais vespertino que matutino
☐ Do tipo vespertino

46. Você faz uso algum medicamento frequentemente?

☐ Sim. Qual(is)? _____
☐ Não

47. Você considera satisfatório o ambiente da sua casa para o sono?

☐ Não ☐ Sim

48. Quais os fatores que costumam atrapalhar seu sono em sua casa? (PODE MARCAR MAIS DE UMA RESPOSTA)

☐ Nenhum fator me atrapalha

☐ Barulho do trânsito

☐ Barulho de pessoas ou telefone na casa

☐ Calor

☐ Frio

☐ Iluminação (claridade)

☐ Cheiro ou odor desagradável

☐ Outro, qual (is):_____

49. Você fuma?

☐ Não, nunca fumei (VÁ PARA QUESTÃO 51)

☐ Não, fumei no passado, mas parei de fumar (VÁ PARA QUESTÃO 51)

☐ Sim

50. Se sim, quanto cigarros? _____ por dia

51. Você consome bebidas alcoólicas?
☐ Não, nunca consumi (VÁ PARA QUESTÃO 53)
☐ Não, mas consumi no passado (VÁ PARA QUESTÃO 53)
☐ Sim, consumo em ocasiões especiais, como festas, aniversários, churrascos, etc.
52. Com que frequência você bebe nessas ocasiões?
☐ Menos de 1 vez por mês
☐ 1 vez por mês
☐ A cada 15 dias
☐ 1 a 2 vezes por semana
☐ 3 a 5 vezes por semana
☐ 6 a 7 vezes por semana

As perguntas estão relacionadas ao tempo que você gasta fazendo atividade física em uma semana NORMAL/HABITUAL.
Para responder as questões lembre que:

Atividades físicas VIGOROSAS são aquelas que precisam de um grande esforço físico e que fazem respirar MUITO mais forte que o normal.
Atividades físicas MODERADAS são aquelas que precisam de algum esforço físico e que fazem respirar UM POUCO mais forte que o normal.

53. Em quantos dias de uma semana normal, você realiza atividades VIGOROSAS **por pelo menos 10 minutos seguidos**, como, por exemplo, correr, fazer ginástica/musculação, jogar futebol, pedalar rápido na bicicleta, fazer serviços domésticos pesados em casa, no quintal ou no jardim, carregar pesos elevados ou qualquer atividade que faça você suar bastante ou aumente MUITO sua respiração ou batimentos do coração.
_____Dias por SEMANA
 Nenhum

54. Nos dias em que você faz essas atividades vigorosas **por pelo menos 10 minutos seguidos**, quanto tempo, no total, você gasta fazendo essas atividades **por dia?**
_____ minutos
 Não faço atividades vigorosas

55. Em quantos dias de uma semana normal, você realiza atividades MODERADAS **por pelo menos 10 minutos seguidos**, como, por exemplo, pedalar leve na bicicleta, nadar, dançar, fazer ginástica/musculação leve, jogar vôlei, carregar pesos leves, fazer serviços domésticos em casa, no quintal ou no jardim como varrer, aspirar, ou qualquer atividade que faça você suar leve ou aumente MODERADAMENTE sua respiração ou batimentos do coração **(por favor, não inclua caminhada)**
_____Dias por SEMANA
 Nenhum

56. Nos dias em que você faz essas atividades moderadas **por pelo menos 10 minutos seguidos**, quanto tempo, no total, você gasta fazendo essas atividades **por dia?**
_____ minutos
 Não faço atividades moderadas

57. Em quantos dias de uma semana normal, você caminha **por pelo menos 10 minutos seguidos** em casa ou no trabalho, como forma de transporte para ir de um lugar para outro, por lazer, por prazer ou como forma de exercício?
_____Dias por SEMANA
 Nenhum

58. Nos dias em que você caminha **por pelo menos 10 minutos seguidos**, quanto tempo, no total, você gasta caminhando **por dia**?
_____ minutos
 Não faço caminhadas

59. Quanto tempo no total, você gasta sentado durante **um dia de trabalho**?
_____ horas _____minutos/dia

60. Quanto tempo no total, você gasta sentado durante **um dia de folga**?
_____ horas _____minutos/dia

QUESTÕES SOBRE O SONO

Você vivenciou alguma das situações seguintes nos últimos 6 meses?		Nunca	Raramente (Ocasio-nalmente)	Às vezes (Algumas vezes por mês)	Muitas vezes (1-2 vezes por semana)	Frequen-temente (3-4 vezes por semana)	Sempre (5 ou mais vezes por semana)
61	Dificuldades para adormecer	0	1	2	3	4	5
62	Acordou diversas vezes e teve dificuldades para dormir	0	1	2	3	4	5
63	Acordou antes do necessário (despertar precoce)	0	1	2	3	4	5
64	Sono agitado / perturbado	0	1	2	3	4	5
65	Dificuldades para acordar	0	1	2	3	4	5
66	Sensação de estar exausto ao acordar	0	1	2	3	4	5
67	Sentiu-se cansado quando acordou	0	1	2	3	4	5

68. Durante o mês passado, a que horas você foi deitar à noite na maioria das vezes?
HORÁRIO DE DEITAR NOS DIAS DE TRABALHO: _____:_____
HORÁRIO DE DEITAR NOS DIAS DE FOLGA: _____:_____

69. Durante o mês passado, quanto tempo (em minutos) você demorou para pegar no sono, na maioria das vezes?
QUANTOS MINUTOS DEMOROU PARA PEGAR NO SONO NOS DIAS DE TRABALHO: _____
QUANTOS MINUTOS DEMOROU PARA PEGAR NO SONO NOS DIAS DE FOLGA: _____

70. Durante o mês passado, a que horas você acordou de manhã, na maioria das vezes?
HORÁRIO DE ACORDAR NOS DIAS DE TRABALHO:_____:_____
HORÁRIO DE ACORDAR NOS DIAS DE FOLGA:_____:_____

71. Durante o mês passado, quantas horas de sono por noite você dormiu? (pode ser diferente do número de horas que você ficou na cama)
HORAS DE SONO POR NOITE NOS DIAS DE TRABALHO: _____
HORAS DE SONO POR NOITE NOS DIAS DE FOLGA: _____

Para cada uma das questões seguintes escolha uma única resposta, que você ache mais correta. Por favor, responda a todas as questões.

Durante o mês passado, quantas vezes você teve problemas para dormir por causa de:

72. Demorar mais de 30 minutos para pegar no sono:
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

73. Acordar no meio da noite ou de manhã muito cedo:
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

74. Levantar-se para ir ao banheiro:
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

75. Ter dificuldade para respirar:
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

76. Tossir ou roncar muito alto:
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

77. Sentir muito frio
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

78. Sentir muito calor
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

79. Ter sonhos ruins ou pesadelos
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

80. Sentir dores
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

81.Outra razão, por favor, descreva:
_____

82 Quantas vezes você teve problemas para dormir por esta razão, durante o mês passado?
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

83. Durante o mês passado, como você classificaria a qualidade do seu sono?
(0) Muito boa (1) Boa
(2) Ruim (3) Muito ruim

84. Durante o mês passado, você tomou algum remédio para dormir, receitado pelo médico, ou indicado por outra pessoa (farmacêutico, amigo, familiar) ou mesmo por sua conta?
(0) Nenhuma vez (1) Menos de uma vez por semana

(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais Qual(is)? _____ 85. Durante o mês passado, se você teve problemas para ficar acordado enquanto estava dirigindo, fazendo suas refeições ou participando de qualquer outra atividade social, quantas vezes isso aconteceu? (0) Nenhuma vez (1) Menos de uma vez por semana (2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais
86. Durante o mês passado, você sentiu indisposição ou falta de entusiasmo para realizar suas atividades diárias? (0) Nenhuma indisposição nem falta de entusiasmo (1) Indisposição e falta de entusiasmo pequenas (2) Indisposição e falta de entusiasmo moderadas (3) Muita indisposição e falta de entusiasmo
87. Para você, o sono é: □ Um prazer □ Uma necessidade □ Outro – Qual? _____
88. Você cochila? □ Não □ Sim
89. Caso Sim – Você cochila intencionalmente, ou seja, por que quer cochilar? □ Não □ Sim
90. Para você, cochilar é: □ Um prazer □ Uma necessidade □ Outro – Qual? _____

MEDIDAS DA COMPOSIÇÃO CORPORAL

91. Circunferência da cintura: _____ cm	92. Estatura: _____ metros
93. Circunferência cervical: _____ cm	94. Circunferência do quadril: _____ cm
95. Pulsação:_____bpm	95. PA: _____ mmHg

Obrigada pela sua participação,

Equipe de pesquisa

APÊNDICE 14 - INSTRUMENTO DE AVALIAÇÃO APÓS CADA FASE DO PROTOCOLO

INSTRUMENTO DE COLETA DE DADOS – TÉRMINO DE FASE

Universidade de São Paulo
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PESQUISA: "EFEITO DA MELATONINA NO SONO E NO METABOLISMO DE TRABALHADORAS NOTURNAS COM EXCESSO DE PESO"

Código da participante	

QUESTÕES SOBRE O TRABALHO

1. No seu horário de "descanso" durante o plantão noturno nesse hospital, você diria que na maior parte das vezes:
 Somente descansa (não consegue dormir)
 Dorme. Se sim, quantas vezes por plantão? _____ vezes
 Quanto tempo você dorme durante o plantão (tempo total)? ____h ____minutos
 Não dorme, nem descansa
 Não quer responder

2. Nos dias do plantão noturno nesse hospital, qual horário habitualmente você costuma jantar?
_____:_____ horário

3. Além do jantar, você costuma comer outras coisas durante o plantão (incluindo lanches, petiscos, bolos, chocolates, bolachas, sucos, iogurtes, refrigerantes, etc)?
 Não
 Sim. Em média, quantas vezes durante o plantão? _____vezes

4. Suponha que a sua melhor capacidade para o trabalho tem um valor igual a 10 pontos. Assinale com X um número na escala de zero a dez, quantos pontos você daria para sua capacidade de trabalho atual.

0	1	2	3	4	5	6	7	8	9	10

Estou incapaz para o trabalho

Estou na minha melhor capacidade para o trabalho

5. Como você classificaria sua capacidade atual para o trabalho em relação às **exigências físicas** do seu trabalho? (por exemplo, fazer esforço físico com partes do corpo)
 Muito boa
 Boa
 Moderada
 Baixa
 Muito baixa

6. Como você classificaria sua capacidade atual para o trabalho em relação às **exigências mentais** de seu trabalho? (por exemplo, interpretar fatos, resolver problemas, decidir a melhor forma de fazer)
 Muito boa
 Boa
 Moderada
 Baixa
 Muito baixa

7. Sua lesão ou doença é um impedimento para seu trabalho atual? (você pode marcar mais de uma resposta nesta pergunta)
☐ Não há impedimento / eu não tenho doenças
☐ Eu sou capaz de fazer meu trabalho, mas ele me causa alguns sintomas
☐ Algumas vezes preciso diminuir meu ritmo de trabalho ou mudar meus métodos de trabalho
☐ Frequentemente preciso diminuir meu ritmo de trabalho ou mudar meus métodos de trabalho
☐ Por causa de minha doença sinto-me capaz de trabalhar apenas em tempo parcial
 Na minha opinião estou totalmente incapacitado para trabalhar

8. Quantos dias inteiros você esteve fora do trabalho devido a problema de saúde, consulta médica ou para fazer exame durante os últimos três meses?
 Nenhum
 Até 9 dias
 De 10 a 24 dias
 De 25 a 99 dias
 De 100 a 365 dias

9. Considerando sua saúde, você acha que será capaz de daqui a 2 anos fazer seu trabalho atual?
☐ É improvável
☐ Não estou muito certo
☐ Bastante provável

10. Recentemente você tem conseguido apreciar suas atividades diárias?
☐ Sempre
☐ Quase sempre
☐ Ás vezes
☐ Raramente
☐ Nunca

11. Recentemente você tem-se sentido ativo e alerta?
☐ Sempre
☐ Quase sempre
☐ Às vezes
☐ Raramente
☐ Nunca

12. Recentemente você tem se sentido cheio de esperança para o futuro?
☐ Continuamente
☐ Quase sempre
☐ Às vezes
☐ Raramente
☐ Nunca

13. Na sua opinião, quais das lesões por acidentes ou doenças, citadas abaixo, você possui atualmente. Marque também aquelas que foram confirmadas pelo médico que você possui atualmente.

		Em minha opinião	Diagnóstico médico
01	Lesão nas costas		
02	Lesão nos braços / mãos		
03	Lesão nas pernas / pés		
04	Lesão em outras partes do corpo. Onde? Que tipo de lesão? _____		
05	Doença da parte superior das costas ou região do pescoço, com dores frequentes.		
06	Doença na parte inferior das costas com dores frequentes		
07	Dor nas costas que se irradia para perna (ciática)		
08	Doença musculoesquelética afetando os membros (braços e pernas) com dores frequentes		
09	Artrite reumatóide		
10	Outra doença musculoesquelética. Qual? _____		
11	Hipertensão arterial (pressão alta)		
12	Doença coronariana, dor no peito durante exercício (angina pectoris)		
13	Infarto do miocárdio, trombose coronariana		
14	Insuficiência cardíaca		
15	Outra doença cardiovascular. Qual? _____		
16	Infecções repetidas do trato respiratório (incluindo sinusite aguda, amigdalite, bronquite aguda)		
17	Bronquite crônica		
18	Sinusite crônica		
19	Asma		
20	Enfisema		
21	Tuberculose pulmonar		
22	Outra doença respiratória. Qual? _____		
23	Distúrbio emocional severo (exemplo, depressão severa)		
24	Distúrbio emocional leve (exemplo, depressão leve, tensão, ansiedade, insônia)		
25	Problema ou diminuição da audição		
26	Doença ou lesão da visão (não assinale se apenas usa óculos e/ou lentes de contato de grau)		
27	Doença neurológica (acidente vascular cerebral ou “derrame”, neuralgia, enxaqueca, epilepsia)		
28	Outra doença neurológica ou dos órgãos dos sentidos. Qual? _____		
29	Pedras ou doenças da vesícula biliar		
30	Doença do pâncreas ou do fígado		
31	Úlcera gástrica ou duodenal		
32	Gastrite ou irritação duodenal		
33	Colite ou irritação do cólon		
34	Outra doença digestiva. Qual? _____		
35	Infecção das vias urinárias		
36	Doença dos rins		
37	Doença dos genitais e aparelho reprodutor (exemplo, problema nas trompas ou ovários, ou na próstata)		
38	Outra doença geniturinária. Qual? _____		
39	Alergia, eczema		
40	Outra erupção. Qual? _____		
41	Outra doença na pele. Qual? _____		
42	Tumor benigno		
43	Tumor maligno (câncer). Onde? _____		
44	Obesidade		
45	Diabetes		
46	Bócio ou outra doença da tireóide		
47	Outra doença endócrina ou metabólica. Qual? _____		
48	Anemia		
49	Outra doença do sangue. Qual? _____		
50	Defeito de nascimento. Qual? _____		
51	Outro problema ou doença. Qual? _____		

QUESTÕES SOBRE SAÚDE E ESTILO DE VIDA

14. Você faz uso algum medicamento frequentemente?
 Sim. Qual(is)? _____
 Não

As perguntas estão relacionadas ao tempo que você gasta fazendo atividade física em uma semana NORMAL/HABITUAL.
Para responder as questões lembre que:

- **Atividades físicas VIGOROSAS** são aquelas que precisam de um grande esforço físico e que fazem respirar MUITO mais forte que o normal.
- **Atividades físicas MODERADAS** são aquelas que precisam de algum esforço físico e que fazem respirar UM POUCO mais forte que o normal.

15. Em quantos dias de uma semana normal, você realiza atividades VIGOROSAS **por pelo menos 10 minutos seguidos**, como, por exemplo, correr, fazer ginástica/musculação, jogar futebol, pedalar rápido na bicicleta, fazer serviços domésticos pesados em casa, no quintal ou no jardim, carregar pesos elevados ou qualquer atividade que faça você suar bastante ou aumente MUITO sua respiração ou batimentos do coração.
_____Dias por SEMANA
◎ Nenhum

16. Nos dias em que você faz essas atividades vigorosas **por pelo menos 10 minutos seguidos**, quanto tempo, no total, você gasta fazendo essas atividades **por dia?**
_____ minutos
◎ Não faço atividades vigorosas

17. Em quantos dias de uma semana normal, você realiza atividades MODERADAS **por pelo menos 10 minutos seguidos**, como, por exemplo, pedalar leve na bicicleta, nadar, dançar, fazer ginástica/musculação leve, jogar vôlei, carregar pesos leves, fazer serviços domésticos em casa, no quintal ou no jardim como varrer, aspirar, ou qualquer atividade que faça você suar leve ou aumente MODERADAMENTE sua respiração ou batimentos do coração **(por favor, não inclua caminhada)**
_____Dias por SEMANA
◎ Nenhum

18. Nos dias em que você faz essas atividades moderadas **por pelo menos 10 minutos seguidos**, quanto tempo, no total, você gasta fazendo essas atividades **por dia?**
_____ minutos
◎ Não faço atividades moderadas

19. Em quantos dias de uma semana normal, você caminha **por pelo menos 10 minutos seguidos** em casa ou no trabalho, como forma de transporte para ir de um lugar para outro, por lazer, por prazer ou como forma de exercício?
_____Dias por SEMANA
◎ Nenhum

20. Nos dias em que você caminha **por pelo menos 10 minutos seguidos**, quanto tempo, no total, você gasta caminhando **por dia**? _____ minutos ◎ Não faço caminhadas
21. Quanto tempo no total, você gasta sentado no dia que realiza **o plantão noturno, incluindo o tempo que está trabalhando à noite**? _____ horas _____minutos/dia
22. Quanto tempo no total, você gasta sentado durante o dia após **o seu plantão noturno**? _____ horas _____minutos/dia
23. Quanto tempo no total, você gasta sentado durante **o seu dia de folga**? _____ horas _____minutos/dia

QUESTÕES SOBRE O SONO

	Você vivenciou alguma das situações seguintes nos últimos 3 MESES?	Nunca	Raramente (Ocasionalmente)	Às vezes (Algumas vezes por mês)	Muitas vezes (1-2 vezes por semana)	Frequentemente (3-4 vezes por semana)	Sempre (5 ou mais vezes por semana)
24	Dificuldades para adormecer	0	1	2	3	4	5
25	Acordou diversas vezes e teve dificuldades para dormir	0	1	2	3	4	5
26	Acordou antes do necessário (despertar precoce)	0	1	2	3	4	5
27	Sono agitado / perturbado	0	1	2	3	4	5
28	Dificuldades para acordar	0	1	2	3	4	5
29	Sensação de estar exausto ao acordar	0	1	2	3	4	5
30	Sentiu-se cansado quando acordou	0	1	2	3	4	5

Para cada uma das questões seguintes escolha uma única resposta, que você ache mais correta. Por favor, responda a todas as questões.

DURANTE OS TRÊS ÚLTIMOS MESES, QUANTAS VEZES VOCÊ TEVE PROBLEMAS PARA DORMIR POR CAUSA DE:

31. Demorar mais de 30 minutos para pegar no sono:
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

32. Acordar no meio da noite ou de manhã muito cedo:
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

33. Levantar-se para ir ao banheiro:

(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

34. Ter dificuldade para respirar:
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

35. Tossir ou roncar muito alto:
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

36. Sentir muito frio
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

37. Sentir muito calor
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

38. Ter sonhos ruins ou pesadelos
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

39. Sentir dores
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

40. Outra razão. Por favor, descreva:
_____

41. Quantas vezes você teve problemas para dormir por esta razão, durante o mês passado?
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

42. Durante o mês passado, como você classificaria a qualidade do seu sono?
(0) Muito boa (1) Boa
(2) Ruim (3) Muito ruim

43. Durante o mês passado, você tomou algum remédio para dormir, receitado pelo médico, ou indicado por outra pessoa (farmacêutico, amigo, familiar) ou mesmo por sua conta?
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

44. Qual(is)?
_____

45. Durante o mês passado, se você teve problemas para ficar acordado enquanto estava dirigindo, fazendo suas refeições ou participando de qualquer outra atividade social, quantas vezes isso aconteceu?
(0) Nenhuma vez (1) Menos de uma vez por semana
(2) Uma ou duas vezes por semana (3) Três vezes por semana ou mais

46. Durante o mês passado, você sentiu indisposição ou falta de entusiasmo para realizar suas atividades diárias?
(0) Nenhuma indisposição nem falta de entusiasmo
(1) Indisposição e falta de entusiasmo pequenas
(2) Indisposição e falta de entusiasmo moderadas
(3) Muita indisposição e falta de entusiasmo

47. Após essa fase da pesquisa/intervenção, assinale como você se sentiu em relação aos seguintes aspectos:

Aspectos	**Melhorou**	**Piorou**	**Não modificou / Indiferente**
Qualidade do sono			
Hábitos alimentares			
TPM			
Ansiedade			
Humor			
Disposição física			
Disposição mental			

48. Durante esse primeiro período da intervenção, há algum aspecto ou observação que você queira comentar sobre o uso da melatonina/placebo?

