

Universidade Federal do Rio Grande do Sul  
Faculdade de Medicina  
Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento

Débora Barrogi Constantino

INVESTIGAÇÃO DE PARÂMETROS CIRCADIANOS ASSOCIADOS A  
ALTERAÇÕES METABÓLICAS E OBESIDADE

Porto Alegre, 2021

Débora Barrogi Constantino

INVESTIGAÇÃO DE PARÂMETROS CIRCADIANOS ASSOCIADOS A  
ALTERAÇÕES METABÓLICAS E OBESIDADE

Dissertação apresentada como requisito parcial para a obtenção do título de Mestre em Psiquiatria e Ciências do Comportamento, à Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento.

Orientadora: Professora Doutora Maria Paz Loayza Hidalgo

Coorientadora: Doutora Luísa Klaus Pilz

Porto Alegre, 2021

CIP - Catalogação na Publicação

Constantino, Débora  
Investigação de Parâmetros Circadianos Associados a  
Alterações Metabólicas e Obesidade / Débora  
Constantino. -- 2021.  
105 f.  
Orientadora: Maria Paz Loayza Hidalgo.

Coorientadora: Luísa Klaus Pilz.

Dissertação (Mestrado) -- Universidade Federal do  
Rio Grande do Sul, Faculdade de Medicina, Programa de  
Pós-Graduação em Psiquiatria e Ciências do  
Comportamento, Porto Alegre, BR-RS, 2021.

1. Ritmos Circadianos. 2. Metabolismo. 3.  
Obesidade. 4. Urbanização. I. Loayza Hidalgo, Maria  
Paz, orient. II. Klaus Pilz, Luísa, coorient. III.  
Título.

## **Agradecimentos**

À minha mãe, Artemise, pessoa mais importante da minha vida, agradeço por nunca medir esforços para apoiar meus sonhos, por me amparar, por me dar clareza e por ser o maior exemplo de dedicação e amor que eu poderia ter. Ao meu pai, Marcos, por todo apoio e incentivo que sempre recebi e por sempre demonstrar seu orgulho. À Laura, Sylvia, avó Magali por todo companheirismo, paciência e carinho comigo.

À minha orientadora Maria Paz Loayza Hidalgo, por ter me incentivado a acreditar na minha capacidade, por todas as oportunidades e dedicação ao grupo. À minha coorientadora Luísa Klaus Pilz, agradeço por toda a paciência, pela disponibilidade para me ensinar, por confiar em mim e por ter me apresentado caminhos lindos na ciência. Foi um prazer enorme trabalhar contigo!

A todos os colegas do Laboratório de Cronobiologia e Sono que, mesmo fisicamente distantes durante esse período, sempre estiveram presentes, tornando minhas rotinas mais leves e contribuindo imensamente para minha formação. Agradeço especialmente ao André, Nicoli, Mel, Gui e Ju Freitas por sempre estarem sempre “ali” quando precisei. O apoio e companhia de vocês é extremamente importante para mim.

À Laura, Carol e Jé pela amizade de mais de 10 anos. Sou muito grata por poder compartilhar minha vida com vocês há tanto tempo e por toda sinceridade, carinho e amor que temos umas com as outras. À Bruna, Amanda, Júlio e Brenda por terem me acompanhado desde a graduação, dividido comigo todos os momentos bons e ruins que envolvem fazer uma pós-graduação e pelas conversas diárias que sempre melhoram o dia! Morro de saudades de todos. Ao Carlos, que foi uma grata surpresa na minha vida nos últimos tempos, por me querer sempre bem e ser um lugar de calma, conforto e carinho quando eu mais preciso.

À equipe da Unidade de Experimentação Animal do HCPA, por todo ensinamento e apoio durante os experimentos. Ao PPG em Psiquiatria e Ciências do Comportamento da UFRGS por possibilitar a realização do meu mestrado.

A todos amigos, colegas e familiares que não foram contemplados aqui, mas que contribuíram de alguma forma para a minha formação e fizeram parte da minha vida, agradeço a tudo que fizeram por mim. Muito obrigada!

## RESUMO

**Relevância:** Oscilações comportamentais e fisiológicas diárias são uma característica fundamental da biologia de mamíferos. Para permitir que estes ritmos estejam sincronizados com o ambiente externo, mamíferos desenvolveram um relógio circadiano endógeno que responde a pistas ambientais, como o ciclo claro-escuro e os horários de alimentação. No entanto, com a adoção de um estilo de vida moderno, caracterizado por exposição à luz inadequada, jetlag social, hábitos alimentares irregulares, sedentarismo e estresse, a organização dos ritmos circadianos tem sido ameaçada. Estas mudanças ambientais contribuem para o desenvolvimento de diversas patologias, incluindo depressão, obesidade, doenças cardiovasculares e câncer.

**Objetivos:** avaliar a influência de diferentes padrões de iluminação sobre a fisiologia e metabolismo de ratas Wistar. Além disso, identificar associações entre padrões de ritmos de atividade-reposo, exposição à luz e urbanização com índice de massa corporal em comunidades rurais e urbanas.

**Métodos:** Estudo 1: ratas Wistar foram alocadas desde a gestação em quatro padrões de iluminação: combinação de cores RGB (vermelho, *red*; verde, *green*; azul, *blue*) que variam sua composição espectral ao longo do dia (RGB-v; N = 14), RGB durante o fotoperíodo com uma temperatura de cor fixa (RGB-f; N = 13), escuro constante (DD; N = 13) e claro constante (LL; N = 15). Os experimentos foram realizados em fêmeas desde o dia pós-natal (PND) 22 ao 50. Peso corporal, abertura vaginal, ciclicidade estral e parâmetros metabólicos séricos foram mensurados. Estudo 2: Foram coletados dados antropométricos de moradores de comunidades quilombolas rurais e urbanas para calcular o índice de massa corporal, que foi categorizado em:  $\geq 18.5$  kg/m<sup>2</sup> to  $< 25$  kg/m<sup>2</sup> = peso normal;  $\geq 25$  kg/m<sup>2</sup> to  $< 30$  kg/m<sup>2</sup> = sobrepeso;  $\geq 30$  kg/m<sup>2</sup> = obesidade. Os participantes também foram questionados a respeito das suas rotinas de sono e exposição à luz utilizando o Questionário de Cronotipos de Munique (n = 244). Por fim, foram analisados dados de actimetria de 121 participantes com 7 dias de registros consecutivos.

**Resultados:** Estudo 1: o grupo RGB-f apresentou o primeiro ciclo estral completo mais cedo quando comparado ao grupo RGB-v. Fêmeas expostas ao claro constante apresentaram peso corporal mais baixo do PND 33 ao 47 quando comparadas ao restante dos grupos. Foram observados níveis plasmáticos de triglicerídeos maiores no grupo exposto ao escuro constante, quando comparado aos grupos RGB-v e RGB-f. O colesterol total foi mais baixo no

grupo RGB-v em relação aos outros grupos. Por fim, a gordura visceral foi maior no grupo RGB-f quando comparado ao grupo LL. Estudo 2: Viver em áreas mais urbanizadas e ter maior variabilidade nos ritmos de atividade-repouso foram associados a maior risco para sobrepeso/obesidade, controlando para sexo e idade. **Conclusões:** Este trabalho contribui para o conhecimento sobre o papel da exposição à luz e das características dos ritmos circadianos sobre o desenvolvimento de alterações metabólicas e obesidade. Além disso, estes resultados apontam para novas estratégias na prevenção da obesidade e promoção de perfis metabólicos saudáveis. **Apoio Financeiro:** FIPE/HCPA, CNPq, CAPES.

**Palavras-chave:** Ritmos Circadianos; Metabolismo; Obesidade; Exposição à Luz.

## ABSTRACT

**Relevance:** Daily behavioral and physiological fluctuations are a fundamental feature of the mammalian biology. For synchronizing these rhythms with the external environment, mammals have developed an endogenous circadian clock that responds to environmental cues such as the light-dark cycle and feeding schedules. However, with the adoption of modern lifestyles, characterized by inadequate light exposure, social jetlag, irregular eating habits, sedentary lifestyle and stress, the organization of circadian rhythms has been threatened. These environmental changes contribute to the development of several pathologies, including depression, obesity, cardiovascular disease and cancer.

**Objectives:** to evaluate the influence of different lighting patterns on the physiology and metabolism of Wistar rats. Furthermore, to identify associations between patterns of activity-rest rhythms, light exposure and urbanization with body mass index in rural and urban communities.

**Methods:** Study 1: Female Wistar rats were allocated from gestation into four lighting patterns: RGB color combination (red, red; green, green; blue, blue) varying their spectral composition throughout the day (RGB-v; N = 14), RGB during the photoperiod with a fixed color temperature (RGB-f; N = 13), constant dark (DD; N = 13) and constant light (LL; N = 15). The experiments were performed on females from postnatal day (PND) 22 to 50. Body weight, vaginal opening, estrous cyclicity and serum metabolic parameters were measured. Study 2: Anthropometric data were collected from residents of rural and urban quilombolas communities to calculate the body mass index, which was then categorized into:  $\geq 18.5 \text{ kg/m}^2$  to  $< 25 \text{ kg/m}^2$  = normal weight;  $\geq 25 \text{ kg/m}^2$  to  $< 30 \text{ kg/m}^2$  = overweight;  $\geq 30 \text{ kg/m}^2$  = obesity. Participants were also asked about their sleep routines and light exposure using the Munich Chronotype Questionnaire (n = 244). Finally, actimetry data from 121 participants with 7 days of consecutive records were analyzed.