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

MEDIDAS DA COMPOSIÇÃO CORPORAL

49. Massa corporal: _____ kg	50. Estatura: _____ metros
51. Circunferência da cintura: _____ cm	52. Circunferência do quadril: _____ cm
53. Circunferência cervical: _____ cm	54. Pressão arterial:
55. Pulsação: _____bpm	

Obrigada pela sua participação,

Equipe de pesquisa

APÊNDICE 15 - DIÁRIO DE CONTROLE DO USO DO SUPLEMENTO

Código da participante	**PESQUISA "EFEITO DA MELATONINA NO SONO E NO METABOLISMO DE TRABALHADORAS NOTURNAS COM EXCESSO DE PESO"**
Código do suplemento	Dúvidas – entrar em contato com: Elaine Marqueze (11) 98758-6384 e Pollyanna Pellegrino (13) 99736-9761

Data: _______/_______ **Dia da semana:** ( ) 2ª ( ) 3ª ( ) 4ª ( ) 5ª ( ) 6ª ( ) Sab ( ) Dom

( ) Dia de saída do plantão às 7 h ( ) Dia de folga

Horário que tomou suplemento: ______:______ **Horário que dormiu:** ______:______ **Horário que acordou:** ______:______

Como foi a qualidade do sono principal?
Muito ruim — Muito boa

Você dormiu o suficiente hoje?
Não — Sim

Data: _______/_______ **Dia da semana:** ( ) 2ª ( ) 3ª ( ) 4ª ( ) 5ª ( ) 6ª ( ) Sab ( ) Dom

( ) Dia de saída do plantão às 7 h ( ) Dia de folga

Horário que tomou suplemento: ______:______ **Horário que dormiu:** ______:______ **Horário que acordou:** ______:______

Como foi a qualidade do sono principal?
Muito ruim — Muito boa

Você dormiu o suficiente hoje?
Não — Sim

Data: _______/_______ **Dia da semana:** ( ) 2ª ( ) 3ª ( ) 4ª ( ) 5ª ( ) 6ª ( ) Sab ( ) Dom

( ) Dia de saída do plantão às 7 h ( ) Dia de folga

Horário que tomou suplemento: ______:______ **Horário que dormiu:** ______:______ **Horário que acordou:** ______:______

Como foi a qualidade do sono principal?
Muito ruim — Muito boa

Você dormiu o suficiente hoje?
Não — Sim

APÊNDICE 16 - DIÁRIO DE SONO E DE ATIVIDADES

PESQUISA "EFEITO DA MELATONINA NO SONO E NO METABOLISMO DE TRABALHADORAS NOTURNAS COM EXCESSO DE PESO"

Código da participante	**Dúvidas - entrar em contato com: Elaine Marqueze (11) 98758-6384**

No: _____ Data: _____/_____/_____ Dia da semana: _____ ( ) Dia de folga ( ) Dia de trabalho

Hora 0	0	1	2	3	4	5	6	7	8	9	10	11	12
Trabalhando nesse hospital													
Trabalhando em outra atividade remunerada													
Sono / Cochilo													
Outras atividades													

Hora 1	12	13	14	15	16	17	18	19	20	21	22	23	24
Trabalhando nesse hospital													
Trabalhando em outra atividade remunerada													
Sono / Cochilo													
Outras atividades													

Como foi a qualidade do sono principal?

Muito ruim Muito boa

Você dormiu o suficiente hoje?

Não Sim

ANEXOS

ANEXO 1 - Coautoria em artigo original publicado na *Nutrition*

EATING HABITS, SLEEP AND A PROXY FOR CIRCADIAN DISRUPTION ARE CORRELATED WITH DYSLIPIDEMIA IN OVERWEIGHT NIGHT WORKERS

Ananda Laís Felix Garrido[a], Adriana de Sousa Duarte[a], Patrícia Teixeira de Santana[b], Gabriella Habib Rodrigues[c], Pollyanna Pellegrino[d], Luciana Fidalgo Ramos Nogueira[d], José Cipolla-Neto[g], Claudia Roberta de Castro Moreno[e,f], Elaine Cristina Marqueze[d,e]

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[d]Department of Epidemiology, Public Health Graduate Program, Catholic University of Santos (SP), Brazil.
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(4413 words; 4 figures and 2 tables)

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ABSTRACT: Objective: To evaluate the relationship between proxy for circadian disruption, eating habits, sleep characteristics and dyslipidemic parameters. **Methods:** This is a randomized, double-blind, crossover controlled clinical trial, and for this study, only baseline data were used. The sample was composed of 36 overweight female nurses who worked on

a fixed night shift (12hx36h). Linear regression models were used to assess the relationship between the mentioned variables. **Results:** The participants' average age was 39.4 years (SE 1 year) and the average night sleep duration was 5.76 h (SE 0.16 h). The average chronotype indicated a moderate morning type (03:03 h; SE 20 min) and the average social jetlag was 03:42 h (SE 10 min). It was found that one hour less of night sleep increased VLDL-cholesterol levels by 2.75 mg/dl and triglyceride levels by 3.62 mg/dl. In addition, higher social jetlag was associated with higher LDL-cholesterol levels. On the other hand, each additional hour in the chronotype increased HDL-cholesterol levels by 3.06 mg/dl and a time interval longer than two hours between the last meal and sleep onset was associated with higher levels of HDL- cholesterol. **Conclusion:** Short duration of night sleep and high social jetlag are risk factors for dyslipidemia, while the evening type and the longer time interval between the last meal and the sleep onset seem to be protective factors for dyslipidemia.

Keywords: night work; dyslipidemia; sleep; nursing; nutrition; eating behavior.

INTRODUCTION

Studies have shown that night shift workers have a shorter sleep, presenting a chronic sleep debt [1-3]. Short duration and poor quality o f sleep can increase the risk of obesity and lead to metabolic disorders [4]. Moreover, an association between night shift work and hypertriglyceridemia, as well as an increased risk of developing dyslipidemia have been described [5]. Holanda et al. [6] found a higher prevalence of risk factors for the diagnosis of metabolic syndrome, including hypertriglyceridemia, in nurses who worked night shifts compared to day and former night workers. It is worth mentioning that dyslipidemia, which clinically presents as high levels of total cholesterol, triglycerides, low-density lipoprotein (LDL-cholesterol) and reduced levels of high-density lipoprotein (HDL- cholesterol) are among the main components of cardiovascular disorders [7].

The relationship between night work and dyslipidemia, however, is not yet fully understood [8]. The central mechanisms that explain the relationship between shift work and health problems are circadian disruption, melatonin suppression, sleep disorders and lifestyle changes, which affect the so-called peripheral clocks [9,10]. Regarding dyslipidemias, it seems obvious to assume that the content of the diet has a direct effect on its development [11,12]. In addition, time of food intake is a modifiable behavior that can influence metabolic

regulation [13-15]. In an experimental study a high-calorie diet at unfavorable circadian times (during the day) lead the studied mice to gain significantly more weight compared to those fed at night, however, both groups consumed an equivalent number of calories [16]. Persson and Martensson [17] found that night shift could compromise mealtimes and their frequency, as well as food preferences. In a recent study carried out by Kosmadopoulos et al. [14] with shift workers, it was found that the last meal was taken between 2-3 hours before sleep onset. According to a study by Kogevinas et al. [18], an interval of less than two hours between the last meal and sleep onset is considered harmful to metabolic health.

As previously mentioned, in addition to lifestyle changes, sleep disorders and circadian disruption are also mechanisms that affect the health of night workers. The conflict between the worker's chronobiological type (or chronotype) and his/her working hours can compromise both the quality and need of sleep, which can lead to a greater risk of illness [19]. According to Almoosawi et al. [20], the chronotype can modulate health-related physiological processes, such as b l o o d l i p i d concentration.

Social jetlag (SJL), which is characterized by the difference between the mid-phase of sleep on workdays and the mid-phase of sleep on days-off, has been used as a proxy for circadian disruption. Several studies show that a high SJL is an important risk factor for metabolic problems [21-24]. In this context, the aim of this study was to evaluate the relationship between a p r o xy f o r circadian disruption (social jet lag), eating habits (the time interval between the last meal before sleep onset, and food content) and sleep characteristics (chronotype and sleep duration) with dyslipidemic parameters.

METHODS STUDY SAMPLE AND DESIGN

This is a randomized, double-blind, crossover controlled clinical trial. For this study, conducted with 36 overweight nurses who worked in a large hospital in the city of São Paulo/SP, Brazil, only data extracted from the baseline were used. The nurses worked only on fixed night shifts, in the 12x36 hour system (12 hours on night shift, from 19:00 h to 7:00 h, followed by 36 hours off). The power of the sample was calculated a priori; having as a reference for the correlation test, a significance level of 5% (error $\alpha = 0.05$) and a sampling strength of 90% (error Beta). The estimated sample was 34 participants (G*Power software®).

The inclusion criteria were women between 20 and 50 years old, with body mass index (BMI) between ≥ 25 kg/m² and ≤ 40 kg/m², who have worked for at least six months on night shift, and were not under any calorie-restricted diets and have not started new physical activities while participating in the study. Exclusion criteria were: currently pregnant; with a child under one year old; who are in the climacteric or menopausal period; who have a second night job, who make regular use of drugs or food supplements that influence sleep; alertness and the circadian timing system; who have history of neurological or psychiatric illnesses; who are using illicit drugs or alcohol abuse; who have any circadian or sleep disorders; who have metabolic problems (except type 2 diabetes mellitus and treated dyslipidemia); cardiovascular diseases (except treated systemic arterial hypertension), inflammation and/or chronic infections diagnosed by a physician; who have eating disorders (bulimia, anorexia); who have anemia or who donate more than 400 ml of blood in the last three months prior to the study, and who have undergone major surgery in the previous six months of participation in the research.

DATA COLLECTION AND PROCESSING

Baseline data collection was carried out from April to November 2018. The self-administered questionnaire had questions about sociodemographic, work, health and lifestyle characteristics. The participants were assessed on anthropometric parameters, namely: body mass, height, waist and hip circumferences. Subsequently, body mass and height were used to calculate the participants' body mass index and categorized according to World Health Organization criteria [25].

Sleep duration was taken from the Munich ChronoType Questionnaire for shift workers (*MCTQ*$_{shift}$) [26]. This questionnaire contains questions about sleep onsets and sleep

duration, both for workdays and days-off. From the obtained information, the chronotype, social jetlag and sleep duration were calculated.

The time interval between the meal before sleep onset was obtained through a food diary, which was completed on a typical workday and on a typical day-off (from 19:00 h to 19:00 h). On a workday, the last meal was considered to be taken before daytime sleep, since the participants left work at 7:00 h and usually slept by 10:00 h. On the day-off, as sleep was nocturnal, the last evening meal was considered.

From the food diary, information was also obtained on the caloric intake of the last meal before sleep onset (percentages and the amount in grams of the total carbohydrate, protein and fat nutrients). These data were analyzed in the Nutrition Data System for Research [27]. The composition of typically Brazilian foods and preparations was manually added to the software database. For that, the Brazilian Food Composition Table [28] and specific industrialized food labels were used.

To categorize the appropriate percentage of each nutrient, the Recommended Daily Intake established by the U.S. National Academy of Sciences were used. According to the stipulated range for each nutrient, consumption was classified as adequate: total fat: 20-35%, total carbohydrate: 45-65% and total protein: 10-35%, with the percentages that were below or above the stipulated upper limit were classified as inadequate [29].

The dyslipidemic parameters (total cholesterol, low-density lipoprotein (LDL)-cholesterol, highdensity lipoprotein (HDL)-cholesterol, very low-density lipoprotein (VLDL)-cholesterol and triglycerides) were obtained by means of a single 12-hour fasting blood collection, after night sleep, and were categorized according to the criteria of the American Association of Clinical Endocrinologists (AACE) [30]. The categorizations were: Total cholesterol: <190 mg/dl (recommended) and ≥190 mg/dl (high), LDL: <100 mg/dl (recommended) and ≥100 mg/dl (high), HDL:> 40 mg/dl (recommended) and <40 mg/dl (decreased), Triglycerides: <150 mg/dl (recommended) and ≥150 mg/dl (high), VLDL-

cholesterol: values above ≥ 30 mg/dl (high) are negative to health. Although VLDL-cholesterol is not included in the A ACE criteria, this value has been used as a predictor of cardiovascular and metabolic diseases [31], which was adopted in this study.

STATISTICAL ANALYSIS

In order to compare the averages of the sleep duration, the Kruskal-Wallis test (K-W, posthoc Dwass-Steel-Critchlow-Fligner) was performed. To evaluate the relationship between the proxy for circadian disruption, eating habits and sleep characteristics according to each dyslipidemic parameter, linear regression models were performed, with the following adjustment variables: age, function, total time of night work (TTNW) and physical activity (PA). In order to assess the relationship between the food content of the last meal before sleep onset and its time interval with each dyslipidemic parameter, factorial ANOVA (post-hoc Bonferroni) was performed. In all tests, a significance level of 5% and up to 10% was adopted as a trend [32]. The analyses were performed using the software Stata 12.0 and Statistica 7.

ETHICAL ASPECTS

Ethical issues related to research involving human beings have been duly respected. The research project was approved by the L o c a l Ethics Committee of the USP School of Public Health (2.450.682), as well as by the Local Ethics Committee of the hospital were the research was conducted (2.489.636). The clinical trial was registered with the Brazilian Registry of Clinical Trials (ReBEC nº RBR-6pncm9).

RESULTS

The mean age of the participants was 39.4 years (SE 1 year), most of whom were married (61.1%), with children under 14 years old at home (58.3%), of which 52.8% were between 6 and 14 years old. Most of the participants were nurses (52.8%) and the average time of work in the hospital was 8.4 years (SE 0.8 years) and in the night shift 5.6 years (SE 0.7 years).

The mean BMI was 29.9 kg/m² (SE 0.6 kg/m²), in which 55.6% of the participants were classified as overweight and 44.4% as obese. The prevalence of hypercholesterolemia was 33.4%, of high LDL-cholesterol 50%, low HDL-cholesterol 8.3%, high VLDL-cholesterol 8.3% and hypertriglyceridemia 16.7%. Detailed description of sociodemographic characteristics and biochemical parameters of the participants at baseline can be found in supplementary tables 1 and 2.

The mean of sleep duration of the participants after the night shift (daytime sleep 1-A) was short which extends between work shifts (night sleep 1-B) and on days-off (night sleep 1C), however, the participants' mean total of sleep duration was also short (1-D) (KW p <0.001) (Figure 1).

FIGURE 1

The mean chronotype (03:03h, SE 20 min) indicated a moderate morning type, as well as they presented a high SJL (03:42h, SE 10 min). Figures 2-A and 2-B show the chronotype and SJL distributions.

FIGURE 2 A E B

The time interval between the meal closest to sleep onset was more than two hours, both post-shift (2 hours and 24 minutes, SE 16 minutes) and on the day-off (2 hours and 37 minutes, SE 17 min) (Figure 3-A and 3-B, respectively).

FIGURE 3

Table 1 shows the effects of social jetlag, chronotype and time interval between the meal closest to the sleep onset, as predictors of changes in dyslipidemic parameters. The chronotype was positively associated with HDL-cholesterol, that is, each additional hour in the chronotype increases plasma HDL-cholesterol levels by 3.06 mg/dl (adjusted by age, function, TTNW and PA).

Some association trends were also observed: - SJL was positively associated with LDLcholesterol, negatively with HDL-cholesterol and with triglycerides; the chronotype was positively associated with LDL-cholesterol; the time interval between the meal that preceded the sleep onset at rest was positively associated with HDL- cholesterol. The time interval

between the meal that preceded the onset of post-shift sleep was not related to any dyslipidemic parameter.

TABLE 1

Table 2 reports the effects of sleep duration, as an independent predictor, on dyslipidemic parameters. Sleep duration between shifts (night sleep) was negatively associated with VLDL-cholesterol and triglycerides, that is, one hour less in sleep duration increases VLDL-cholesterol and triglyceride plasma levels, by 2.75 and 3.62 mg/dl, respectively (adjusted by TTNW and PA). The total sleep duration was positively associated with LDLcholesterol, showing that every a d d i t i o n a l hour in the total sleep duration causes an increase of 2.02 mg/dl in LDL-cholesterol plasma levels (adjusted by function). The sleep duration after work (daytime sleep) and the sleep duration on day-off were not related to any dyslipidemic parameter.

TABLE 2

When evaluating the dyslipidemic parameters according to the food content of the last meal before the sleep onset and its time interval, both after work and on the day-off, no statistically significant differences were found between the participants who had an adequate consumption nor among those who had an inadequate consumption of the evaluated nutrients (fat, carbohydrate and protein).

DISCUSSION

This study showed that a decrease in the duration of night sleep tends to increase the VLDL-cholesterol and triglyceride plasma levels, on the other hand, a longer total sleep duration tends to increase LDL-cholesterol. Similar data were found in a study conducted by Kanagasabai and Chaput [33] with a population of adults older than 20 years, who found that a decrease in sleep duration was associated with a high risk of developing cardiometabolic problems. In a systematic review carried out by Knutson [34], it was also observed that short sleep (usually < 6 hours a night) was associated with cardiovascular disorders, in line with the data of this study. Backing up these data, Buxton and Marcelli [35] showed that short sleep duration was associated with an increased probability of diagnosis of cardiovascular diseases. However, a meta-analysis carried out by Cappuccio et al. [36] showed that a short

sleep duration was not associated with an increased risk of developing cardiovascular disorders. As the authors explained, this result may have been limited due to the inconsistency of the sleep duration assessment method, which was carried out using different questionnaires.

In this study it was found that the more evening the chronotype, the higher were the HDL-cholesterol plasma levels, and also a tendency for higher LDL- cholesterol. Studies on this association are still contradictory with regard to how evening type can affect cardiometabolic risk. A recent study by Hulsegge et al. [37] suggested that shift workers, with a more evening chronotype, had a higher risk of obesity. However, the authors say it is still not possible to explain the relationship between chronotype and obesity.

In a study conducted Loef et al. [38], no association between chronotype and dyslipidemic parameters was observed. In contrast, Ritonja et al. [39] found an association between the evening chronotype and the increase in LDL-cholesterol levels in day and night workers, although no mechanism has been reported to clarify the relationship between chronotype and LDL-cholesterol levels.

On the other hand, studies conducted with day workers suggest that a more afternoon chronotype are at higher risk for cardiometabolic diseases [40,41]. Wong et al. [42], on the other hand, found an inverse relationship with regard to chronotype and HDL- cholesterol, in which the evening chronotype was associated with low HDL- cholesterol levels, which is opposite to what was observed in our study. However, this contradiction can be explained due to the difference between the studied groups, since the participants were day workers and we have studied night workers. Therefore, it is concluded that the effects of the evening type on cardiometabolic changes are not yet fully understood, and further studies are needed, with different populations and working hours, in order to understand better this relationship.

According to Roenneberg et al. [19], the circadian timing system is in chronic conflict with restrictions of time imposed by society, thus explaining these associations. The studied workers, on average, had a moderately morning chronotype, which was a risk factor for the development of dyslipidemias, which corroborates some studies suggesting that night workers with earlier chronotypes may be in greater risk [26,10].

In this study, there was a tendency towards high social jetlag associated with higher LDLcholesterol levels and lower HDL-cholesterol levels. On the other hand, high social jetlag also tended to lower triglyceride levels.