**Results:** Study 1: the RGB-f group presented the first complete estrous cycle earlier when compared to the RGB-v group. Females exposed to constant light had lower body weight from PND 33 to 47 when compared to the rest of the groups. Higher plasma triglyceride levels were observed in the group exposed to constant dark, when compared to the RGB-v and RGB-f groups. Total cholesterol was lower in the RGB-v group than in the other groups. Finally, visceral fat was higher in the RGB-f group when compared to the LL group. Study 2: Living in more urbanized areas and having higher variability in activity-rest rhythms were associated with a higher risk of overweight/obesity, controlling for sex and age.

**Conclusions:** This work contributes to the knowledge about the role of

light exposure and the circadian rhythms features on the development of metabolic alterations and obesity. Furthermore, these results point to new strategies aiming to prevent obesity and promote healthy metabolic profiles. **Financial Support:** FIPE/HCPA, CNPq, CAPES.

**Keywords:** Circadian Rhythms; Metabolism; Obesity; Light Exposure.



## LISTA DE ILUSTRAÇÕES DA REVISÃO DA LITERATURA

**Figura 1.** Representação dos ritmos circadianos de alguns processos fisiológicos e bioquímicos (página 15). Adaptada de Skene; Arendt, 2006.

**Figura 2.** Corte coronal do núcleo supraquiasmático de um roedor, mostrando a região ventral e a região dorsal (página 16). Adaptado de Karatsoreos et al, 2004.

**Figura 3.** Componentes moleculares do relógio circadiano de mamíferos (página 17). Adaptado de Masri; Sassone-Corsi, 2018.

**Figura 4:** Distribuição de cronotipos na população geral (página 19). Adaptado de Roenneberg et al.

**Figura 5:** Curva de fase-resposta (PRC) da atividade motora de um roedor (página 20). Adaptado de Moore-Ede, M. C., Sulzman, F. M., & Fuller, C. A. (1982). *The clocks that time us: Physiology of the circadian timing system*. Cambridge, MA: Harvard University Press.

**Figura 6:** Sincronização do sistema circadiano pela luz e pela alimentação (página 21). Adaptado de Laermans; Depoortere, 2016.

**Figura 7:** Regulação da síntese de melatonina pela glândula pineal (página 23). Adaptado de Shinari; Loius, 2009.

**Figura 8:** Relógios circadianos regulam o metabolismo de glicose, sensibilidade à insulina e secreção de insulina (página 25). Adaptado de Stenvers et al., 2018.

**Figura 9:** Esquema das alterações metabólicas e circadianas encontradas em roedores submetidos a diferentes protocolos de iluminação (página 31). Adaptado de Plano et al., 2017.

**Figura 10:** Representação do impacto da luz à noite e de padrões alterados de alimentação nos ritmos circadianos e metabolismo de roedores noturnos e humanos (página 32). Adaptado de Plano et al., 2017.

## **LISTA DE ABREVIATURAS EM PORTUGUÊS DA REVISÃO DA LITERATURA**

GSK-3 $\beta$  – glicogênio sintase quinase 3 beta

HPG – hipotálamo-pituitária-gônadas

IMC – índice de massa corporal

LAN – luz à noite

NSQ – núcleo supraquiasmático

SNA – sistema nervoso autônomo

## **LISTA DE ABREVIATURAS EM INGLÊS DA REVISÃO DA LITERATURA**

AANAT - Aralkylamine N-acetyltransferase

BMAL - Brain and muscle ARNT-like protein

CLOCK - Circadian Locomotor Ouptup Cycles Kaput

CRY - Cryptochrome protein

FSH - Follicle stimulating hormone

GnRH - Gonadotropin-releasing hormone

HIOMT - Hydroxyindol-O-metiltransferase

ipRGC - Intrinsically photosensitive retinal ganglion cells

LH - Luteinizing hormone

PER - Period protein

PRC – Phase response curve

ROR - Retinoic acid receptor-related orphan receptors

TRE – Time restricted eating

## SUMÁRIO

<b>1. INTRODUÇÃO</b> .....	13
<b>2. REVISÃO DA LITERATURA</b> .....	15
2.1 O SISTEMA CIRCADIANO E A REGULAÇÃO DOS RITMOS BIOLÓGICOS .....	15
2.2 REGULAÇÃO CIRCADIANA DO SISTEMA REPRODUTIVO.....	24
2.3 REGULAÇÃO CIRCADIANA DO METABOLISMO.....	25
2.4 CRONODISRUÇÃO E METABOLISMO .....	29
<b>3. JUSTIFICATIVA</b> .....	35
<b>4. HIPÓTESE</b> .....	36
<b>5. OBJETIVOS</b> .....	37
5.1 OBJETIVOS GERAIS.....	37
5.2 OBJETIVOS ESPECÍFICOS – ARTIGO 1 .....	37
5.3 OBJETIVOS ESPECÍFICOS – ARTIGO 2 .....	37
<b>6. ARTIGO 1</b> .....	38
<b>7. ARTIGO 2</b> .....	57
<b>8. CONCLUSÕES E CONSIDERAÇÕES FINAIS</b> .....	93
<b>REFERÊNCIAS DA REVISÃO DA LITERATURA</b> .....	95
<b>ANEXO A - Carta de aprovação do projeto pela CEUA/HCPA referente ao Artigo 1</b> .....	104
<b>ANEXO B – Comprovante de recebimento do Artigo 1 pela revista <i>Physiology and Behavior</i></b> .....	105

## 1. INTRODUÇÃO

Durante os últimos 3 bilhões de anos, o sistema circadiano, nome dado à variação nas funções biológicas de diversos seres vivos, evoluiu na presença de luz natural e escuridão. O sucesso evolutivo depende de duas atribuições: prever mudanças regulares que se repetem no ambiente e responder e se adaptar a essas mudanças para garantir a sobrevivência (Bell-Pedersen et al., 2005). Assim, nossos ancestrais recebiam fortes pistas ambientais para sincronizar seus ritmos endógenos e organizar suas rotinas de acordo com as flutuações de fatores externos (ciclo claro-escuro, horários de alimentação e exercício físico). Esta sincronização é essencial para manter a saúde e um bom funcionamento das funções fisiológicas do corpo (Duffy; Czeisler, 2010). Desde a invenção da luz elétrica, há pouco mais de 100 anos atrás, seguida pela invenção da televisão, da Internet e de tecnologias relacionadas, a forma como o ser humano interage com a luz se transformou drasticamente.

Na sociedade moderna, a maioria das pessoas passa a maior parte do tempo em ambientes fechados, expostas a luzes artificiais de baixa intensidade durante o dia e ausência de escuridão durante a noite. Além disso, as sociedades industrializadas também se caracterizam pelo maior consumo de cafeína, maior acesso a alimentos, uso rotineiro de despertadores, agendas de trabalho noturnas (Touitou et al., 2017). Consequentemente, a força das pistas externas que sincronizam nossos ritmos é reduzida e o relógio circadiano da maioria das pessoas sofre mudanças de fase, tornando-as mais vespertinas, enquanto as agendas de trabalho se mantêm similares (Roenneberg; Mellow, 2016).