As found by Wong et al. [42], in a study conducted with fixed daytime workers, a higher SJL was associated with lower HDL-cholesterol levels, a trend also observed in this study, and with an increase in triglyceride levels. In a study conducted by Mota et al. [43] with nonobese, healthy obese and unhealthy obese individuals, in addition to corroborating the fact that a higher SJL was associated with an increase in triglyceride levels, they also found an association with the increase of total cholesterol levels.

Studies addressing how social jetlag influences cardiometabolic health are still scarce, however our results suggest that there is a direct effect of circadian and social disruption on several metabolic parameters, and thus justify the need for prospective studies, with distinct populations and work schedules, to determine the long-term influence of social jetlag on each dyslipidemic parameter.

It is important to emphasize that aspects of lifestyle such as balanced diet and regular physical activity, which act as protectors for dyslipidemic problems, should be encouraged. According to Moreno et al. [44], night work, by itself, is already a considerable risk factor for metabolic problems, as it leads to sleep restriction, causing a decrease in leptin levels and increasing ghrelin and glucose levels upon waking. It also leads to hyperphagia, increased insulin resistance, hyperinsulinemia and increased adipose tissue, factors that may be related to dyslipidemia.

In addition, a longer time interval between the meal before sleep onset showed a tendency to be associated with high levels of HDL-cholesterol, which is beneficial for metabolic health. The time of the last meal before bedtime also seems to be relevant, and according to the study c o nd u c te d by Kogevinas et al. [18] with a population never exposed to night work, with irregular and inadequate eating habits, such as eating late at night, are associated with adverse health effects, a fact that can be aggravated in night workers.

This study found no differences when evaluating the dyslipidemic parameters between the participants who had an adequate consumption and those who had an inadequate consumption of nutrients. This result suggests that both the interval between the last meal

and the sleep onset and the food content were not the factors that directly influenced the dyslipidemic parameters. This finding contradicts the study conducted by Molzof et al. [45] which found an association between time of food consumption, the composition of the nutrients consumed and development factors for cardiometabolic problems. However, this contradiction may be due to the fact that sleep aspects are more important for changes in dyslipidemic parameters than eating habits in the studied group. In a systematic review c o n d u c t e d by Ruger and Scheer [46], it was observed that shift workers have an increased risk for the development of diabetes, obesity and cardiovascular diseases. Corroborating these data, a study conducted by Depner et al. [47] showed that several metabolic consequences, such as dyslipidemias, are influenced by circadian disruption.

Several studies indicate that sleep deprivation, high SJL, evening chronotype and eating and sleeping with an interval less than or equal to two hours, characteristics that are prevalent among night workers, can lead to several metabolic problems, especially dyslipidemia [48,49,2].

This study is of great importance to understand the relationship between aspects of sleeping and eating with dyslipidemic parameters, however it has some limitations, such as: this is a cross-sectional study, which prevents causal inferences. It is also possible that the sample size interferes with the interpretation of the results. In addition, it is important to consider that this study recorded only two days of food consumption. It is worth mentioning that the study was conducted only with fixed night workers, which is of great importance in the interpretation of the presented results. In addition, it should be mentioned that several metabolic mechanisms might mediate the correlation between night shift work and sleep duration with dyslipidemia as glucose intolerance, insulin resistance and hypomelatoninemia that should be addressed in future work.

CONCLUSION

In light of the data found, it is concluded that a reduced night sleep and an elevated social jetlag are risk factors for dyslipidemia, whereas a more evening chronotype and a long interval between the last meal before sleep onset may be protective factors.

AUTHOR CONTRIBUTIONS

- Ananda Laís Felix Garrido: Software, Formal Analysis, Original Draft; Data Curation.
- Adriana de Sousa Duarte: Original Draft, Data Curation.
- Patrícia Teixeira de Santana: Original Draft.
- Gabriella Habib Rodrigues: Original Draft.
- Pollyanna Pellegrino: Original Draft.
- Luciana Fidalgo Ramos Nogueira: Original Draft, Software.
- José Cipolla-Neto: Funding Acquisition, Review & Editing.
- Claudia Roberta de Castro Moreno: Methodology, Review & Editing, Visualization.
- Elaine Cristina Marqueze: Conceptualization, Project Administration, Supervision, Formal Analysis, Review & Editing, Visualization.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENTS

We thank the hospital staff, and the financial agencies: *Fundação de Amparo à Pesquisa do Estado de São Paulo*- FAPESP (nº 2014/50457-0, 2018/09704-4 and 2019/03624-1); *Conselho Nacional de Desenvolvimento Científico e Tecnológico*-CNPq (nº 150781/2017-2, 144800/2018-7 and 118087/2019-3); *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*-CAPES (nº 88887.356981/2019-00 and 88887.150178/2017-00). ALFG: Software, Formal Analysis, Original Draft; Data Curation; ASD: Original Draft, Data Curation; PTS: Original Draft; GHR: Original Draft; PP: Original Draft; LRFN: Original Draft, Software; JCN: Funding Acquisition, Review & Editing; CRCM: Methodology, Review & Editing, Visualization; ECM: Conceptualization, Project Administration, Supervision, Formal Analysis, Review & Editing, Visualization.

REFERENCES

[1] Wittmann M, Dinich J, Merrow M, Roenneberg T. Social jetlag: misalignment of biological and social time. Chronobiol Int. 2006;23(1-2):497-509. doi:10.1080/07420520500545979.

[2] Reiter R, Tan D, Korkmaz A, Ma S. 2012. Obesity and metabolic syndrome: association with chronodisruption, sleep deprivation, and melatonin suppression. Ann Med. 44(6):564–577. doi:10.3109/07853890.2011.586365.

[3] Moreno CRC, Fischer FM, Rotenberg L. A saúde do trabalhador na sociedade 24 horas. São Paulo Perspec. 2003;17(1):34-46.

[4] Knutson KL, Spiegel K, Penev P, Van Cauter E. The metabolic consequences of sleep deprivation. Sleep Med Rev. 2007;11(3):163-178. doi:10.1016/j.smrv.2007.01.002

[5] Ulhôa MA, Marqueze EC, Burgos LGA, Moreno CRC. Shift work and endocrine disorders. Int J Endocrinol. 2015:1–11. doi: 10.1155/2015/826249.

[6] Holanda NCP, de Castro Moreno CR, Marqueze EC. Metabolic syndrome components: Is there a difference according to exposure to night work?. *Chronobiol Int*. 2018;35(6):801-810. doi:10.1080/07420528.2018.1450267

[7] Vinik AI. The metabolic basis of atherogenic dyslipidemia. Clin Cornerstone. 2005;7(2-3):27- 35. doi:10.1016/s1098-3597(05)80065-1

[8] Tucker P, Marquié JC, Folkard S, Ansiau D, Esquirol Y. Shiftwork and metabolic dysfunction. Chronobiol Int. 2012;29(5):549-555. doi:10.3109/07420528.2012.675259

[9] Rajaratnam SM, Howard ME, Grunstein RR. Sleep loss and circadian disruption in shift work: health burden and management. Med J Aust. 2013;199(8):S11-S15. doi:10.5694/mja13.10561

[10] Reutrakul S, Knutson KL. Consequences of Circadian Disruption on Cardiometabolic Health. Sleep Med Clin. 2015;10(4):455-468. doi:10.1016/j.jsmc.2015.07.005

[11] Mohamed, S. Functional foods against metabolic syndrome (obesity, diabetes, hypertension and dyslipidemia) and cardiovasular disease. Trends Food Sci. Technol. 2014;(35): 114–128. doi: 10.1016/j.tifs.2013.11.001.

[12] Crispim CA, Zimberg IZ, dos Reis BG, Diniz RM, Tufik S, de Mello MT. Relationship between food intake and sleep pattern in healthy individuals. *J Clin Sleep Med*. 2011;7(6):659- 664. doi:10.5664/jcsm.1476

[13] Garaulet M, Gómez-Abellán P. Timing of food intake and obesity: a novel association. *Physiol Behav*. 2014;(134):44-50. doi:10.1016/j.physbeh.2014.01.001

[14] Kosmadopoulos A, Kervezee L, Boudreau P, et al. Effects of Shift Work on the Eating Behavior of Police Officers on Patrol. Nutrients. 2020;12(4):999. Published 2020 Apr 4. doi:10.3390/nu12040999

[15] Flanagan A, Lowson E, Arber S, Griffin BA, Skene DJ. Dietary Patterns of Nurses on Rotational Shifts Are Marked by Redistribution of Energy into the Nightshift. *Nutrients*. 2020;12(4):1053. Published 2020 Apr 10. doi:10.3390/nu12041053

[16] Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW. Circadian timing of food intake contributes to weight gain. Obesity (Silver Spring). 2009;17(11):2100-2102. doi:10.1038/oby.2009.264

[17] Persson M, Mårtensson J. Situations influencing habits in diet and exercise among nurses working night shift. J Nurs Manag. 2006;14(5):414-423. doi:10.1111/j.1365-2934.2006.00601.x.

[18] Kogevinas M, Espinosa A, Castelló A, et al. Effect of mistimed eating patterns on breast and prostate cancer risk (MCC-Spain Study). Int J Cancer. 2018;143(10):2380-2389. doi:10.1002/ijc.31649

[19] Roenneberg T, Pilz LK, Zerbini G, Winnebeck EC. Chronotype and Social Jetlag: A (Self) Critical Review. Biology (Basel). 2019;8(3):54. doi:10.3390/biology8030054

[20] Almoosawi S, Vingeliene S, Gachon F, et al. Chronotype: Implications for Epidemiologic Studies on Chrono-Nutrition and Cardiometabolic Health. Adv Nutr. 2019;10(1):30-42. doi:10.1093/advances/nmy070

[21] Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms. 2003;18(1):80-90. doi:10.1177/0748730402239679

[22] Hidalgo MP, Caumo W, Posser M, Coccaro SB, Camozzato AL, Chaves ML. Relationship between depressive mood and chronotype in healthy subjects. *Psychiatry Clin Neurosci*. 2009;63(3):283-290. doi:10.1111/j.1440-1819.2009.01965.x

[23] Levandovski R, Dantas G, Fernandes LC, et al. Depression scores associate with chronotype and social jetlag in a rural population. Chronobiol Int. 2011;28(9):771-778. doi:10.3109/07420528.2011.602445.

[24] Roenneberg T, Allebrandt KV, Merrow M, Vetter C. Social jetlag and obesity [published correction appears in Curr Biol. 2013 Apr 22;23(8):737]. Curr Biol. 2012;22(10):939-943. doi:10.1016/j.cub.2012.03.038

[25] World Health Organization (WHO). BMI classifications, 2006.

[26] Juda M, Vetter C, Roenneberg T. The Munich ChronoType Questionnaire for Shift-Workers ($MCTQ_{Shift}$). *J Biol Rhythms*. 2013;28(2):130-140. doi:10.1177/0748730412475041.

[27] Nutrition Data System for Research Software [cd-rom]. Nutrition Coordinating Center/Division of Epidemiology/NDS. Minessota: 2007.

[28] Universidade Estadual de Campinas: The Brazilian Food Composition Table (TACO), http://www.nepa.unicamp.br/taco/tabela.php?ativo=tabela; 4th ed., 2011 [accessed 15 jun 2020].

[29] Institute of Medicine. Dietary Reference Intakes. Estimated Average Requirements for Groups. Washington (DC): National Academy Press, 2006.

[30] American Association of Clinical Endocrinologists and American College of Endocrinology (2017): guidelines for management of dyslipidemia and prevention of cardiovascular disease. Endocr Pract. 23(Supplement 2), 1–87. doi: 10.4158/EP171764.APPGL.

[31] Grundy SM. Richard Havel, Howard Eder, and the evolution of lipoprotein analysis [published correction appears in J Clin Invest. 2004 Dec;114(11):1687]. *J Clin Invest*. 2004;114(8):1034-1037. doi:10.1172/JCI23198

[32] Thiese M, Ronna B, Ott U. P value interpretations and considerations. Journal of Thoracic Disease. 2016;8:E928-E931. doi:10.21037/jtd.2016.08.16

[33] Kanagasabai T, Chaput JP. Sleep duration and the associated cardiometabolic risk scores in adults. Sleep Health. 2017;3(3):195-203. doi:10.1016/j.sleh.2017.03.006

[34] Knutson KL. Sleep duration and cardiometabolic risk: a review of the epidemiologic evidence. Best Pract Res Clin Endocrinol Metab. 2010;24(5):731-743. doi:10.1016/j.beem.2010.07.001

[35] Buxton OM, Marcelli E. Short and long sleep are positively associated with obesity, diabetes, hypertension, and cardiovascular disease among adults in the United States. *Soc Sci Med*. 2010;71(5):1027-1036. doi:10.1016/j.socscimed.2010.05.041

[36] Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *Eur Heart J*. 2011;32(12):1484-1492. doi:10.1093/eurheartj/ehr007

[37] Hulsegge G, Loef B, van Kerkhof LW, Roenneberg T, van der Beek AJ, Proper KI. Shift work, sleep disturbances and social jetlag in healthcare workers. J Sleep Res. 2019;28(4):e12802. doi:10.1111/jsr.12802

[38] Loef B, Baarle DV, van der Beek AJ, Beekhof PK, van Kerkhof LW, Proper KI. The association between exposure to different aspects of shift work and metabolic risk factors in health care workers, and the role of chronotype. *PLoS One*. 2019;14(2):e0211557. doi:10.1371/journal.pone.0211557

[39] Ritonja J, Tranmer J, Aronson KJ. The relationship between night work, chronotype, and cardiometabolic risk factors in female hospital employees. Chronobiol Int. 2019;36(5):616-628. doi:10.1080/07420528.2019.1570247

[40] Knutson KL, von Schantz M. Associations between chronotype, morbidity and mortality in the UK Biobank cohort. Chronobiol Int. 2018;35(8):1045-1053. doi:10.1080/07420528.2018.1454458

[41] Merikanto I, Lahti T, Puolijoki H, et al. Associations of chronotype and sleep with cardiovascular diseases and type 2 diabetes. Chronobiol Int. 2013;30(4):470-477. doi:10.3109/07420528.2012.741171

[42] Wong PM, Hasler BP, Kamarck TW, Muldoon MF, Manuck SB. Social Jetlag, Chronotype, and Cardiometabolic Risk. J Clin Endocrinol Metab. 2015;100(12):4612-4620. doi:10.1210/jc.2015-2923

[43] Mota MC, Silva CM, Balieiro LCT, Fahmy WM, Crispim CA. Social jetlag and metabolic control in non-communicable chronic diseases: a study addressing different obesity statuses. Sci Rep. 2017;7(1):6358. doi:10.1038/s41598-017-06723-w

[44] Moreno CRC, Marqueze EC, Sargent C, Wright Jr KP Jr, Ferguson SA, Tucker P. Working Time Society consensus statements: Evidence-based effects of shift work on physical and mental health. Ind Health. 2019;57(2):139-157. doi:10.2486/indhealth.SW-1

[45] Molzof HE, Wirth MD, Burch JB, et al. The impact of meal timing on cardiometabolic syndrome indicators in shift workers. Chronobiol Int. 2017;34(3):337-348. doi:10.1080/07420528.2016.1259242

[46] Rüger M, Scheer FA. Effects of circadian disruption on the cardiometabolic system. Rev Endocr Metab Disord. 2009;10(4):245-260. doi:10.1007/s11154-009-9122-8

[47] Depner CM, Stothard ER, Wright KP Jr. Metabolic consequences of sleep and circadian disorders. *Curr Diab Rep*. 2014;14(7):507. doi:10.1007/s11892-014-0507-z

[48] Alonso-Vale MI, Andreotti S, Mukai PY, et al. Melatonin and the circadian entrainment of metabolic and hormonal activities in primary isolated adipocytes. J Pineal Res. 2008;45(4):422- 429. doi:10.1111/j.1600-079X.2008.00610.x

[49] Van Cauter E, Knutson KL. Sleep and the epidemic of obesity in children and adults. Eur J Endocrinol. 2008;159 Suppl 1(1):59-66. doi:10.1530/EJE-08-0298

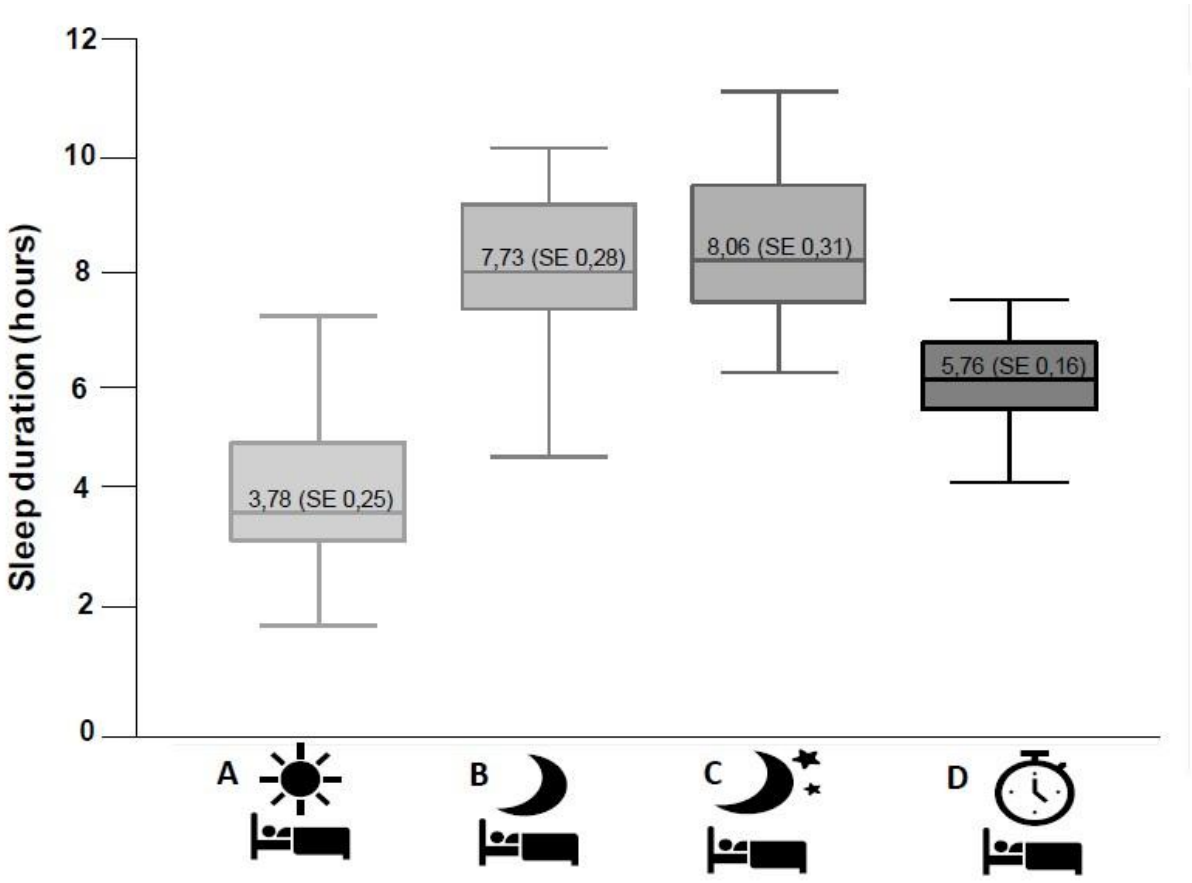

Figure 1- Sleep duration of overweight night shift nurses:1-A Daytime sleep,1B Nightsleep,1-C Nightsleep,1-D Mean total sleep (n=36). Post-hoc *Dwass-Steel-Critchlow-Fligne* r$p<0.001$: A<B,C,D;B and C>D.

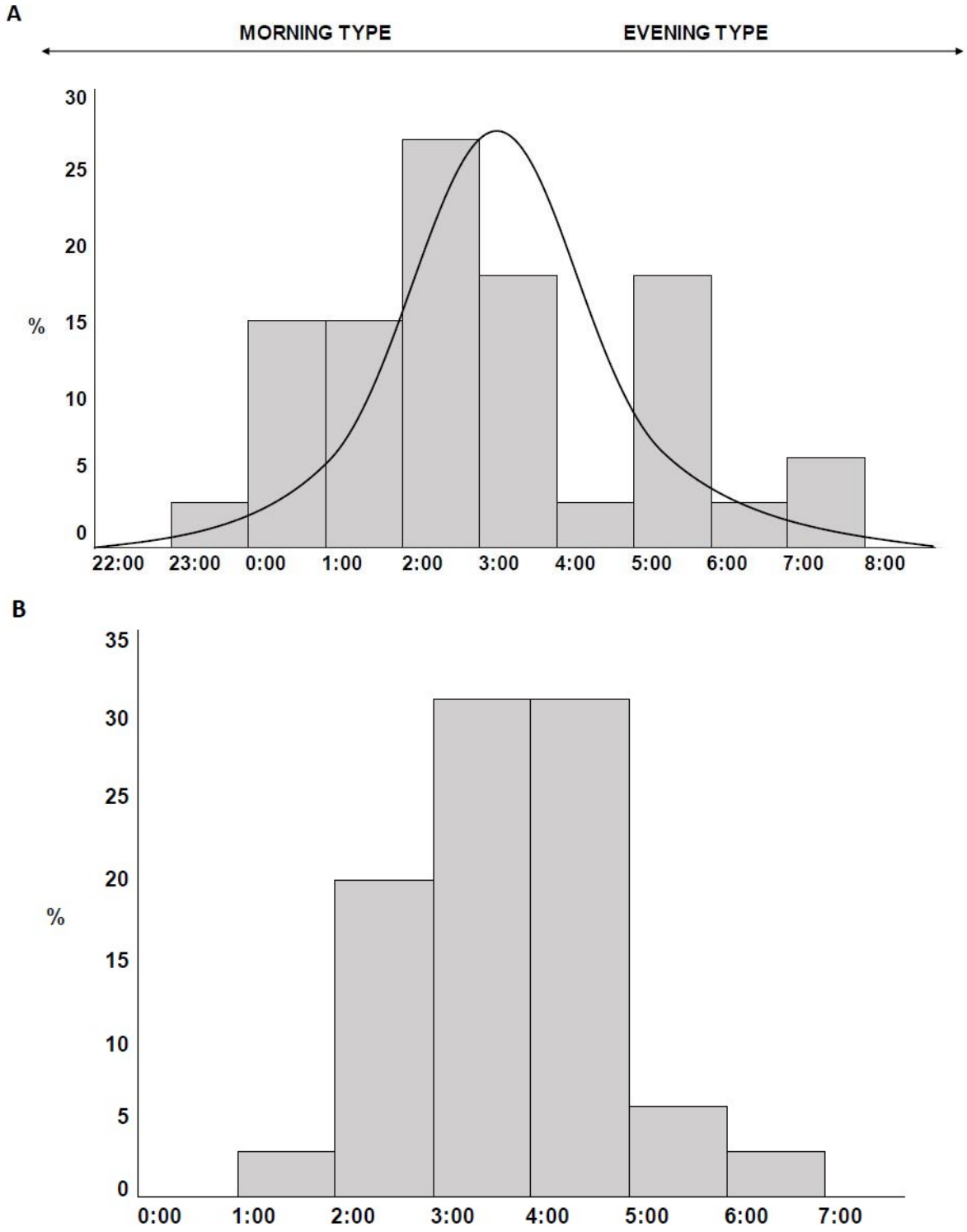

Figure 2- Distribution of chronotype (A) and social jetlag (B) among overweight night shift nurses (n=36).

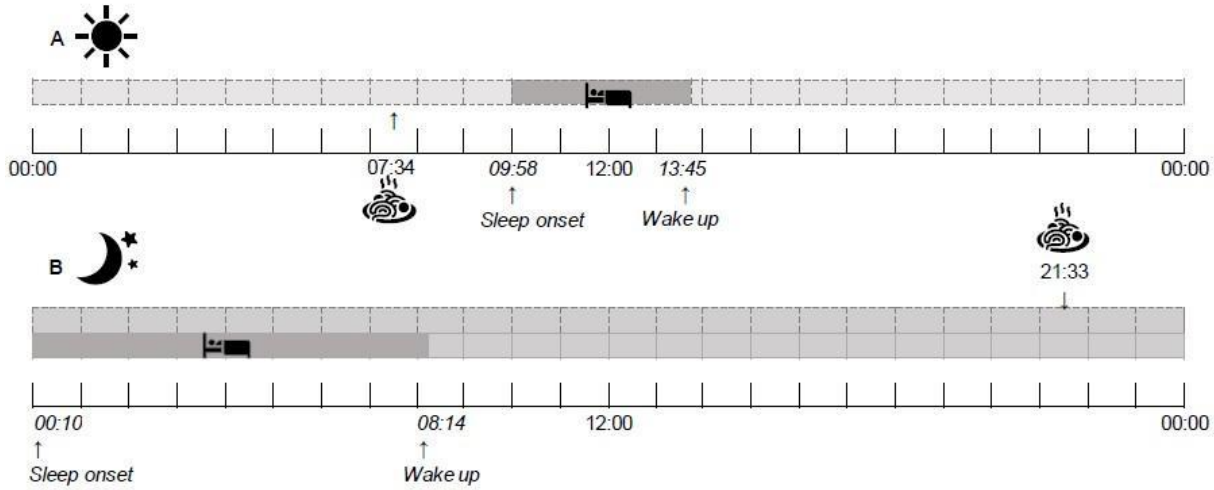

Figure 3- Time interval (hours) between the meal closest to sleep onset after night work(A) and time interval (hours) between the meal closest to sleep onset on day off (B) of overweight night shift nurses (n=36).

Table 1- Linear regressions between social jetlag, chronotype and time interval between the meal that preceded the sleep o nse t by overweight night shift nurses, depending on each dyslipidemic parameter (n=36).

Predictors	Adjustment variables	Dyslipidemic variables									
		Total cholesterol		LDL		HDL		VLDL		Triglycerides	
		β	p	β	p	β	p	β	p	β	p
Social jet lag	- Model 1	1.20	0.24	1.82	0.08**	-1.91	0.07**	0.71	0.48	0.26	0.80
	- Model 2	1.31	0.20	1.89	0.07**	-1.27	0.21	0.42	0.68	-0.00	1.00
	- Model 3	0.16	0.88	0.35	0.74	0.19	0.85	-1.69	0.39	-2.03	0.09**
	- Model 4	1.14	0.27	1.79	0.08**	-1.86	0.07**	0.70	0.49	0.27	0.79
Chronotype	- Model 1	0.13	0.37	1.74	0.09**	-0.34	0.73	0.15	0.88	-0.31	0.76
	- Model 3	-0.20	0.85	-0.33	0.75	1.28	0.24	-1.39	0.20	-1.50	0.17
	- Model 4	1.39	0.17	1.54	0.13	-0.30	0.77	0.17	0.87	-0.26	0.79
	- Model 5	-0.39	0.71	-0.72	0.50	3.06	**0.03***	-0.90	0.41	-0.73	0.50
Time interval between the meal before the post-shift sleep onset	Model 1	-0.96	0.35	-0.31	0.76	-1.09	0.29	-1.60	0.12	-1.57	0.13
	Model 3	-0.21	0.84	-0.16	0.88	-0.35	0.74	0.24	0.82	0.21	0.84
	- Model 4	-1.37	0.18	-0.71	0.48	-1.00	0.33	-1.64	0.11	-1.56	0.13
	- Model 5	0.45	0.68	0.43	0.69	-0.43	0.70	0.95	0.41	0.18	0.87
Time interval between the meal before the day-off sleep onset	Model 1	1.19	0.25	1.16	0.26	-0.28	0.79	0.83	0.42	0.55	0.59
	Model 3	-0.44	0.67	-0.42	0.68	0.24	0.82	-0.44	0.67	-0.51	0.63
	- Model 4	0.84	0.41	0.75	0.46	-0.16	0.87	0.91	0.37	0.68	0.50
	- Model 5	-0.99	0.38	-1.21	0.29	2.25	0.09**	-1.22	0.29	-1.00	0.37

Model 1 adjusted by Age.
Model 2 adjusted by Function.
Model 3 adjusted by Physical Activity (PA).
Model 4 adjusted by Age and Function.
Model 5 adjusted by Age, Position, Total time of night work (TTNW) and PA.
*p<0.05; **p<0.10

Table 2- Linear regressions between post-shift sleep duration, sleep duration between shifts, day-off sleep duration and total sleep duration of overweight night shift nurses, depending on each dyslipidemic parameter (n=36).

Predictors	Adjustment variables	Dyslipidemic variables: Total β	Total p	LDL β	LDL p	HDL β	HDL	HDL	HDL p			VLDL β	VLDL p	Triglycerides β	Triglycerides p
Post-shift sleep duration (daytime sleep)	-Model 1	0,83	0,41	0,57	0,57	1,21			0,24			-0,64			0,42
	-Model 2	0,30	0,77	0,06	0,95	0,85			0,40				0,53		0,62
	-Model 3	0,88	0,38	0,70	0,49	0,84			0,41			-0,39	0,70	β	0,50
												-0,48	0,64	-0,81	
														-0,51	
														-0,69	
	-Model 4	1,08	0,33	0,87								0,56	0,60	0,31	0,77
Sleep duration between shifts (night sleep)	-Model 1	0,46	0,65	0,42								1,06	0,30	0,78	0,44
	-Model 2	1,51	0,14	1,51								0,87	0,39	0,45	0,65
	-Model 3	0,43	0,67	0,33		~~-0,37~~ -0,40	~~0,72~~ 0,69					0,99	0,33	0,76	0,46
					0,14	-0,26			0,80						
					0,74	-0,22			0,83						
	-Model 4	~~-0,53~~ -0,12	~~0,61~~ 0,91												**0,01***
Day-off sleep duration (night sleep)	-Model 1														
	-Model 2	0,35	0,73	0,40											0,38
	-Model 3	-0,12	0,91	-0,22					~~0,65~~ 0,81	~~-2,75~~ 1,07	~~**0,03***~~ 0,29	~~-3,62~~ 0,90			0,26
					0,69	-0,85			0,40				1,44	0,16	1,15	0,35
					0,83	-0,35			0,73				1,12	0,27	0,94	
	-Model 4	-0,55	0,60			~~-0,16~~ -0,68	~~0,88~~ 0,50	~~-1,50~~ 1,27						0,18	-1,72	0,13
Total sleep duration	-Model 1	1,19	0,24											0,21	0,77	0,45
	-Model 2	1,93	0,06**	2,02	**0,05***	-0,50			0,62				1,05	0,30	0,49	0,63
	-Model 3	1,11	0,21	1,17	0,22	-0,21			0,76				-0,04	0,32	-0,39	0,59
	-Model 4	0,80	0,45	0,99	0,35	-1,21			0,27				0,55	0,60	0,21	0,84

Model 1 adjusted by Age.
Model 2 adjusted by Function.
Model 3 adjusted by Total time of night work (TTNW).
Model 4 adjusted by TTNW and Physical Activity (PA).
*p<0,05; **p<0,10

ANEXO 2 - Coautoria em artigo original publicado na *Journal of Pineal Research*

Exogenous melatonin decreases circadian misalignment and body weight among early types

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Funding information
National Council for Scientific and Technological Development, CNPq; The São Paulo Research Foundation, FAPESP

Abstract

Shift workers experience chronic circadian misalignment, which can manifest itself in reduced melatonin production, and has been associated with metabolic disorders. In addition, chronotype modulates the effect of night shift work, with early types presenting greater circadian misalignment when working night shift as compared to late types. Melatonin supplementation has shown positive results reducing weight gain in animal models, but the effect of exogenous melatonin in humans on body weight in the context of shift work remains inconsistent. The aim of this study was thus to evaluate the effects of exogenous melatonin on circadian misalignment and body weight among overweight night shift workers, according to chronotype, under real- life conditions. We conducted a double-b lind, randomized, placebo- controlled, crossover trial where melatonin (3 mg) or placebo was administered on non- night shift nights for 12 weeks in 27 female nurses (37.1 yo, ±5.9 yo; BMI 29.9 kg/m^2, ±3.3 kg/m^2). Melatonin (or placebo) was only taken on nights when the participants did not work night shifts, that is, on nights when they slept (between night shifts and on days off). Composite Phase Deviations (CPD) of actigraphy- based mid- sleep timing were calculated to measure circadian misalignment. The analyses were performed for the whole group and by chronotype. We found approximately 20% reduction in circadian misalignment after exogenous melatonin administration considering all chronotypes. Moreover, melatonin supplementation in those who presented high circadian misalignment, as observed in early chronotypes, reduced body weight, BMI, waist circumference, and hip circumference, without any change in the participants' calorie intake or physical activity levels.

KEYWORDS

circadian rhythm disorders, dietary supplements, melatonin, night shift work, nursing staff, overweight, working women

1 | INTRODUCTION

The integrity of the circadian timing system is one of the major determinants of health and body weight homeostasis; sleeping well and enough at night is essential for metabolic health.[1] Circadian misalignment and reduced production of melatonin due to light exposure during night shifts[2] chronically affect shift workers, contributing to the development of diseases, including obesity.[3-5] Moreover, chronotype modulates the effect of shift work, with early types presenting greater circadian misalignment when working night shifts compared to late types.[6,7]

Melatonin, a hormone produced and secreted primarily by the pineal gland in humans, is an endogenous synchronizer of circadian rhythms. It plays an important role in the regulation of energy metabolism (body weight control) and energy balance, including energy intake, energy flow to and from storage and energy expenditure.[8-12]

It is known that light exposure at night results in suppression of melatonin synthesis and its amplitude,[13-16] which is thought to modify several physiological and behavioral processes that contribute to the development or aggravation of pre- existing diseases.[17,18] Animal models have shown that melatonin supplementation reduces visceral obesity, decreases and/or limits body weight gain and reduces dietary intake,[19-21] and this is likely due to the action of melatonin on hypothalamic food intake circuits, intensifying the anorexigenic signals and decreasing the orexigenic signals.[19-22] However, it is important to mention that extrapolating findings from animal models, which are mostly performed with rats (nocturnal species) to humans (diurnal species), may not be directly translatable, since exogenous melatonin supplementation may act differently on body weight in distinct photoperiodic species.[23,24]

Melatonin supplementation in humans has been used since the mid- 1980s in order to treat circadian rhythm sleep disorders by shifting the timing of the circadian.[25-27] Both the reviews by Skene et al[25] and Arendt et al[26] presented results of melatonin studies that were successful in phase shifting and entraining the circadian system including the sleep- wake cycle, as well as improving symptoms of sleep disorders. In a meta-analysis that evaluated the effects of exogenous melatonin in treating primary sleep disorders, Auld et al[27] found robust evidence that melatonin administration reduced sleep latency, improved delayed sleep phase syndrome and regulated the sleep-w ake cycle in blind people.

Taken together, exogenous melatonin can be considered a chronobiotic capable of phase shifting the circadian timing in humans according to a phase response curve.[14,21,28,29] Although Cipolla- Neto and Amaral[21] claim that research on the clinical use of exogenous melatonin in energy metabolism has a promising future, the results of studies with humans remain inconsistent, with some studies reporting decreased weight gain, while

other studies reported weak or no effects of melatonin on body weight. Mostafavi et al[30] conducted a meta- analysis of controlled trials on the effects of melatonin administration on the body weight of human participants, including seven clinical trials, with only three of them showing a decrease in body weight. However, the authors discussed how the quality of the studies may influence the results. Although there are studies with simulated shift work,[31] only two studies have evaluated the effects of exogenous melatonin on night workers in real-l ife conditions.[14,32] These studies assessed the effect of melatonin on sleep, alertness and performance, but did not assess melatonin's effect on the shift workers' body weight. Therefore, the present study aimed to evaluate the effects of exogenous melatonin on circadian misalignment and body weight among overweight night shift workers, according to chronotype, under real-l ife conditions. Our hypothesis was that melatonin supplementation would reduce circadian misalignment by its chronobiotic effect and, consequently, contribute to the reduction of body weight, regardless to a direct effect of food content (kcal) and physical activity.

2 | MATERIALS AND METHODS

2.1 | ETHICS

The experimental protocol was approved by the Research Ethics Committee of the School of Public Health, University of São Paulo, Brazil (process 2.450.682) and by the Ethics Committee of the Hospital (process 2.489.636), and all research participants gave their written informed consent to participate in the trial. The protocol of the clinical trial is registered at the World Health Organization's International Clinical Trials Registry Platform (UTN U1111- 1238- 7395) and the Brazilian Registry of Clinical Trials (ReBEC– RBR-6pncm9). Our study was developed in accordance with
CONSORT 2010.[33]

2.2 | OVERVIEW OF STUDY DESIGN

A 24- week double- blind, randomized, placebo- controlled crossover trial was implemented under real- life conditions, in a large private hospital in São Paulo/Brazil. The study population comprised overweight nurses and nursing technicians (all women) who work permanent night shifts from 19:00 to 07:00 h (12 h work, 36 h no work), with a day off every 15 days (see Figure 2).

For the sample size calculation, we estimated that by week 12, the circadian misalignment would be reduced by 25% after melatonin supplementation and 0% after the placebo, with 80% power to detect a significant difference (n = 27) (G*Power software®). The study participants were recruited from March 2018 to June 2019. A total of 46 subjects were eligible and agreed to participate in the protocol. Of these, 19 (41.3%) stopped participating after initiating the protocol because they had started a second night job,

got pregnant, changed the shift schedule, or left the job.

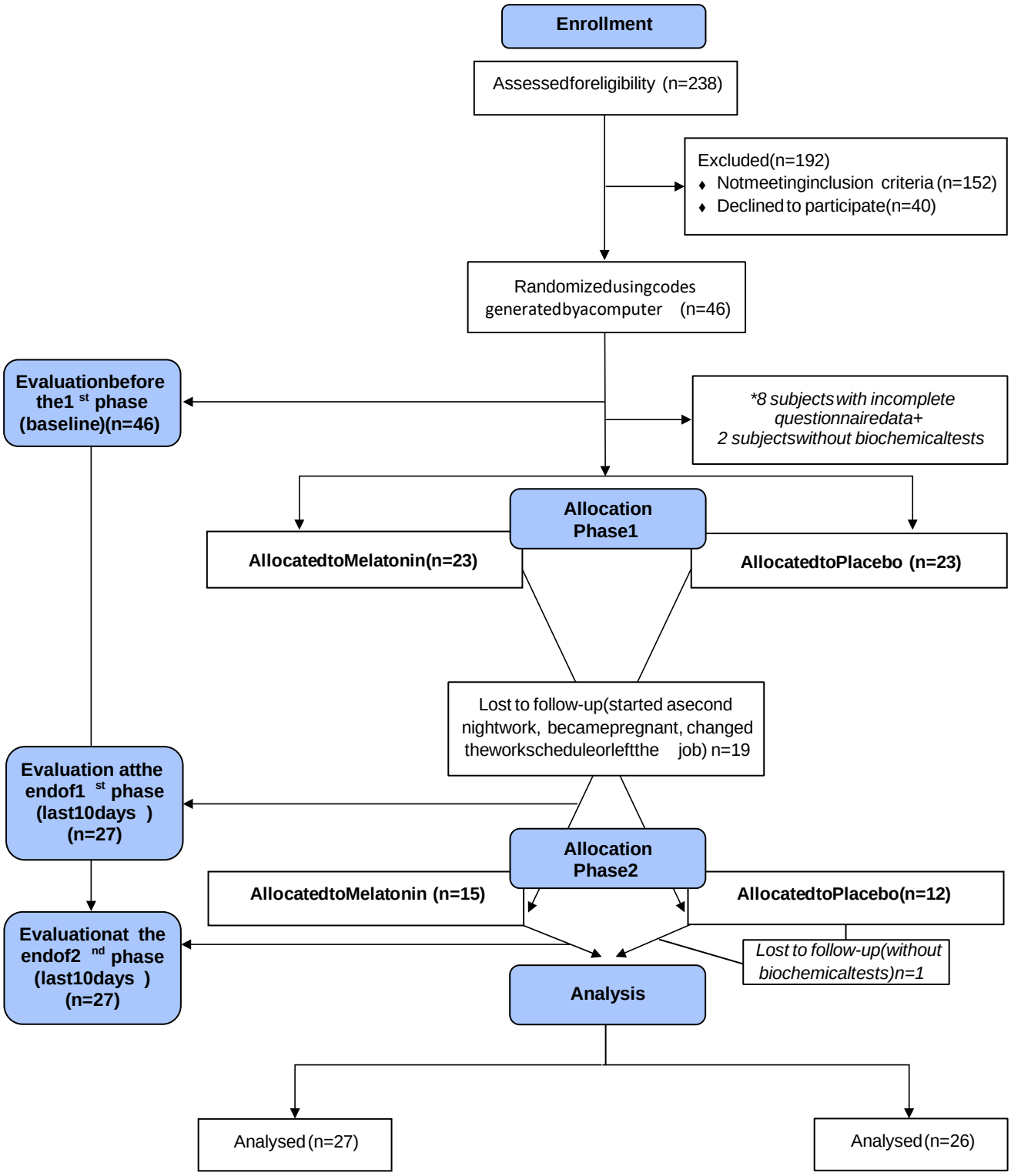


FIGURE 1 Study flow chart

Thus, the final sample was composed of 27 participants (Figure 1). Each participant took part in the protocol for 25 weeks (12 weeks for melatonin and 12 weeks for placebo plus one week for baseline). The participants did not all

start the protocol at the same time, and the study was conducted for 18 months.