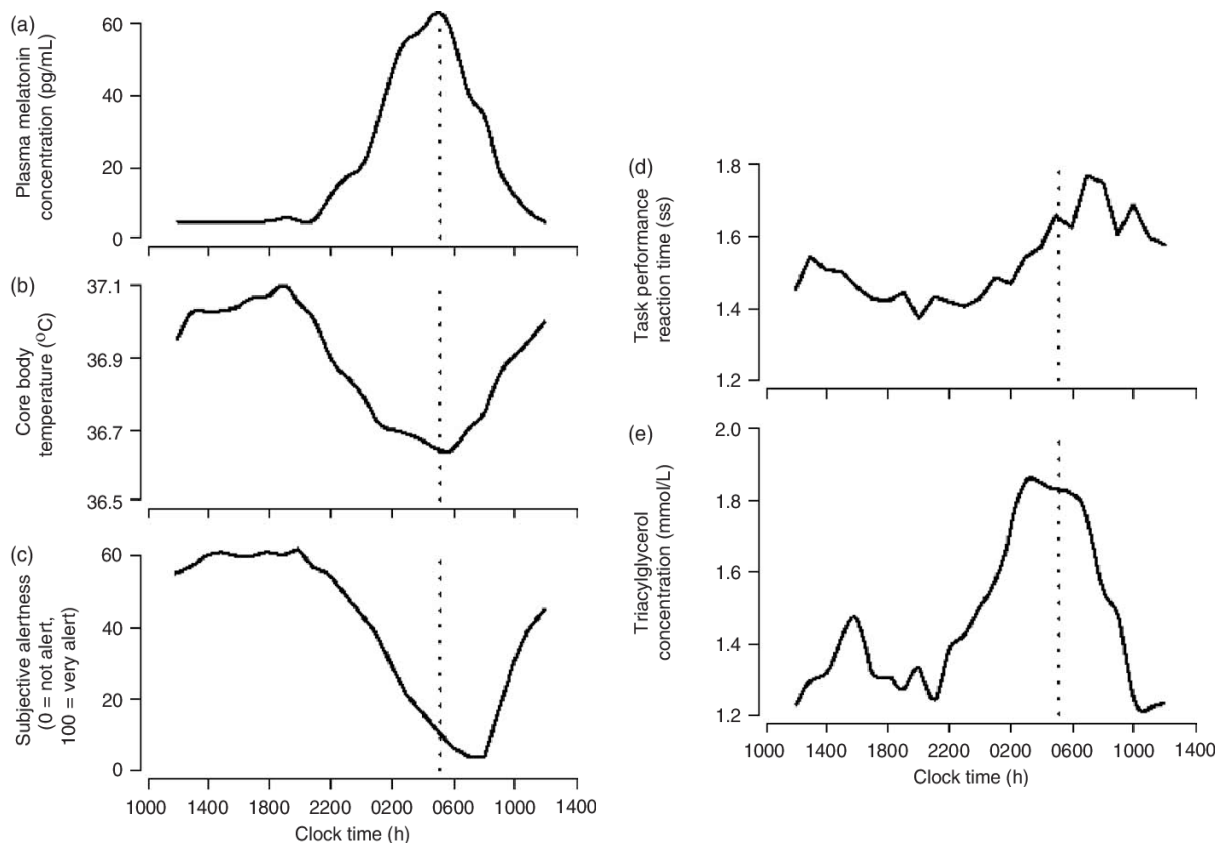
Em condições naturais, os ritmos endógenos são sincronizados com as variações de fatores externos (ciclo claro-escuro, horário de alimentação e rotinas sociais). No entanto, sob rotinas sociais que vão contra o relógio endógeno, a sincronização dos ritmos internos é afetada, podendo causar prejuízos à saúde (Albrecht; Rippeger, 2018). De fato, diversos estudos mostram que a cronodisrupção, isto é, o estado de desalinhamento entre ritmos circadianos internos e pistas externas, está associado a uma série de doenças, como depressão, diabetes, obesidade, doenças neurodegenerativas e câncer (Rüger; Scheer, 2009). Ainda não se sabe quais os mecanismos por trás da relação entre cronodisrupção e ganho de peso, mas diversos estudos experimentais e epidemiológicos demonstram que exposição irregular à luz e padrões alterados nos ritmos de atividade-reposo associam-se a piores desfechos metabólicos (Rybnikova et al., 2016; Froy, 2012). Deste modo,

destaca-se a importância de entender como a exposição à luz e o estilo de vida de centros urbanos impactam a fisiologia e o metabolismo, de modo a auxiliar na busca por estratégias que busquem prevenir o aumento nas taxas de obesidade e doenças metabólicas.

## 2. REVISÃO DA LITERATURA

### 2.1 O SISTEMA CIRCADIANO E A REGULAÇÃO DOS RITMOS BIOLÓGICOS

A rotação da Terra em torno do seu próprio eixo causa alterações cíclicas e previsíveis na luz e na temperatura de ambientes naturais. Como resposta adaptativa a estas mudanças dinâmicas, a seleção natural favoreceu a evolução de organismos capazes de antecipar as mudanças ambientais diárias e, assim, adaptar o seu comportamento e a sua fisiologia para os horários apropriados do dia (Panda et al., 2002). A temperatura corporal, o fluxo sanguíneo, a produção de urina, os níveis hormonais, as taxas metabólicas e o ciclo sono-vigília são exemplos de processos fisiológicos e bioquímicos que apresentam flutuações, isto é, elevam-se e declinam-se de acordo com os horários do dia (Aschoff, 1981; Skene; Arendt, 2006) (**Figura 1**). Todos estes ritmos são orquestrados por uma organização temporal endógena, conhecida como sistema circadiano (do Latim *circa* = “cerca de” e *diem* = “dia”), que está presente em praticamente todas as formas de vida; desde bactérias a plantas, invertebrados e mamíferos. Os ritmos circadianos são os mais estudados na literatura, embora existam outros ritmos na natureza que se repetem com um ciclo maior de 24 horas, os infradianos, enquanto outros se repetem com um ciclo menor, os ultradianos (Laje et al., 2018).