Inclusion criteria were as follows: women; aged between 20 and 50 years old; working night shifts for at least six months; BMI between 25 and 40 kg/m^2; and did not have plans to change eating or physical activity habits while participating in the project. Exclusion criteria were as follows: pregnancy; breastfeeding; children under 1 year old; premenopause or postmenopause (absence of menses for more than 6 months); having a second nocturnal job; regular use of medications or dietary supplements that influence sleep, alertness, and the circadian timing system; past history of neurological or psychiatric disorders, drug and alcohol abuse, diagnosed circadian or sleep disorder, metabolic disorders, cardiovascular disease, inflammation and/or physician- diagnosed chronic infections, and eating disorders; current or past episode of anemia; and having undergone major surgery in the last 6 months prior the study.

2.3 | PROTOCOL

The participants were randomly assigned into two groups using codes generated by a computer: One group received melatonin first and then placebo, and the other group

melatonin and placebo tablets were identical in appearance (Aché Pharmaceutics, Brazil). They took 3 mg of oral melatonin, fast-r elease formulation, or a placebo, according to each study phase. Melatonin was only ingested on the nights when the participants slept at night, that is, on nights between working night shifts and on days off. The instructions were to take the tablet one hour before preferred sleep onset.[21] It was not administered prior to daytime sleep or during the night shifts.

The average number of days of melatonin supplementation was 45 days (±10.3 days), and the use of placebo was 44.3 days (±8.2 days) across the 24 weeks of intervention (12 weeks for melatonin and 12 weeks for placebo). It is noteworthy that the number of days was not the same in both study phases due to the difference in the number of days off among the participants. In the transition period between the two study phases: melatonin/placebo and placebo/melatonin, a washout was not performed. In the last 10- 15 days of the first and second study phases, anthropometric assessments were carried out and the questionnaires for monitoring the protocol were completed.

2.4 | *OUTCOME MEASURES*

2.4.1 | Primary outcome — Circadian misalignment

ıate the circadian misalignment, we used Composite Phase Deviations (CPD)

proposed by Fischer et al,[34] which quantifies| Physical activity and food intake

For physical activity assessment, the participants completed the International Physical Activity Questionnaire (IPAQ- short version).37 The short version of the IPAQ has four questions related to vigorous and moderate physical activities performed in a typical week. To categorize the level of physical activity, we used the recommendations from the Centers for Disease Control and Prevention and mistiming of sleep relative to the usual sleep timing, and relative to sleep timing on the previous day. CPD of actigraphy- based mid- sleep were calculated to measure circadian misalignment. Actigraphs were worn during 10 consecutive days (Condor Instruments ActTrust and Basic Motionlogger Actigraph®). From the sleep onset and sleep offset data, the mid- sleep was calculated. To validate the data recorded by the actigraphy, the participants filled concurrently in sleep/activity diaries. The MSF^{N}_{sc} was obtained from Munich Chronotype Questionnaire for shift work ($MCTQ_{shift}$)[35]:

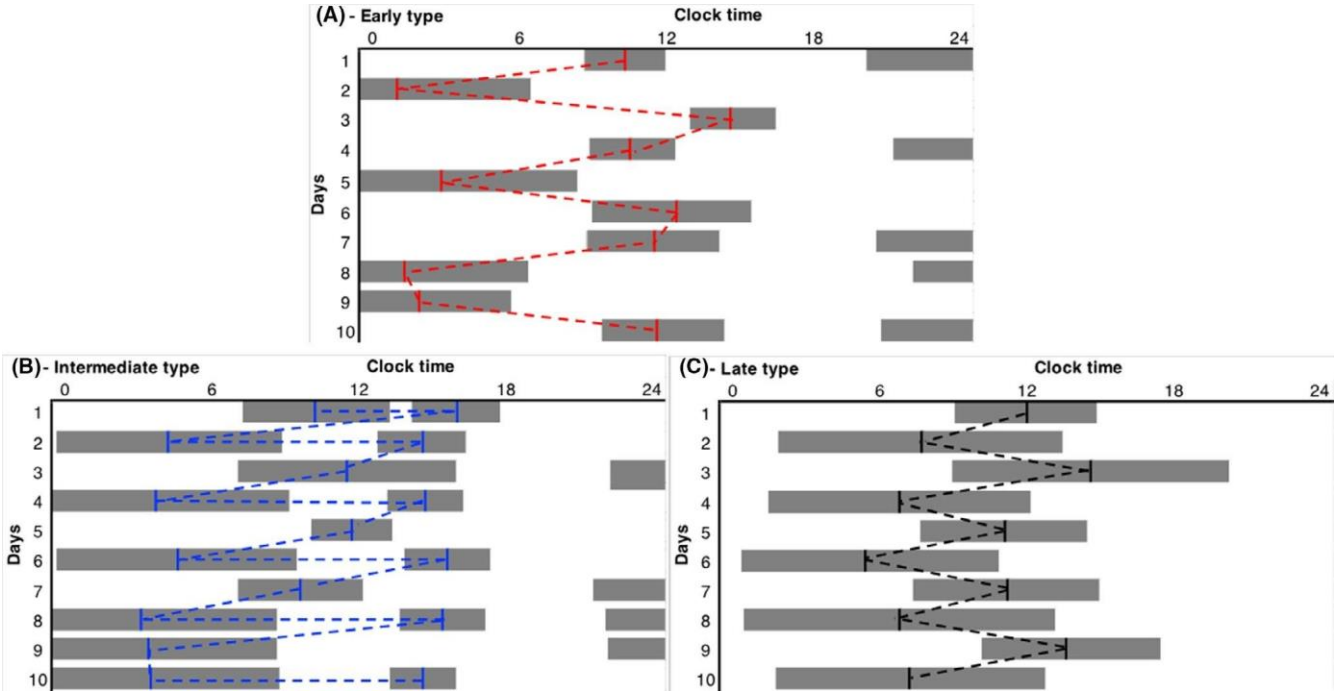
$$\text{Composite phasedeviation: } |CPD_i| = \left|\left[\begin{matrix} x_i \\ y_i \end{matrix}\right]\right| = \sqrt{x_i^2 + y^2_i}$$

CPD_i = composite phase deviation on day i; x_i = distance of mid- sleep on day i to chronotype (MSF^{N}_{sc})*; y_i = distance of mid- sleep on day i to previous day $i - 1$; $*MSF^{N}_{sc}$ = chronotype (mid- sleep on free days after night shifts, corrected for over- sleep).

2.4.2 | Secondary outcome — Anthropometric measures

Body weight was assessed to the nearest 0.1 kg without shoes and in light clothing using a digital balance. Height was assessed using a wall-m ounted portable stadiometer to the nearest 0.1 cm. Weight and height measurements were performed according to the standardization method proposed by Lohman et al.[36] Body mass index (BMI, kg/m^2) was calculated as the weight (kg) divided by the height squared (m^2). Waist (between top edge of iliac crest and 12th rib- medial portion) and hip (at maximum extension of buttocks on anterior- posterior and lateral plane) circumferences were measured using a flexible anthropometric tape (Gulick, Mabis®) with scale increments of 0.1 cm. Abdominal and hip circumferences were used to calculate waist- hip ratio (WHR). Cervical circumference (at the cricoid cartilage) was also measured. All anthropometric assessments were performed at baseline, after melatonin supplementation and after placebo.

FIGURE 2 Sleep times measured derived by actigraphy according to participants' chronotype: early (A), intermediate (B), and late (C) types. Each figure corresponds to one individual. Gray area corresponds to sleep and dashed lines link each day mid-sleep points



the American College of Sports Medicine[38] and the World Health Organization.[39] Participants were classified as being physically active if they performed at least 150 min of vigorous and/or moderate physical activities per week. Participants who undertook 10-1 49 min of physical activity per week were classified as moderately active, and those who practiced less than 10 min per week were classified as insufficiently active. These data were used to assess whether there was a change in physical activity during the intervention.

Food intake was assessed by food diaries, which were completed on one workday and one typical day-o ff (from 19:00 to 19:00 h), once a month while participating in the study (baseline plus 24 weeks- 7 months in the total). The average of the three records (3 months) was calculated during the melatonin supplementation and placebo. These data were analyzed in the Nutrition Data System for Research[40] and were used to assess whether there was a change in calorie intake during the intervention.

2.4.4 | Work Ability Index

Work ability was assessed by the translated[41] and validated Brazilian version[42] of the Work Ability Index.[43] It allows evaluation of the ability to work from the perception of the workers themselves, through 10 questions summarized in seven dimensions. The results of the seven dimensions provide a score that varies from 7 to 49 points.

2.4.5 | Sufficient sleep duration for rest

Self- reported data of "sufficient sleep duration for rest" were available through the visual analogue scale, ranging from 0 to 10 points (0 = no; 10 = yes). The scale was completed for

10 consecutive days in the three stages of the study (baseline, at the end of melatonin administration, and at the end of the placebo administration).

2.4.6 | Descriptive variables

The participants first underwent a 2 week prescreening period in which they completed a self-a dministered questionnaire about socio-demographic aspects (age, marital status, education level), work characteristics (position, previous night work, reason to work at night, duration of working at night, second job), health and lifestyle (smoking, alcohol consumption, physical activity, chronotype, food, sleep), and were assessed anthropometrically. Chronotype was assessed using mid- point of sleep on free days after night shifts, corrected for over- sleep (MSF^{N}_{sc}) derived from the Munich Chronotype Questionnaire for shift work ($MCTQ^{shift}$),[33] being categorized by the tercile (1st early type, 2nd intermediate type, 3rd late type).

2.5 | *STATISTICAL ANALYSES*

All data are presented as mean and ±standard deviation. Statistical analyses were performed using Statistica®.

A repeated-measures analysis of variance (ANOVA) was performed to compare the Composite Phase Deviations (CPD) according to the intervention stage, as well as to compare the CPD according to the chronotype, for each intervention stage. Two-w ay repeated-measures ANOVA to compare the differences in anthropometric measures, according to the intervention stage (melatonin and placebo), and chronotype was used. It is important to note that we tested the order effects, and the results were the comparable.

In addition, repeated- measures ANOVA was performed to see whether chronotype changed across intervention stages. In addition, Spearman correlation coefficient between CPD and chronotype was calculated. Moreover, in order to see the influence of confounding factors, we performed a Fisher's exact hypothesis test to compare participants according to physical activity and a two- way repeated- measures ANOVA to compare the differences in caloric intake, according to the intervention stage (melatonin and placebo) and chronotype. Due to missing data, there is variation in some analyzes. Post hoc comparisons were performed for all ANOVA using LSD's correction to determine differences between group means; P- value of less than 0.05 was considered statistically significant.

3 | RESULTS

This all female night shift work cohort (n = 27) had a mean age of 37.1 years old (±5.9 years), of which 63% were married. Among those, 59.2% had complete or incomplete postgraduation degree, 51.9% were nursing technicians, 29.6% reported that this was their first night job, and the main reason for working

at night was to reconcile work with home and/or children care (40.7%). Lifetime night work exposure was 9.2 years (±6.4 years), with 63% working night shifts for more than 5 years, and only two participants had another job on the day shift (7.4%). None were smokers; the consumption of alcoholic beverages on special occasions was reported by 63%, and the others reported not consuming alcohol at all. While 37% said they undertook physical activity during leisure time (1.8 h per week, ±32 min), the majority of the workers reported being sedentary. The mean BMI at baseline was 29.9 kg/m^2 (±3.3 kg/m^2).

The mean chronotype (based on mid-s leep on days off) at baseline was at 3:18 h (±2 h). Questionnaire- based chronotype (MCTQ^{shift}) did not change after melatonin supplementation (03:19 h, ±1.28 h) or after the placebo (03:17 h, ±1.33 h) (repeated-m easures ANOVA P =.87). Figure 2 shows examples of actograms according to the participant's chronotype (early, intermediate or late).

The Work Ability Index significantly improved after melatonin supplementation (38.2 points, EP 0.70 points) in comparison with baseline (mean 34.6 points, EP 0.92 points, repeated-measures ANOVA P < .0001) and also in comparison with placebo (36.9 points, EP 0.87 points, repeated- measures ANOVA P < .0001). Early (7.94 points) and late types (5 points) reported getting enough sleep after melatonin administration in comparison with baseline (6.77 points and 4.20 points, respectively) (repeated-m easures ANOVA—Chronotype F(2, 21) = 2.35, P = .12; Intervention F(2, 42) = 7.67, P < .01; Chronotype*Intervention F(4, 42) = 0.72, P = .58).

Late types had a Composite Phase Deviations (CPD) 52.5% and 46.8% lower than early and intermediate types, respectively (Figure 3A, LSD P < .01). After melatonin supplementation for 12 weeks (45 nights) there was a decrease of more than 20% in CPD in comparison with the baseline considering all chronotypes (Figure 3B, LSD P = .01). We did not find an interaction between chronotype and intervention (F(4, 34) = 1.022, P = .41).

As expected, we observed strong correlations between chronotype and CPD at baseline (rho = −0.71, P < .0001), after melatonin supplementation (rho = −0.72, P < .001) and after the use of placebo (rho = −0.74, P < .001). This result is in line with earlier study[44] demonstrating higher levels of circadian misalignment for earlier chronotypes working the night shift as compared to later types (Figure S1).

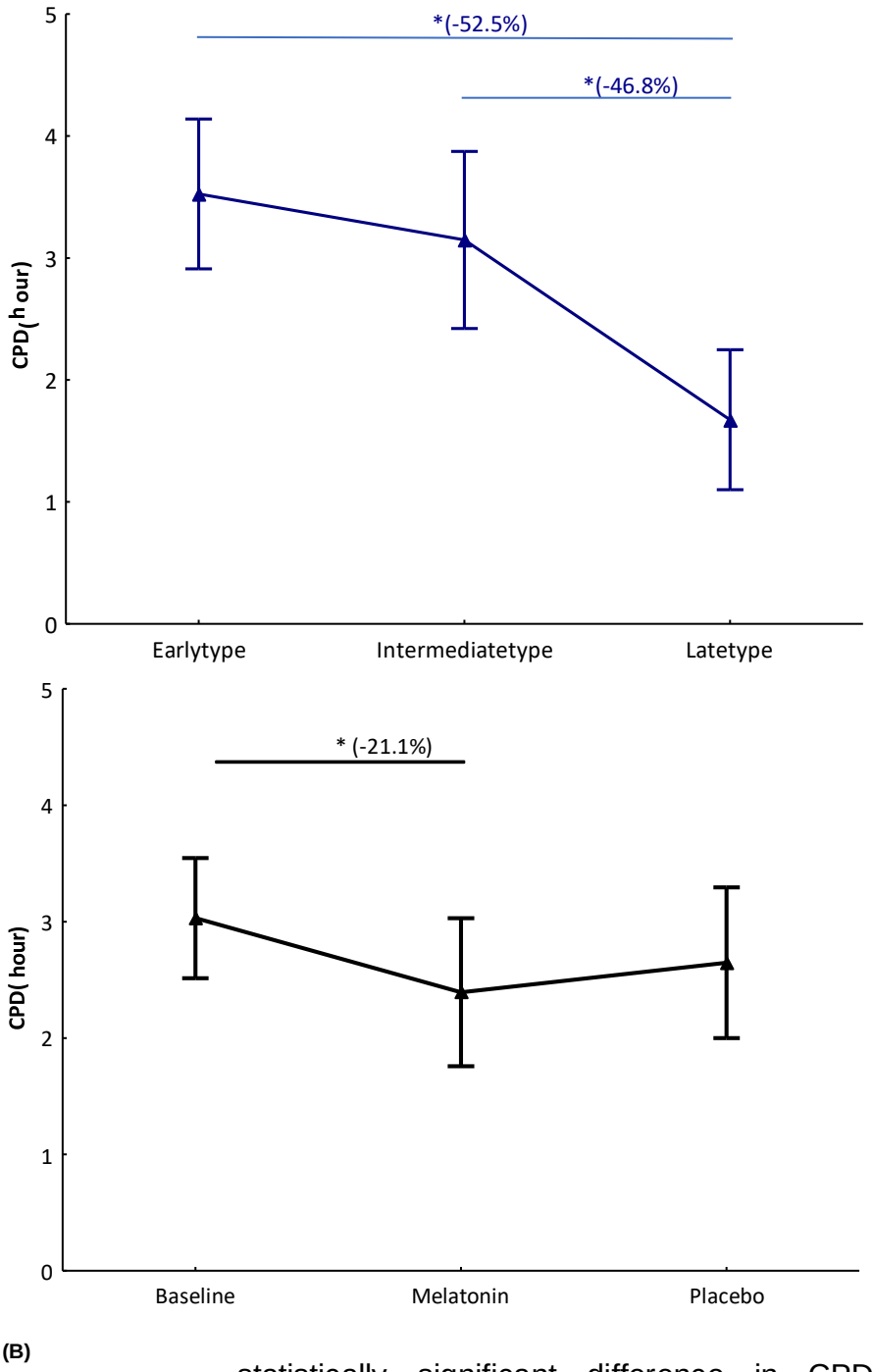
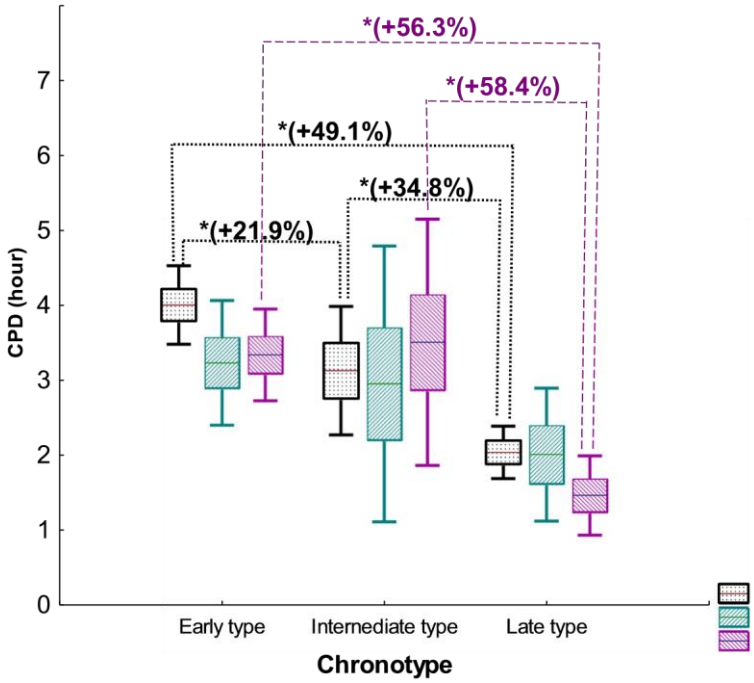


FIGURE 3 Repeated-measures **A)** ANOVA for Composite Phase Deviations (CPD) according to the chronotype and intervention phase. A, Chronotype $F(2, 17) = 11.97$, $P < .01$; B, Intervention $F(2, 34) = 3.23$, $P = .05$; Chronotype*Intervention $F(4, 34) = 1.02$, $P = .41$.*: LSD $P < .05$. Vertical bars denote 0.95 confidence intervals

When evaluating the CPD according to the chronotype for each phase of intervention, we found that, in the baseline, early types had higher levels of CPD as compared to intermediate (LSD $P = .04$) and late types (LSD $P < .01$), as well as intermediate types in comparison with late types (LSD $P = .01$). After melatonin supplementation, there was no statistically significant difference in CPD between chronotypes. In the placebo condition, though, early (LSD $P < .01$) and intermediate types (LSD $P < .01$) remained at higher CPD levels in comparison with late types (Figure 4).