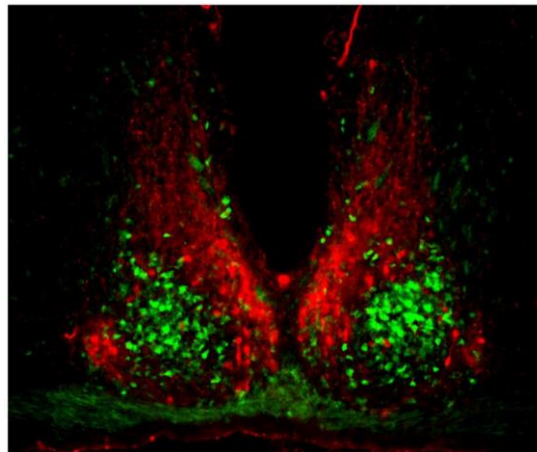


**Figura 1:** Representação dos ritmos circadianos de melatonina plasmática (a), temperatura corporal (b), estado de alerta subjetivo (c), tempo de reação no desempenho de tarefa (d) e concentração de triacilgliceróis (e). Em condições normais, estes ritmos são caracterizados por um horário de pico e nadir em um intervalo de tempo definido (relação de fase) entre os diferentes ritmos. Adaptada de Skene; Arendt, 2006.

Inicialmente, o sistema circadiano era visto como uma capacidade difusa de medição do tempo do organismo como um todo. A partir de 1960, Pittendrigh divulgou o conceito de que existe um oscilador sensível à luz capaz de servir como um “marcapasso” do organismo (Saunders, 2016). A partir de então, começou-se uma investigação, por meio de experimentos com lesões cerebrais em roedores, de estruturas que poderiam ser responsáveis por sincronizar as funções fisiológicas. Estes experimentos levaram a identificação do núcleo supraquiasmático (NSQ), estrutura composta por um grupo de neurônios especializados e localizada no hipotálamo anterior, logo acima do quiasma óptico (Stephan; Zucker, 1972) (**Figura 2**). O papel desta estrutura foi demonstrado quando neurofisiologistas submeteram roedores à lesão cirúrgica deste núcleo e registraram sua atividade locomotora. Como resultado, detectou-se o desaparecimento da ritmicidade, ou seja, os momentos de atividade locomotora tornaram-se aleatórios. Os



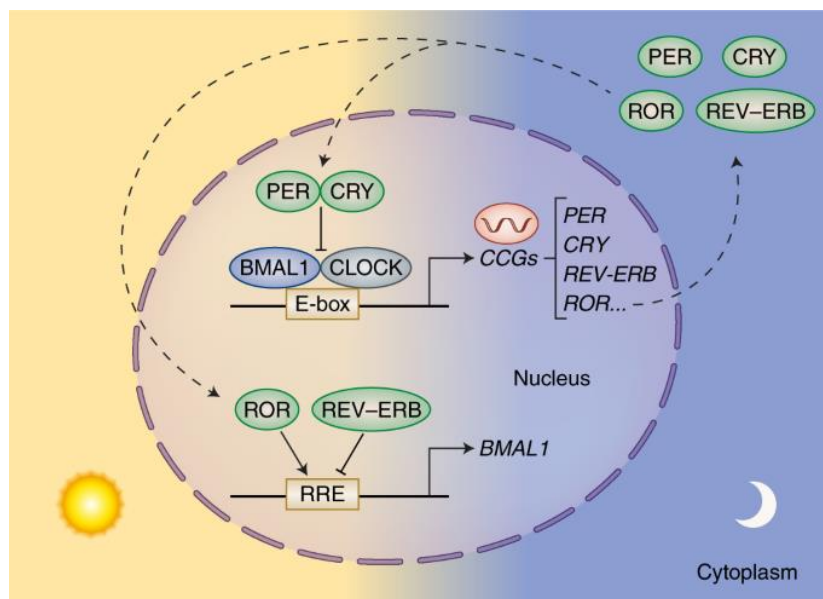
mesmos resultados são obtidos para outras funções circadianas além da atividade locomotora; ritmos endócrinos também são abolidos (Moore; Eichler, 1972). Desta forma, concluiu-se que o núcleo supraquiasmático é o local de regulação primária da ritmicidade circadiana, conferindo periodicidade às diversas funções fisiológicas e comportamentais dos mamíferos.



**Figura 2:** Corte coronal do núcleo supraquiasmático de um roedor, mostrando a região ventral (em verde) e a região dorsal (em vermelho). Adaptado de Karatsoreos et al, 2004.