The proportion of moderately to highly active participants at baseline and after using the placebo was 37%, respectively, and after melatonin supplementation was 22.3%, with no statistically significant difference between

proportions (Fisher's exact test P = .70). The caloric intake appeared to be lower after melatonin supplementation and placebo compared with the baseline; however, the analysis revealed only a borderline significance (P = .05) (Figure 5). No differences in caloric intake among the different chronotypes were found.



After melatonin supplementation, no statistically significant changes in body weight (−0.38 kg, F[2, 50] = 0.55, P = .58), BMI (−0.14 kg/m^2, F[2, 50] = 0.44, P = .65), waist circumference (0.13 cm, F[2, 50] = 0.03, P = .97), hip circumference (−0.98 cm, F[2, 50] = 1.38, P = .26), WHR (0.01, F[2, 50] = 1.10, P = .34), and cervical circumference (−0.10 cm, F[2, 50] = 0.19, P = .83) were observed in comparison with baseline as well as in comparison with placebo (−0.29 kg, −0.09 kg/m^2, 0.10 cm, −0.44 cm, 0.0 and −0.08 cm, respectively).

On the other hand, when evaluating these parameters according to chronotype, significant differences were observed. Among the early types, there was a significant reduction in body weight (Figure 6A, LSD P = .02), BMI (Figure 6B, LSD P = .02), waist circumference (Figure 6C, LSD P = .03), and hip circumference (Figure 6D, LSD P = .03) after melatonin supplementation in comparison with baseline. After the use of placebo in comparison with melatonin, an increase in hip circumference among the early types was observed (Figure 6D, LSD P = .02). A reduction in cervical circumference in the late types was also observed after melatonin supplementation compared with baseline (Figure 6F, LSD P = .04). Still, among the intermediate types, there was an increase in waist circumference (Figure 6C, LSD P = .01) and WHR (Figure 6E, LSD P = .02) after

melatonin supplementation in comparison with baseline, as well as a reduction in waist circumference (Figure 6C, LSD P = .03) after placebo in comparison with melatonin. It is important to highlight that there were no changes after the placebo in the other anthropometric parameters (Figure 6A-F).

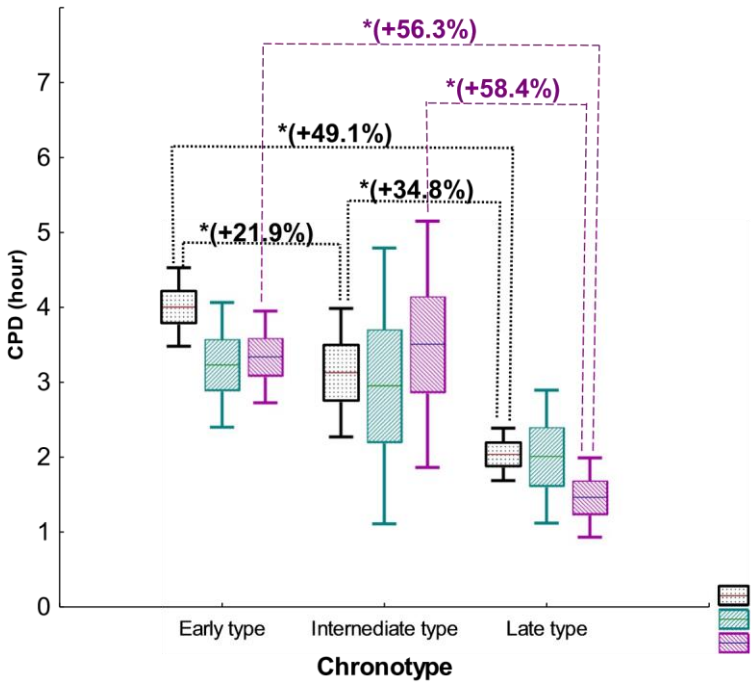


FIGURE 4 Repeated- measures ANOVA for the Composite Phase Deviations (CPD) according to the chronotype, for each intervention stage. CPD baseline $F(2, 24) = 14.15$, $P < .001$; PD melatonin $F(2, 21) = 1.76$, $P = .195$; CPD placebo $F(2, 18) = 9.66$, $P = .001$. **: LSD $P < .05$*. Middle point line: mean; box plot: stand error; whiskers: confidence intervals 95%. [The percentage in parentheses indicates the decrease (−) or increase (+) in the CPD]. More details in Table S1

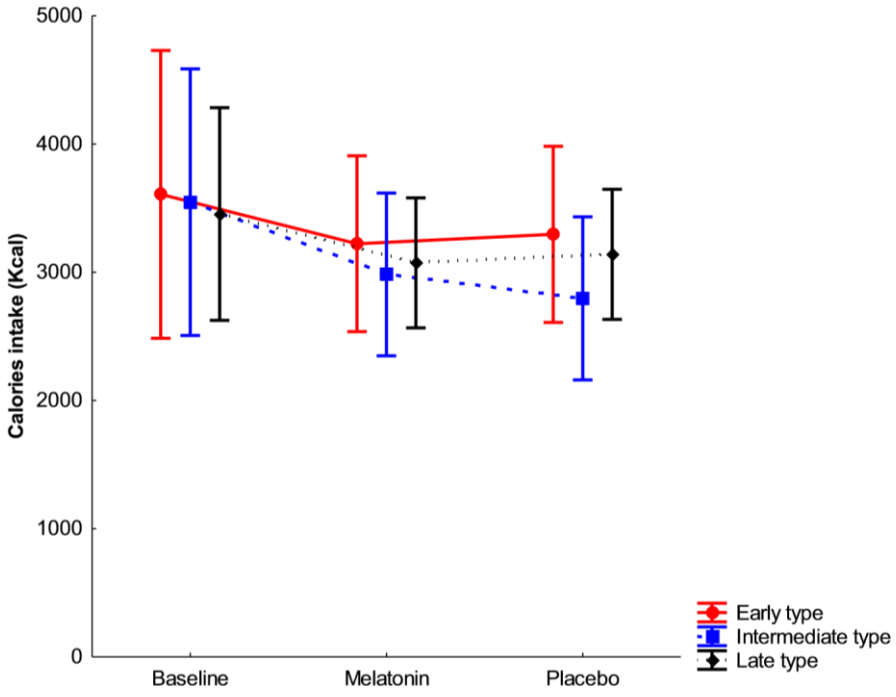


FIGURE 5 Repeated- measures ANOVA for the anthropometric measures according to the intervention phase and chronotype. A, Body weight— Chronotype $F(2, 23) = 0.26$, $P = .774$; Intervention $F(2, 46) = 0.92$, $P = .405$; Chronotype*Intervention $F(4, 46) = 2.51$, $P = .054$; B, body mass index (BMI)— Chronotype $F(2, 23) = 0.24$, $P = .786$; Intervention $F(2, 46) = 0.78$, $P = .465$; Chronotype*Intervention $F(4, 46) = 2.63$, $P = .046$; C, waist circumference— Chronotype $F(2, 23) = 1.03$, $P = .371$; Intervention $F(2, 46) = 0.00$, $P = .996$; Chronotype*Intervention $F(4, 46) = 3.46$, $P = .014$; D, hip circumference— Chronotype $F(2, 23) = 0.05$, $P = .949$; Intervention $F(2, 46) = 1.76$, $P = .183$; Chronotype*Intervention $F(4, 46) = 1.51$, $P = .214$; E, waist- hip ratio (WHR)— Chronotype $F(2, 23) = 1.97$, $P = .163$; Intervention $F(2, 46) = 0.93$, $P = .400$; Chronotype*Intervention $F(4, 46) = 1.28$, $P = .292$; F, cervical circumference— Chronotype $F(2, 23) = 0.51$, $P = .606$; Intervention $F(2, 46) = 0.07$, $P = .936$; Chronotype*Intervention $F(4, 46) = 1.40$,

P = .250. *: LSD P < .05. Vertical bars denote 0.95 confidence intervals. The percentage in parentheses indicates the decrease (−) or increase (+) in the anthropometric measures. More details in Table S2

4 | DISCUSSION

The current study is the first study to evaluate the effect of exogenous melatonin on body weight in night shift workers, taken intermittently (on every second night, when they slept at night, that is, on nights between working night shifts, and on days off). Our results show that exogenous melatonin supplementation in overweight female night workers reduced body weight in early types and also reduced the circadian misalignment of the participants. The group with highest baseline CPD levels was the early types, which showed a greater reduction in CPD after the melatonin intervention. In short, the present results show that the exogenous melatonin supplementation performed in this study (including dose, time of administration, and type of melatonin) was effective in reducing 21.1% circadian misalignment considering all chronotypes.

Some studies suggest that night shift workers with early chronotypes are the ones who suffer the most under this work schedule, and consequently are more susceptible to develop health problems.[7,34] Considering that the CPD assesses the circadian misalignment from the usual sleep time relative to the previous day, it makes sense to observe greater misalignment of early types who work at night. Because chronotype is prone to modifications according to the strength of the *zeitgeber* (light-d ark cycle), it may undergo changes after exogenous melatonin supplementation. In other words, chronotype is dependent on light-d ark cycle exposure. Thus, it would be interesting to investigate whether chronic melatonin supplementation would shift early types working night shifts later, since sleep phase-d erived chronotype can be considered a proxy for phase of entrainment.[5,45] Moreover, the optimal administration time should be better investigated in order to assess the best timing for advances or delays of phase.[46]

Almost 20 years ago, Burgess et al[28] discussed the effects of melatonin to improve the circadian adaptation of shift workers and night workers. The authors presented results from three field studies that administered melatonin after two to six nights of work, in which a decrease in self- reported awakenings during sleep was observed without alterations in sleep duration. However, the authors highlight that none of these studies measured the circadian phase of the participants, which did not allow a change in the rhythm phase to be assessed. Burgess et al[28] also presented findings from a simulated night work study, which melatonin was taken before afternoon or evening sleep. The authors observed that

exogenous melatonin was able to advance the phase of sleep.

Sack and Lewi,[14] also mentioned in this review, conducted the only study in which melatonin was administered to night workers/shift workers and, simultaneously, measured circadian phase. In this study, the participants worked 7 night followed by 7 days off and received 0.5 mg of melatonin at night during the week off, and during the day throughout the working week. The findings revealed phase shifting in both directions (advance and delay) and the magnitude of the effect varied individually.

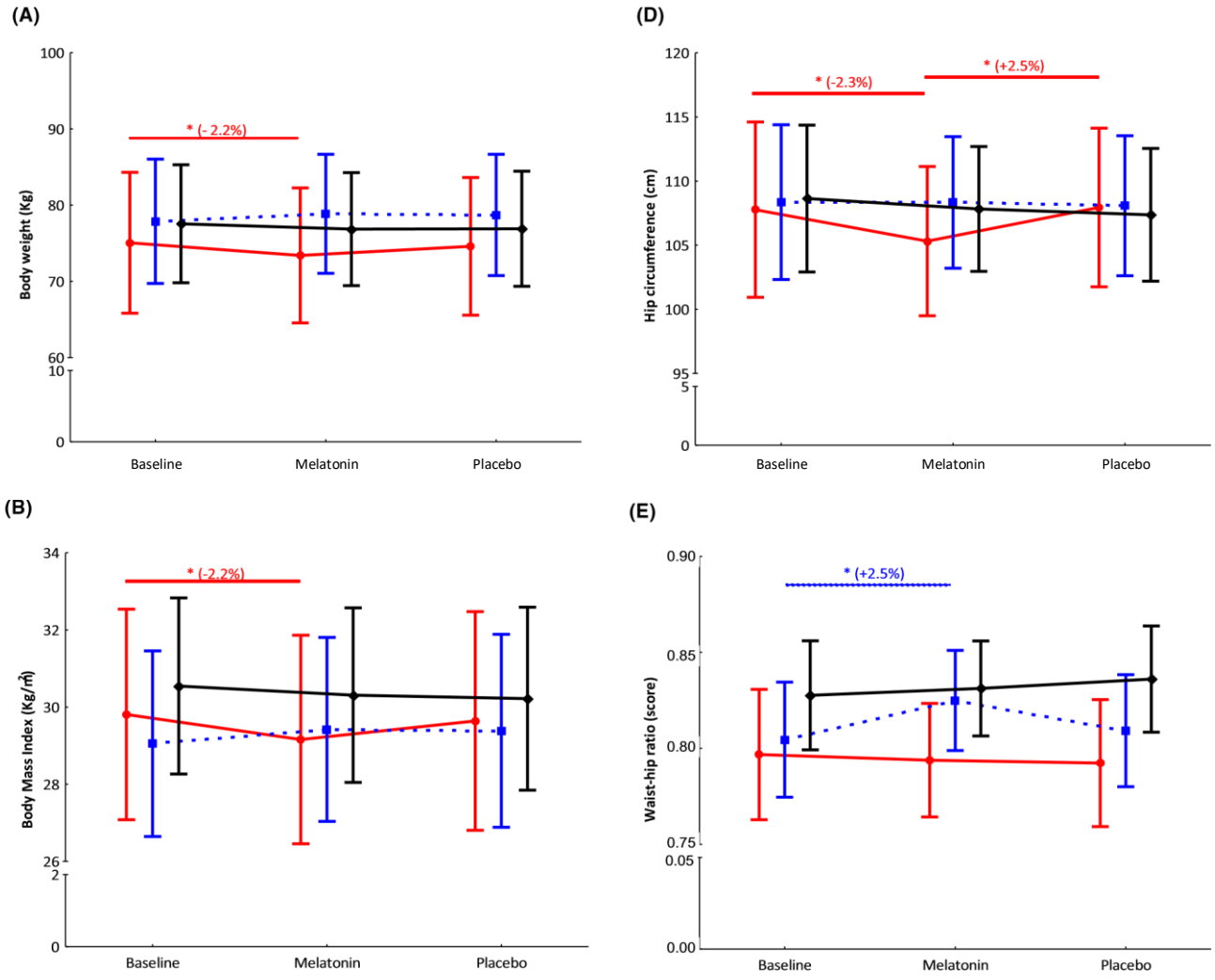
Melatonin is a powerful circadian chronobiotic agent; that is, it is responsible for encoding time for the internal environment, being able to phase shift and synchronize the circadian rhythms of several organs and their functions.[1,47] Among night workers, melatonin production is compromised, since it decreases or does not occur during work nights.[3] As a result, this misalignment in the activity/rest and feeding/fasting cycle experienced by night workers contributes to the development of metabolic disorders.[3,4,48]

However, we also observed that the strongest reduction in CPD occurred in the late types in the placebo group. In a review of the placebo effect in drug trials, Enck and Klosterhalfen[49] observed that, in crossover trials, the more frequent the researchers' contact with the participants is, the greater the odds of a placebo effect. According to the authors, this occurs due to manipulation of participants' expectations and is independent of the nature of the outcome assessed. These results are in agreement with the present study, since the placebo effect observed in the late types was not observed in those in which melatonin was more effective.

In the current study, the supplementation of exogenous melatonin improved the anthropometric parameters among early types, with a decrease in four out of six anthropometric parameters evaluated (body weight, BMI, waist and hip circumferences). It was also observed that early and late types met their sleep needs, measured by a visual analogue scale, after melatonin supplementation. According to McMahon et al,[50] early types had a higher percentage of fat and higher waist- to- hip ratio than individuals with intermediate type. McMahon et al[50] concluded that poor sleep quality is more strongly associated with obesity among people with early type.

It is possible that the study participants became more concerned with their diet and exercises by being part of the study. Nevertheless, the analyses showed that there was no significant change in energy intake nor in the levels of physical activity after the intervention, reinforcing the hypothesis that it was melatonin supplementation that was effective in reducing body weight. This hypothesis is in line with a recent systematic

review on the effects of exogenous melatonin supplementation on eating habits and appetite-r egulating hormones.[51] The authors observed that melatonin's metabolic effects may occur independently, since the improvement in endocrine- metabolic disorders seems to occur independently of energy intake. In addition to a putative increased metabolic energy expenditure due to brown adipose tissue activity,[1] we suppose that this result is due to the chronobiotic action of melatonin, evidenced by the reduction in circadian misalignment (measured by CPD) (of course, we cannot disregard the soporific effects of melatonin).



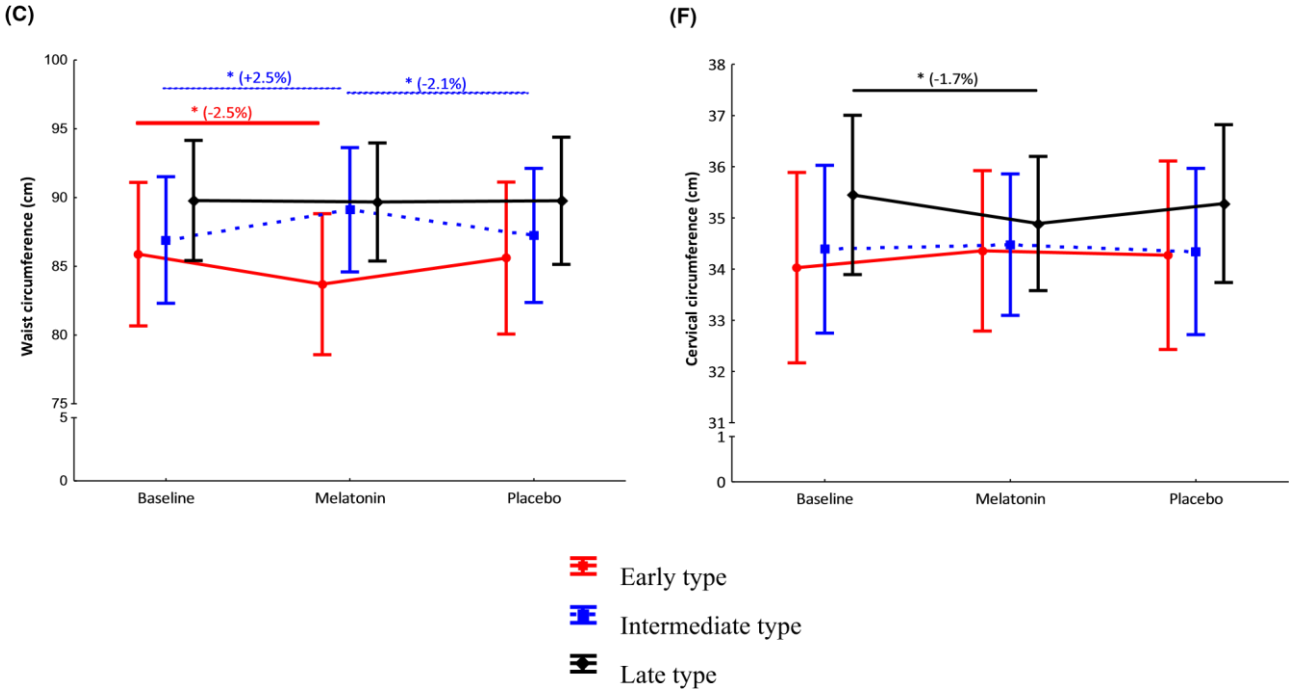


FIGURE 6 Repeated- measures ANOVA for caloric intake according to the intervention phase and chronotype. Chronotype $F(2, 21) = 0.16$, $P = 0.85$; Intervention $F(2, 42) = 3.23$, $P = .05$; Chronotype*Intervention $F(4, 42) = 0.25$, $P = .91$. Vertical bars denote 0.95 confidence intervals

It is important to highlight that the participants work every second night, which means there are also subsequent effects caused by the endogenous production of melatonin during every second night. For instance, insulin production early in the morning is influenced by nocturnal melatonin.[52] Thus, insulin production may be compromised by the light- induced suppression of melatonin every second night of work. Prolonged effects of melatonin, such as those controlled by clock genes, for example, are affected by the reduction in melatonin production during night work.[1] The administration of exogenous melatonin at the night at home might have reinforced the chain of events the next day. In other words, the night at home reinforced by the exogenous melatonin might have contributed to the control peripheral and central cellular functions throughout the next day.