A regulação do sistema circadiano a nível molecular é bem conhecida e seu esclarecimento rendeu o Prêmio Nobel de Medicina e Fisiologia em 2017 para três pesquisadores da área (The Nobel Prize in Physiology or Medicine 2017. NobelPrize.org. Nobel Prize Outreach AB 2021. Wed. 28 Jul 2021. <https://www.nobelprize.org/prizes/medicine/2017/summary/>). A maquinaria circadiana dos mamíferos é controlada por dois ‘*loops de feedback*’ (alças de retroalimentação) positivos e negativos que interagem a nível de transcrição gênica, tradução e pós-tradução (**Figura 3**). Estes *loops* consistem em proteínas que estimulam ou inibem a sua própria síntese a partir da sua interação com os genes-relógio. Foram identificados 14 genes centrais do relógio circadiano de mamíferos, pertencentes às famílias *Per*, *Cry*, *Bmal*, *Clock*, *Ror* e *Rev-Erb* (Ye et al., 2018). As proteínas CLOCK e BMAL (sintetizadas a partir dos genes *Clock* e *Bmal*) associam-se como heterodímeros e ativam a síntese de duas famílias de proteínas PER e CRY. Estas últimas são reguladores negativos, que inibem a sua própria síntese a partir dos genes *Per* (*Per1*, *Per2*, *Per3*) e *Cry* (*Cry 1*, *Cry 2*). A síntese das proteínas PER e CRY só reinicia (sob a influência de BMAL e CLOCK) quando seus níveis intracelulares são baixos o suficiente, ou seja, após

a degradação destas proteínas. BMAL1 e CLOCK também promovem a transcrição dos genes precursores da proteína REV-ERB, que inibe a transcrição do gene *Bmal1* (Eide et al., 2005; Ko; Takahashi, 2006; He et al., 2016). Estes eventos moleculares são regulados por uma série de mecanismos. As proteínas PER e CRY são fosforiladas por várias enzimas, como por exemplo a GSK-3B (Glicogênio sintase quinase – 3 beta), o que influencia sua estabilidade e sua taxa de entrada no núcleo (Hirota et al., 2008). Estes *loops* ocorrem de forma concomitante produzindo oscilações com período de 24 horas (Balsalobre et al, 1998).

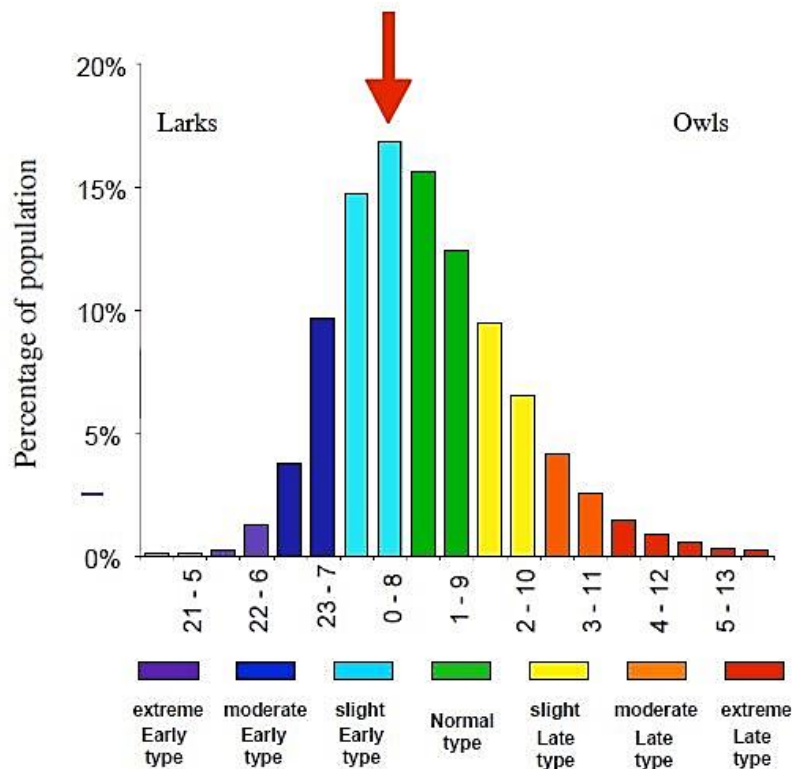


**Figura 3:** Componentes moleculares do relógio circadiano de mamíferos e regulação da ritmicidade pelos ‘*loops de feedback*’ de transcrição e tradução dos genes do relógio dentro de um período de 24 horas. Adaptado de Masri; Sassone-Corsi, 2018.

A sincronia entre o relógio interno e fatores ambientais externos é fundamental para a sobrevivência e bem-estar dos animais. Para isso, as células osciladoras do núcleo supraquiasmático devem receber sinalização de células que detectam as variações ambientais para integrar esta informação e enviar sinais apropriados aos tecidos (Morse; Sassone-Corsi, 2002). Assim, pela ação de vias de sinalização de entrada (aférentes), marcapassos e vias de sinalização de saída (eferentes), os relógios biológicos são ajustáveis ao ambiente. Estes são os três componentes principais do sistema circadiano que permitem a operação de funções e comportamentos em ritmos sincronizados com os ciclos externos (Dibner et al., 2010). Os sinais ou pistas provenientes do ambiente são conhecidos como *zeitgebers* (do alemão “doador de tempo”).