According to Reiter et al[3] and Loloei et al,[53] melatonin plays an important role in phase shifting/synchronizing the circadian rhythm of energy metabolism, in which the anti-obesogenic effect of melatonin can lead to a reduction in body weight.[17,54] Therefore, the melatonin anti-o besogenic effect would be linked to its phase-s hifting effect. Preclinical and clinical trials have revealed the beneficial effects of melatonin as a nutraceutical for body weight regulation, validating its powerful anti-i nflammatory and antioxidant effects.[17,55- 57]

Although studies with shift workers are scarce, there are several studies with animal models that support the hypothesis that exogenous melatonin reduces body weight and other positive anthropometric results.[58- 60] The absence or reduction of melatonin or its membrane receptors, in addition to induced hypothalamic leptin resistance and reduction in brown adipose tissue energy expenditure,[61- 63] seems to prevent the typical circadian rhythmic metabolic synchronization to periods of activity and rest. This circadian misalignment, which leads to metabolic disorders and obesity, disappears in animals treated daily with melatonin.[17]

For Mesri Alamdari et al,[64] exogenous melatonin supplementation may prevent the adverse health consequences of obesity. The authors conducted a trial in order to study the effects of melatonin (6 mg, 40 days, 2 h before bedtime) on oxidative stress and inflammatory parameters of obese women. Although both the placebo and the melatonin groups experienced reductions in anthropometric parameters, only the melatonin group significantly reduced inflammatory responses through decreasing proinflammatory cytokines. Szewczyk- Golec et al[65] assessed the effect of melatonin

supplementation (10 mg, 30 days, 1 h before bedtime) on the antioxidant status and levels of circulating adipokines involved in the energy homeostasis in obese human subjects (male and female). The authors found a significant reduction in body weight after melatonin supplementation compared with the placebo. It is worth mentioning that both groups underwent supplementation (melatonin or placebo) and a calorie-r estricted diet. A similar result was observed by Bahrami et al[66] in a study of 12 weeks of supplementation with 6 mg of melatonin (one hour before the desired time to sleep) in 70 individuals with metabolic syndrome. The authors found a significant reduction in body weight, waist, and abdominal circumference, in addition to a tendency to reduce BMI in comparison with the placebo.

In a review, Loloei et al[53] analyzed the effect of melatonin supplementation on anthropometric parameters and found no statistically significant reduction in BMI, body weight, and waist circumference after melatonin supplementation. Of a total of six reviewed articles, only one, performed with postmenopausal women, reported a decrease in BMI with melatonin (5 mg, 24 weeks). Regarding body weight, a reduction was also found in a single study carried out with schizophrenic patients (3 mg, 8 weeks). Regarding waist circumference, a reduction was found in a study carried out with menopausal women, with a melatonin dosage of 3 g/day for 24 weeks. These findings support Cipolla-N eto et al[17] who stated that the action of melatonin in the regulation of body weight depends on different mechanisms, and its supplementation (depending on the correct dose, formulation, and time) can prevent and/or contribute to body weight reduction and elimination of metabolic disorders.

This study is a phase II clinical trial, which limits generalizability to a larger population. Individual variability in tolerance for shift work[67] was also not evaluated in this study and should be taken into account before prescribing exogenous melatonin. However, the longitudinal crossover design in real- life conditions is the strength of this study. Moreover, exogenous melatonin administration may be a less harmful alternative to health than controlled medications among shift workers who have circadian misalignment problems, and consequently metabolic disorders.

In summary, we showed that exogenous melatonin decreased approximately 20% of circadian misalignment among overweight night workers. In addition, exogenous melatonin reduced body weight among early types, without any change in the participants' calorie intake or physical activity levels. Individualized dose, type of

melatonin (fast or time release), different groups of shift workers, and time of should be investigated before recommending melatonin to shift workers.

ACKNOWLEDGEMENTS

We thank the hospital staff, and the funding agencies: FAPESP (no 2014/50457- 0) and CNPq (no 150781/2017- 2).

CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHORS' CONTRIBUTIONS

Elaine C. Marqueze involved in concept/design and acquisition, analyzed and interpreted the data, drafted, and reviewed the manuscript. **Luciana FR Nogueira** interpreted the data, drafted, and reviewed the manuscript. **Celine Vetter involved in** concept/design, drafted, and reviewed the manuscript. **Debra J. Skene** involved in concept/design, drafted, and reviewed the manuscript. **José Cipolla- Neto** involved in concept/design and acquisition, drafted, and reviewed the manuscript. **Claudia RC Moreno** involved in concept/ design, analyzed and interpreted the data, drafted, and reviewed the manuscript. All authors have read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Amaral FGD, Andrade-S ilva J, Kuwabara WMT, Cipolla-Neto J. New insights into the function of melatonin and its role in metabolic disturbances. *Expert Rev Endocrinol Metab*. 2019;14(4):293- 300.
2. Thorne H, Hampton S, Morgan L, Skene DJ, Arendt J. Differences in sleep, light, and circadian phase in offshore 18:00–0 6:00 h and 19:00– 07:00 h shift workers. *Chronobiol Int*. 2008;25(2):225- 235.
3. Reiter RJ, Tan DX, Korkmaz A, Ma S. Obesity and metabolic syndrome: association with chronodisruption, sleep deprivation, and melatonin suppression. *Ann Med*. 2012;44(6):564- 577.
4. Ulhôa MA, Marqueze EC, Burgos LG, Moreno CR. Shift work and endocrine disorders. *Int J Endocrinol*. 2015;2015: 826249.
5. Vetter C. Circadian disruption: What do we actually mean? *Eur J Neurosci*. 2020;51(1):531- 550.
6. Vetter C, Fischer D, Matera JL, Roenneberg T. Aligning work and circadian time in shift workers improves sleep and reduces circadian disruption. *Curr Biol*. 2015;25(7):907- 911.
7. Juda M, Vetter C, Roenneberg T. Chronotype modulates sleep duration, sleep quality, and social jet lag in shift-workers. *J Biol Rhythms*. 2013;28(2):141- 151.
8. Agil A, Navarro- Alarcón M, Ruiz R, Abuhamadah S, El-M ir MY, Vázquez GF. Beneficial effects of melatonin on obesity and lipid profile in young Zucker diabetic fatty rats. *J Pineal Res*. 2011;50(2):207- 212.

9. Hardeland R. Melatonin in aging and disease -m ultiple consequences of reduced secretion, options and limits of treatment. *Aging Dis.* 2012;3(2):194- 225.
10. Navarro- Alarcón M, Ruiz-O jeda FJ, Blanca-Herrera RM, et al. Melatonin and metabolic regulation: a review. *Food Funct*.
2014;5(11):2806- 2832.
11. Amaral FGD, Cipolla- Neto J. A brief review about melatonin, a pineal hormone. *Arch Endocrinol Metab*. 2018;62(4):472- 479.
12. Garaulet M, Qian J, Florez JC, Arendt J, Saxena R, Scheer FAJL. Melatonin effects on glucose metabolism: time to unlock the controversy. *Trends Endocrinol Metab*. 2020;31(3):192- 204.
13. Bojkowski CJ, Aldhous ME, English J, et al. Suppression of nocturnal plasma melatonin and 6-s ulphatoxymelatonin by bright and dim light in man. *Horm Metab Res.* 1987;19(9):437- 440.
14. Sack RL, Lewy AJ. Melatonin as a chronobiotic: treatment of circadian desynchrony in night workers and the blind. *J Biol Rhythms*. 1997;12:595- 603.
15. Dijk DJ, Duffy JF, Silva EJ, Shanahan TL, Boivin DB, Czeisler CA. Amplitude reduction and phase shifts of melatonin, cortisol and other circadian rhythms after a gradual advance of sleep and light exposure in humans. *PLoS One*. 2012;7(2):e30037.
16. Phillips AJK, Vidafar P, Burns AC, et al. High sensitivity and interindividual variability in the response of the human circadian system to evening light. *Proc Natl Acad Sci USA*. 2019;116(24):12019- 12024.
17. Cipolla- Neto J, Amaral F, Afeche S, Tan D, Reiter R. Melatonin, energy metabolism, and obesity. *J Pineal Res.* 2014;56:371- 381.
18. Daugaard S, Garde AH, Bonde JPE, et al. Night work, light exposure and melatonin on work days and days off. *Chronobiol Int*. 2017;34(7):942- 955.
19. Rasmussen DD, Boldt BM, Wilkinson CW, Yellon SM, Matsumoto AM. Daily melatonin administration at middle age suppresses male rat visceral fat, plasma leptin, and plasma insulin to youthful levels [published correction appears in Endocrinology 2002; 143(4):1269]. *Endocrinology*. 1999;140(2):1009- 1012.
20. Cardinali DP, Pagano ES, Scacchi Bernasconi PA, Reynoso R, Scacchi P. Disrupted chronobiology of sleep and cytoprotection in obesity: possible therapeutic value of melatonin. *Neuro Endocrinol Lett*. 2011;32(5):588- 606.
21. Cipolla- Neto J, Amaral FGD. Melatonin as a hormone: new physiological and clinical insights. *Endocr Rev*. 2018;39(6):990- 1028.
22. Piccinetti CC, Migliarini B, Olivotto I, Coletti G, Amici A, Carnevali O. Appetite regulation: the central role of melatonin in Danio rerio. *Horm Behav*. 2010;58(5):780- 785.
23. Wolden- Hanson T, Mitton DR, McCants RL, et al. Daily melatonin administration to middle-a ged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. *Endocrinology*. 2000;141(2):487- 497.
24. Helfer G, Barrett P, Morgan P. A unifying hypothesis for control of body weight and reproduction in seasonally breeding mammals. *J Neuroendocrinol*. 2019;31(3):e12680.
25. Skene DJ, Deacon S, Arendt J. Use of melatonin in circadian rhythm disorders and following phase shifts. *Acta Neurobiol Exp (Wars).* 1996;56(1):359- 362.
26. Arendt J, Skene DJ, Middleton B, Lockley SW, Deacon S. Efficacy of melatonin treatment in jet lag, shift work, and blindness. *J Biol Rhythms.* 1997;12(6):604- 617.
27. Auld F, Maschauer EL, Morrison I, Skene DJ, Riha RL. Evidence for the efficacy of melatonin in the treatment of primary adult sleep disorders. *Sleep Med Rev*. 2017;34:10- 22.
28. Burgess HJ, Sharkey KM, Eastman CI. Bright light, dark and melatonin can promote circadian adaptation in night shift workers.
Sleep Med Rev. 2002;6(5):407- 420.
29. Li T, Jiang S, Han M, et al. Exogenous melatonin as a treatment for secondary sleep disorders: a systematic review and meta-a nalysis. *Frontiers Neuroendocrinol*. 2019;52:22- 28.
30. Mostafavi SA, Akhondzadeh S, Mohammadi MR, et al. Role of melatonin in body weight: a systematic review and meta-a nalysis. *Curr Pharm Des*. 2017;23(23):3445- 3452.
31. Smith MR, Eastman CI. Shift work: health, performance and safety problems, traditional countermeasures, and innovative management strategies to reduce circadian misalignment. *Nat Sci Sleep*. 2012;27(4):111- 132.
32. Folkard S, Arendt J, Clark M. Can melatonin improve shift workers' tolerance of the night shift? Some preliminary findings. *Chronobiol Int*. 1993;10:315- 320.
33. Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *PLoS Medicine*. 2010;7(3):e1000251.
34. Fischer D, Vetter C, Roenneberg T. A novel method to visualise and quantify circadian misalignment. *Sci Rep*. 2016;6:38601.

35. Juda M, Vetter C, Roenneberg T. The Munich ChronoType Questionnaire for shift- workers ($MCTQ_{shift}$). *J BiolRhythm*. 2013;28(2):130- 140.
36. Lohman TG, Roche AF, Martorell R. *Anthropometric Standardization Reference Manual*. Champaign: Human Kinetics Books; 1988.
37. Matsudo S, Araújo T, Matsudo V, et al. Questionário Internacional de Atividade Física (IPAQ): estudo e validade e reprodutibilidade no Brasil. *Rev Bras de Ativ Fis Saúde*. 2001;6:5- 18.
38. Pate RR, Pratt M, Blair SN, et al. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA*. 1995;273:402- 407.
39. Guilbert JJ. The world health report 2002 – reducing risks, promoting healthy life. *Educ Health (Abingdon)*. 2003;16:230.
40. Nutrition Data System for Research Software [CD- ROM]. Nutrition Coordinating Center, Division of Epidemiology. Minnesota: NDS; 2007.
41. Fischer FM. Breve histórico desta tradução. In: *Fischer FM. Índice de capacidade para o trabalho*. p. 9– 10. Ed.FSCar, 2005.
42. Martinez MC, Latorre MRDO, Fischer FM. Validade e confiabilidade da versão brasileira do Índice de Capacidade para o Trabalho. *Rev Saude Publica*. 2009;43(3):525- 532.
43. Tuomi K, Ilmarinen J, Jahkola A, Katajarinne L, Tulkki A. *Índice de capacidade para o trabalho*. Institute of Occupational Health. São Paulo: FSPUSP; 1997.
44. Vetter C, Devore EE, Ramin CA, Speizer FE, Willett WC, Schernhammer ES. Mismatch of sleep and work timing and risk of type 2 diabetes. *Diabetes Care*. 2015;38(9):1707- 1713.
45. Roenneberg T, Pilz LK, Zerbini G, Winnebeck EC. Chronotype and social jetlag: A (self-) critical review. *Biology (Basel)*. 2019;8(3):54.
46. Burgess HJ, Revell VL, Molina TA, Eastman CI. Human phase response curves to three days of daily melatonin: 0.5 mg versus 3.0 mg. *J Clin Endocrinol Metab*. 2010;95(7):3325- 3333.
47. Kennaway DJ. A critical review of melatonin assays: past and present. *J Pineal Res*. 2019;67(1):e12572.
48. Moreno CRC, Marqueze EC, Sargent C, Wright KP Jr, Ferguson SA, Tucker P. Working time society consensus statements: evidence-b ased effects of shift work on physical and mental health. *Ind Health*. 2019;57(2):139- 157.
49. Enck P, Klosterhalfen S. Placebos and the placebo effect in drug trials. *Handb Exp Pharmacol*. 2019;260:399- 431.
50. McMahon DM, Burch JB, Youngstedt SD, et al. Relationships between chronotype, social jetlag, sleep, obesity and blood pressure in healthy young adults. *Chronobiol Int*. 2019;36(4):493- 509.
51. Nogueira LFR, Marqueze EC. Effects of melatonin supplementation on eating habits and appetite- regulating hormones: a systematic review of randomized controlled clinical and preclinical trials. *Chronobiol Int*. 2021;2:1- 14.
52. Costes S, Boss M, Thomas AP, Matveyenko AV. Activation of melatonin signaling promotes beta-c ell survival and function. *Mol Endocrinol*. 2015;29(5):682- 692.
53. Loloei S, Sepidarkish M, Heydarian A, et al. The effect of melatonin supplementation on lipid profile and anthropometric indices: A systematic review and meta- analysis of clinical trials. *Diabetes Metab Syndr*. 2019;13(3):1901- 1910.
54. Genario R, Cipolla- Neto J, Bueno AA, Santos HO. Melatonin supplementation in the management of obesity and obesity- associated disorders: a review of physiological mechanisms and clinical applications. *Pharmacol Res*. 2020;163:e105254.
55. Tan DX, Manchester L, Fuentes- Broto L, Paredes S, Reiter R. Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. *Obes Rev*. 2011;12:167- 188.
56. Jiménez- Aranda A, Fernández- Vázquez G, Campos D, et al. Melatonin induces browning of inguinal white adipose tissue in Zucker diabetic fatty rats. *J Pineal Res*. 2013;55:416- 423.
57. Mauriz JL, Collado PS, Veneroso C, Reiter RJ, González- Gallego J. A review of the molecular aspects of melatonin's anti- inflammatory actions: recent insights and new perspectives. *J Pineal Res*. 2013;54:1- 14.
58. Prunet- Marcassus B, Desbazeille M, Bros A, et al. Melatonin reduces body weight gain in Sprague Dawley rats with diet- induced obesity. *Endocrinology*. 2003;144(12):5347- 5352.
59. Ríos- Lugo MJ, Jiménez-O rtega V, Cano-Barquilla P, et al. Melatonin counteracts changes in hypothalamic gene expression of signals regulating feeding behavior in high-f at fed rats. *Horm Mol Biol Clin Investig*. 2015;21(3):175- 183.
60. Farias TSM, Cruz MM, Sá RCC, et al. Melatonin supplementation decreases hypertrophic obesity and inflammation induced by high- fat diet in mice. *Front Endocrinol*. 2019;10:750.
61. Buonfiglio D, Tchio C, Furigo I, et al. Removing melatonin receptor type 1 signaling leads to selective leptin resistance in the arcuate nucleus. *J Pineal Res*. 2019;67(2):e12580.
62. de Souza CAP, Gallo CC, de Camargo LS, et al. Melatonin multiple effects on brown adipose tissue molecular machinery. *J Pineal Res*. 2019;66(2):e12549.
63. Halpern B, Mancini MC, Bueno C, et al. Melatonin increases brown adipose tissue volume and activity in patients with melatonin deficiency: a proof-o f- concept study. *Diabetes*. 2019;68(5):947- 952.

64. Mesri Alamdari N, Mahdavi R, Roshanravan N, Lofti Yaghin N, Ostadrahimi AR, Faramarzi E. A double- blind, placebo- controlled trial related to the effects of melatonin on oxidative stress an inflammatory parameters of obese women. *Horm Metab Res*. 2015;47(7):504- 508.
65. Szewczyk- Golec K, Rajewski P, Gackowski M, et al. Melatonin supplementation lowers oxidative stress and regulates adipokines in obese patients on a calorie-r estricted diet. *Oxid Med Cell Longev*. 2017;2017:e8494107.
66. Bahrami M, Cheraghpour M, Jafarirad S, Alavinejad P, Cheraghian B. The role of melatonin supplement in metabolic

ANEXO 6 - Parecer do CEP da Faculdade de Saúde Pública da Universidade de São Paulo - FSP-USP

USP - FACULDADE DE SAÚDE PÚBLICA DA UNIVERSIDADE DE SÃO PAULO - FSP/USP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito da melatonina no sono e no metabolismo de trabalhadoras noturnas com excesso de peso

Pesquisador: ELAINE CRISTINA MARQUEZE

Área Temática:

Versão: 1

CAAE: 79452217.6.0000.5421

Instituição Proponente: Faculdade de Saúde Pública da Universidade de São Paulo - FSP/USP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.450.682

Apresentação do Projeto:

Trata-se de um ensaio clínico duplo cego a ser realizado em Hospital da rede privada de saúde com profissionais de enfermagem que trabalham à noite em turnos de 12X 36.

Objetivo da Pesquisa:

O objetivo da pesquisa é o de avaliar os efeitos da melatonina sintética nas variaveis antropometricas, nos aspectos de sono, hormonais, fisiologicos e bioquimicos de trabalhadoras sobrepesas e obesas que trabalham em turnos noturnos fixos de 12x36 horas

Avaliação dos Riscos e Benefícios:

Riscos mínimos, já que a melatonina e um hormonio naturalmente produzido pelo organismo, e o uso da melatonina sintetica nao altera a producao endogena. Há também o desconforto da coleta de sangue. Benefícios presentes e descritos.

Comentários e Considerações sobre a Pesquisa:

O projeto é bem apresentado e fundamentado. Metodologia bem desenhada.

Sem problemas éticos

Considerações sobre os Termos de apresentação obrigatória:

Apresentados e adequados

Endereço: Av. Doutor Arnaldo, 715
Bairro: Cerqueira Cesar **CEP:** 01.246-904
UF: SP **Município:** SAO PAULO
Telefone: (11)3061-7779 **Fax:** (11)3061-7779 **E-mail:** coep@fsp.usp.br

Recomendações:

Sem recomendações

Conclusões ou Pendências e Lista de Inadequações:

Aprovado

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1016199.pdf	20/10/2017 17:55:14		Aceito
Folha de Rosto	Folha_de_rosto_assinada.pdf	20/10/2017 17:53:39	ELAINE CRISTINA MARQUEZE	Aceito
Outros	Procedimentos_para_coleta_de_dados.pdf	20/10/2017 17:52:02	ELAINE CRISTINA MARQUEZE	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_pesquisa_ECM.pdf	20/10/2017 17:51:20	ELAINE CRISTINA MARQUEZE	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_ECM.pdf	18/10/2017 21:09:13	ELAINE CRISTINA MARQUEZE	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Documentos_CEP_ECM.pdf	18/10/2017 21:08:51	ELAINE CRISTINA MARQUEZE	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

SAO PAULO, 20 de Dezembro de 2017

Assinado por:
Maria Regina Alves Cardoso
(Coordenador)

Endereço: Av. Doutor Arnaldo, 715
Bairro: Cerqueira Cesar **CEP:** 01.246-904
UF: SP **Município:** SAO PAULO
Telefone: (11)3061-7779 **Fax:** (11)3061-7779 **E-mail:** coep@fsp.usp.br

ANEXO 7 - PARECER DO CEP DO HOSPITAL ALEMÃO OSWALDO CRUZ

HOSPITAL ALEMÃO OSWALDO CRUZ - SP

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito da melatonina no sono e no metabolismo de trabalhadoras noturnas com excesso de peso

Pesquisador: ELAINE CRISTINA MARQUEZE

Área Temática:

Versão: 3

CAAE: 79452217.6.3001.0070

Instituição Proponente: HOSPITAL ALEMAO OSWALDO CRUZ

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.489.636

Apresentação do Projeto:

O presente estudo tem por objetivo avaliar os efeitos da melatonina nas variáveis antropométricas, nos aspectos de sono, hormonais, fisiológicos e bioquímicos de trabalhadoras sobrepesas e obesas que trabalham em turnos noturnos fixos de 12x36 horas. Será realizado um ensaio clínico randomizado duplo cego, do tipo crossover com mulheres profissionais de enfermagem, que trabalham apenas em turnos noturnos fixos, no sistema

de 12x36 horas (12 horas de trabalho noturno e 36 horas de folga), no município de São Paulo/SP. Dentre as aptas a participarem do estudo e que aceitarem participar voluntariamente, será realizada uma randomização estratificada pelo índice de massa corporal (1º estrato com IMC de 25 a 29,9kg/m2; 2º estrato com IMC 30 a 40kg/m2). Dentro de cada estrato, serão randomizadas as participantes do grupo de intervenção e do grupo de

controle da primeira etapa do estudo, com duração de três meses. Posteriormente, será realizada a segunda etapa do estudo (três meses de duração), em que as que foram intervenção na primeira etapa, serão controle na segunda etapa, e vice-versa. Os estratos serão pareados pela faixa etária e função atual de trabalho no hospital. A intervenção consiste no uso da melatonina (dose de 3 mg) somente nos dias de folgas das enfermeiras, ou seja, nos dias que as mesmas realizarem o sono durante a noite. Nos dias de trabalho noturno, a melatonina não será administrada pelas participantes. O grupo-controle será orientado a fazer uso de um comprimido idêntico a melatonina, mas esse será placebo, recebendo as mesmas orientações de uso do grupo

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Bairro: Paraíso **CEP:** 01.323-903
UF: SP **Município:** SAO PAULO
Telefone: (11)3549-0863 **Fax:** (11)3549-0862 **E-mail:** cep@haoc.com.br

intervenção. Por se tratar de um estudo duplo cego, nem as participantes, nem a pesquisadora responsável, saberão quando estarão fazendo parte do grupo intervenção ou do grupo controle. Tendo como referência para cálculo da amostra a realização do teste de comparação de duas médias (amostras relacionadas), um nível de significância de 5% (err prob=0,05), efetividade de 0,3 nos aspectos metabólicos e de sono avaliados e o tamanho mínimo para uma força amostral de 80%, a amostra calculada foi de 70 pessoas. Considerando uma perda de 12%, a amostra será composta por 80 pessoas, sendo 20 em cada grupo (força amostral de 85%). Será utilizado o teste de comparação de duas médias (amostras relacionadas) das variáveis metabólicas e de sono, antes e após intervenção e também para testar a diferença das médias entre os grupos controle e o grupos intervenção. E o teste de proporções para comparar os dois grupos. Em todos os testes será considerado significante o valor de "p" menor que 0,05. Para as análises estatísticas serão utilizados os programas Statistica 12.0 e STATA 12.0 (Stata corp, Texas, USA).

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar os efeitos da melatonina nas variáveis antropométricas de trabalhadoras sobrepesas e obesas que trabalham em turnos noturnos fixos de 12x36 horas.

Objetivo Secundário:

Avaliar os efeitos da melatonina nos aspectos de sono, hormonais, fisiológicos e bioquímicos de trabalhadoras sobrepesas e obesas que trabalham em turnos noturnos fixos de 12x36 horas.

Avaliação dos Riscos e Benefícios:

Riscos:

O presente estudo oferece riscos mínimos à saúde dos participantes, uma vez que a melatonina é um hormônio naturalmente produzido pelo organismo, e o uso da melatonina sintética não altera a produção endógena (Cipolla-Neto et al., 2014).A melatonina pode ser considerada um indutor de sono leve, porém, na dose e horário utilizados no estudo, não se esperam efeitos colaterais na dosagem utilizada no presente estudo (Campos, 2004). Estudos recentes têm demonstrado que a melatonina regula aspectos que influenciam o metabolismo energético, as lipidemias, o peso corporal e o sono; bem como, que o uso da melatonina não está associado a reações adversas ou toxicidade (Medeiros, 2005).

Benefícios:

A prevalência de excesso de peso é elevada, sendo essa ainda maior entre os trabalhadores em

turnos e noturno. Além das comorbidades associadas ao excesso de peso, que possuem um elevado custo de tratamento, a obesidade per se, acarreta inúmeros prejuízos, desde efeitos deletérios à saúde física e sono, como também danos psicossociais e elevado custo médico. Diversos são os tratamentos medicamentosos para o tratamento do excesso de peso, no entanto, esses possuem efeitos colaterais adversos, e nem sempre com resultados satisfatórios. A contribuição científica deste estudo está em avaliar os efeitos do uso da melatonina no metabolismo e no sono de pessoas com excesso de peso e que trabalham em turno noturno, em situação real de vida, apresentando novas evidências, uma vez que nunca foi realizado estudo semelhante no meio científico.

Comentários e Considerações sobre a Pesquisa:

"Será realizado um ensaio clínico randomizado duplo cego, do tipo crossover (Gordis, 2010), para avaliar a efetividade da melatonina nos aspectos metabólicos e de sono de trabalhadoras com excesso de peso que trabalham em turnos noturnos fixos de 12x36 horas".

Considerações sobre os Termos de apresentação obrigatória:

Os termos de apresentação obrigatória encontram-se de acordo.

Conclusões ou Pendências e Lista de Inadequações:

O Comitê de Ética em Pesquisa do Hospital Alemão Oswaldo Cruz de acordo com as atribuições definidas na Resolução CNS nº 466 de 2012 e na Norma Operacional nº 001 de 2013 do CNS, manifesta-se pela APROVAÇÃO deste Projeto de Pesquisa.

Considerações Finais a critério do CEP:

"Lembramos que os pesquisadores deverão enviar relatórios semestrais e relatório final ao CEP/HAOC, via Plataforma Brasil.

"O CEP deverá ser informado sobre qualquer "alteração", "emenda" e sobre quaisquer eventos adversos relacionados ao projeto.

"Cabe ao CEP revisar todos os protocolos de pesquisa envolvendo seres humanos, inclusive os multicêntricos, cabendo-lhe a responsabilidade primária pelas decisões sobre a ética da pesquisa a ser desenvolvida na instituição, de modo a garantir e resguardar a integridade os direitos dos voluntários participantes nas referidas pesquisas.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação

Endereço: Rua João Julião, 331
Bairro: Paraíso **CEP:** 01.323-903
UF: SP **Município:** SAO PAULO
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Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1057150.pdf	07/02/2018 12:41:34		Aceito
Declaração de Pesquisadores	Declaracao_sobre_armazenamento_material_biologico.pdf	07/02/2018 12:18:28	ELAINE CRISTINA MARQUEZE	Aceito
Declaração de Pesquisadores	CEP_HAOC_Respostas_pendencias2.docx	07/02/2018 12:15:13	ELAINE CRISTINA MARQUEZE	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_ECM3.pdf	07/02/2018 12:14:42	ELAINE CRISTINA MARQUEZE	Aceito
Declaração de Pesquisadores	Declaracoes_Prof_Cipolla.pdf	01/02/2018 15:39:26	ELAINE CRISTINA MARQUEZE	Aceito
Cronograma	Cronograma_execucao2.pdf	01/02/2018 15:37:51	ELAINE CRISTINA MARQUEZE	Aceito
Declaração de Pesquisadores	Procedimentos_coleta_dados2.pdf	01/02/2018 15:37:14	ELAINE CRISTINA MARQUEZE	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_pesquisa_Elaine_Marqueze2.pdf	01/02/2018 15:36:27	ELAINE CRISTINA MARQUEZE	Aceito
Declaração de Pesquisadores	Termo_compromisso.pdf	20/12/2017 15:39:28	ELAINE CRISTINA MARQUEZE	Aceito
Declaração de Pesquisadores	Dec_Vinculo.pdf	20/12/2017 15:38:32	ELAINE CRISTINA MARQUEZE	Aceito
Declaração de Pesquisadores	Dec_divulgacao_resultados.pdf	20/12/2017 15:38:13	ELAINE CRISTINA MARQUEZE	Aceito
Declaração de Pesquisadores	Curriculo.pdf	20/12/2017 15:36:33	ELAINE CRISTINA MARQUEZE	Aceito
Declaração de Pesquisadores	Criterio_encerramento.pdf	20/12/2017 15:36:13	ELAINE CRISTINA MARQUEZE	Aceito
Declaração de Instituição e Infraestrutura	CartaAnuenciaProjetoMelatonina.pdf	20/12/2017 15:35:49	ELAINE CRISTINA MARQUEZE	Aceito
Outros	Procedimentos_para_coleta_de_dados.pdf	20/10/2017 17:52:02	ELAINE CRISTINA MARQUEZE	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_pesquisa_ECM.pdf	20/10/2017 17:51:20	ELAINE CRISTINA MARQUEZE	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_ECM.pdf	18/10/2017 21:09:13	ELAINE CRISTINA MARQUEZE	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Documentos_CEP_ECM.pdf	18/10/2017 21:08:51	ELAINE CRISTINA MARQUEZE	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO PAULO, 07 de Fevereiro de 2018

Assinado por:
João Carlos Campagnari
(Coordenador)

Endereço: Rua João Julião, 331
Bairro: Paraíso **CEP:** 01.323-903
UF: SP **Município:** SAO PAULO
Telefone: (11)3549-0863 **Fax:** (11)3549-0862 **E-mail:** cep@haoc.com.br

ANEXO 8 - PARECER DO CEP DO HOSPITAL ALEMÃO OSWALDO CRUZ - PRORROGAÇÃO

HOSPITAL ALEMÃO OSWALDO CRUZ - SP

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito da melatonina no sono e no metabolismo de trabalhadoras noturnas com excesso de peso

Pesquisador: ELAINE CRISTINA MARQUEZE

Área Temática:

Versão: 1

CAAE: 79452217.6.3001.0070

Instituição Proponente: HOSPITAL ALEMAO OSWALDO CRUZ

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.079.085

Apresentação do Projeto:

Apresentação do Projeto:

O presente estudo tem por objetivo avaliar os efeitos da melatonina nas variáveis antropométricas, nos aspectos de sono, hormonais, fisiológicos e bioquímicos de trabalhadoras sobrepesas e obesas que trabalham em turnos noturnos fixos de 12x36 horas. Será realizado um ensaio clínico randomizado duplo cego, do tipo crossover com mulheres profissionais de enfermagem, que trabalham apenas em turnos noturnos fixos, no sistema

de 12x36 horas (12 horas de trabalho noturno e 36 horas de folga), no município de São Paulo/SP. Dentre as aptas a participarem do estudo e que aceitarem participar voluntariamente, será realizada uma randomização estratificada pelo índice de massa corporal (1o estrato com IMC de 25 a 29,9kg/m2; 2o estrato com IMC 30 a 40kg/m2). Dentro de cada estrato, serão randomizadas as participantes do grupo de intervenção e do grupo de controle da primeira etapa do estudo, com duração de três meses. Posteriormente, será realizada a segunda etapa do estudo (três meses de duração), em que as que foram intervenção na primeira etapa, serão controle na segunda etapa, e vice-versa. Os estratos serão pareados pela faixa etária e função atual de trabalho no hospital. A intervenção consiste no uso da melatonina (dose de 3 mg) somente nos dias de folgas das enfermeiras, ou seja, nos dias que as mesmas realizarem o sono durante a noite. Nos dias de trabalho noturno, a melatonina não será administrada pelas participantes. O grupo-controle será orientado a fazer uso de um comprimido idêntico a melatonina, mas esse será placebo, recebendo

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Bairro: Paraiso **CEP:** 01.323-903
UF: SP **Município:** SAO PAULO
Telefone: (11)3549-0863 **Fax:** (11)3549-0862 **E-mail:** cep@haoc.com.br

as mesmas orientações de uso do grupo
intervenção. Por se tratar de um estudo duplo cego, nem as participantes, nem a pesquisadora responsável, saberão quando estarão fazendo parte do grupo intervenção ou do grupo controle. Tendo como referência para cálculo da amostra a realização do teste de comparação de duas médias (amostras relacionadas), um nível de significância de 5% (err prob=0,05), efetividade de 0,3 nos aspectos metabólicos e de sono avaliados e o tamanho mínimo para uma força amostral de 80%, a amostra calculada foi de 70 pessoas. Considerando uma perda de 12%, a amostra será composta por 80 pessoas, sendo 20 em cada grupo (força amostral de 85%). Será utilizado o teste de comparação de duas médias (amostras relacionadas) das variáveis metabólicas e de sono, antes e após intervenção e também para testar a diferença das médias entre os grupos controle e o grupos intervenção. E o teste de proporções para comparar os dois grupos. Em todos os testes será considerado significante o valor de "p" menor que 0,05. Para as análises estatísticas serão utilizados os programas Statistica 12.0 e STATA 12.0 (Stata corp, Texas, USA).

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar os efeitos da melatonina nas variáveis antropométricas de trabalhadoras sobrepesas e obesas que trabalham em turnos noturnos fixos de 12x36 horas.

Objetivo Secundário:

Avaliar os efeitos da melatonina nos aspectos de sono, hormonais, fisiológicos e bioquímicos de trabalhadoras sobrepesas e obesas que trabalham em turnos noturnos fixos de 12x36 horas.

Avaliação dos Riscos e Benefícios:

Riscos:

O presente estudo oferece riscos mínimos à saúde dos participantes, uma vez que a melatonina é um hormônio naturalmente produzido pelo organismo, e o uso da melatonina sintética não altera a produção endógena (Cipolla-Neto et al., 2014).A melatonina pode ser considerada um indutor de sono leve, porém, na dose e horário utilizados no estudo, não se esperam efeitos colaterais na dosagem utilizada no presente estudo (Campos, 2004). Estudos recentes têm demonstrado que a melatonina regula aspectos que influenciam o metabolismo energético, as lipidemias, o peso corporal e o sono; bem como, que o uso da melatonina não está associado a reações adversas ou toxicidade (Medeiros, 2005).

Benefícios:

A prevalência de excesso de peso é elevada, sendo essa ainda maior entre os trabalhadores em turnos e noturno. Além das comorbidades associadas ao excesso de peso, que possuem um elevado custo de tratamento, a obesidade per se, acarreta inúmeros prejuízos, desde efeitos deletérios à saúde física e sono, como também danos psicossociais e elevado custo médico. Diversos são os tratamentos medicamentosos para o tratamento do excesso de peso, no entanto, esses possuem efeitos colaterais adversos, e nem sempre com resultados satisfatórios. A contribuição científica deste estudo está em avaliar os efeitos do uso da melatonina no metabolismo e no sono de pessoas com excesso de peso e que trabalham em turno noturno, em situação real de vida, apresentando novas evidências, uma vez que nunca foi realizado estudo semelhante no meio científico.

Comentários e Considerações sobre a Pesquisa:

"Será realizado um ensaio clínico randomizado duplo cego, do tipo crossover (Gordis, 2010), para avaliar a efetividade da melatonina nos aspectos metabólicos e de sono de trabalhadoras com excesso de peso que trabalham em turnos noturnos fixos de 12x36 horas".

Considerações sobre os Termos de apresentação obrigatória:

adequados, TCLE detalhado e de fácil compreensão.

Apresentação de novo cronograma :Projeto de pesquisa: Efeito da melatonina no sono e no metabolismo de trabalhadoras noturnas com excesso de peso

Pesquisador(a) Principal: Elaine Cristina Marqueze

Pesquisador(a) Responsável - HAOC: Ellen Maria Hagopian

Identificação da Etapa

Início (mm/aaaa)

Término (mm/aaaa)

Submissão ao CEP

10/2017

02/2018

Início da Coleta de Dados/HAOC

02/2018

06/2109

Análise dos Dados

09/2018

06/2019

Conclusão do Estudo

06/2019

06/2019

Fonte: Plataforma Brasil

Atenciosamente,

Profa. Dra. Elaine Cristina Marqueze

Pesquisadora Principal

Faculdade de Saúde Pública da USP

São Paulo, 29 de outubro de 2018.

Justificativas :São Paulo, 29 de outubro de 2018. Justificativa do novo cronograma junto ao CONEP

O estudo está em andamento, mas infelizmente, ainda não foi possível concluí-lo, sendo os problemas descritos a seguir. A lista das colaboradoras nos foi fornecida pelo hospital no final de 02/2018, a qual continha as informações necessárias para iniciar o campo. Por uma solicitação da coordenação do hospital, a apresentação do projeto de pesquisa às colaboradoras não pode ser realizada via uma palestra para todas, conforme previsto no projeto inicial. A apresentação teve que ocorrer em cada setor, somente no horário das 01:30h às 05:00h, para não atrapalhá-las em seu trabalho (de 02 a 04/2018). Cada setor visitado tinha em torno de duas/três colaboradoras e o tempo médio de cada apresentação foi de 40 minutos. Um total de 238 colaboradoras foram abordadas, dessas 149 (62,6%) não puderam participar do estudo devido aos critérios de inclusão e exclusão, 29 (12,2%) não tiveram interesse em participar, 3 (1,3%) iniciaram o estudo mas desistiram, 27 (11,3%) iniciaram o estudo até esse momento, e 30 (12,6%) aceitaram participar do estudo. Devido ao período de férias das colaboradoras, a participação dessas 30 colaboradoras ocorrerá da seguinte maneira: 5 em Junho/2018, 4 em Julho/2018, 9 em Agosto/2018, 8 em Outubro/2018 e 4 em Novembro/2018. Dessa forma, o final da coleta de dados será em 05/2019, uma vez que o protocolo de estudo tem a duração de seis meses.

Diante do exposto acima, justificamos a prorrogação da pesquisa com o objetivo de atingir o poder amostral conforme previsto no projeto, o que implicaria em continuar coletando dados, assim como realizar todas as etapas previstas, as quais sofreram atrasos devido às exigências impostas pelo local de coleta. Vale ressaltar ainda que a coleta durante a intervenção irá gerar maior volume de dados e, portanto, exigirá maior tempo de análise.

Atenciosamente,

Profa. Dra. Elaine Cristina Marqueze

Pesquisadora Principal

Endereço: Rua João Julião, 331
Bairro: Paraíso **CEP:** 01.323-903
UF: SP **Município:** SAO PAULO
Telefone: (11)3549-0863 **Fax:** (11)3549-0862 **E-mail:** cep@haoc.com.br

Faculdade de Saúde Pública da USP.

Consideradas pertinentes e adequadas

Conclusões ou Pendências e Lista de Inadequações:

Sem pendências ou inadequações

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_ECM3.pdf	29/10/2018 16:49:33	ELAINE CRISTINA MARQUEZE	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_pesquisa_Elaine_Marqueze 2.pdf	29/10/2018 16:49:11	ELAINE CRISTINA MARQUEZE	Aceito
Outros	NovoCronogramaHAOC.pdf	29/10/2018 16:18:05	ELAINE CRISTINA MARQUEZE	Aceito
Outros	Justificativa.pdf	29/10/2018 16:16:30	ELAINE CRISTINA MARQUEZE	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

SAO PAULO, 12 de Dezembro de 2018

Assinado por:
Edmir Felix da Silva Junior
(Coordenador(a))

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