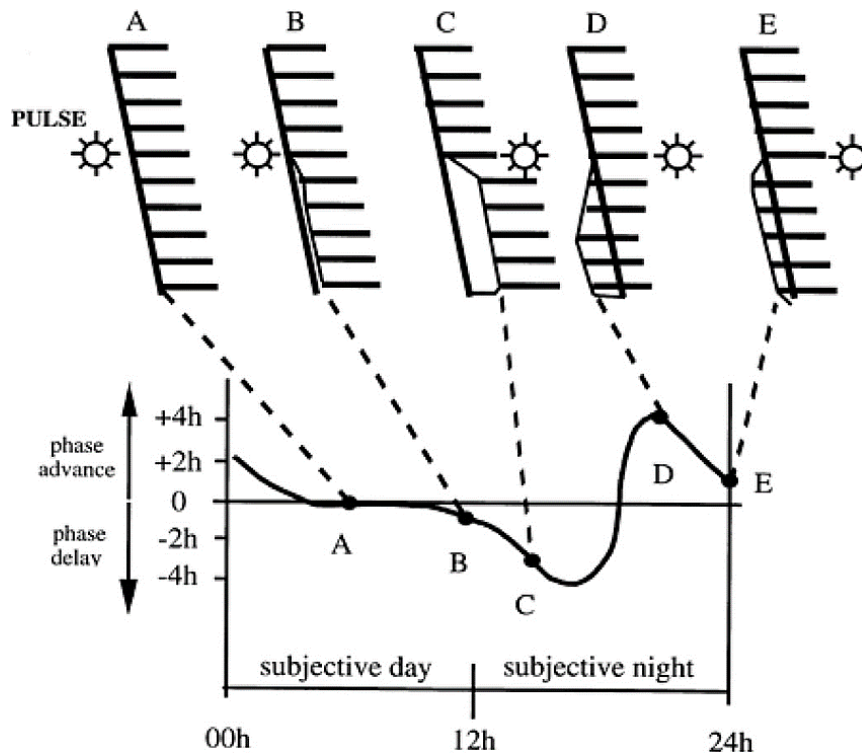
A principal pista responsável pela sincronização do sistema circadiano de mamíferos é a luz. Estes recebem informação luminosa principalmente pela retina, onde as células ganglionares intrinsecamente fotossensíveis (ipRGCs) detectam mudanças de luminosidade do ambiente através de um pigmento fotossensível, chamado melanopsina. A luz é captada pela melanopsina das ipRGCs, cujos axônios (excitatórios) estendem-se até o NSQ, via trato retino-hipotalâmico, e ativam os neurônios do NSQ (Berson et al., 2002; Kuse et al., 2014). Estes, portanto, aumentam sua taxa de disparo durante o dia e diminuem durante a noite (Welsh et al., 1995). Por fim, esta informação é transmitida, via axônios eferentes que emergem do NSQ, para diversas regiões do cérebro, incluindo a área pré-óptica e o núcleo paraventricular do hipotálamo e, via secreção de neuropeptídeos e sinalização humoral, para osciladores presentes em diversos órgãos periféricos (Buijs et al., 2003; Hastings et al., 2003).

Para terem valor adaptativo, os relógios endógenos precisam ser sincronizados aos dias externos, uma vez que o período circadiano intrínseco pode ser de *cerca de* 24 horas, sendo o da maioria das pessoas um pouco mais longo (aproximadamente 11 minutos) (Czeisler et al., 1999). Dependendo do período endógeno e do ciclo claro-escuro externo, a sincronização aos ciclos de 24 horas pode produzir diferentes relações de fase entre relógio endógeno e tempo externo, ou seja, diferentes relações temporais do indivíduo com o *zeitgeber* – ciclo claro-escuro. Esta variabilidade de relações de fase também descreve as diferenças interindividuais nos horários de dormir e acordar e caracteriza o que se chama de cronotipo (Morrow et al., 2003). Os cronotipos variam amplamente dentro da população, criando uma distribuição que varia de cronotipos matutinos extremos até cronotipos vespertinos extremos, com a maioria da população localizada entre estes extremos (**Figura 4**). Os fatores que produzem estas diferenças interindividuais são polimorfismos genéticos em genes do relógio, idade e pistas ambientais, especialmente exposição à luz (Roenneberg; Morrow, 2016).



**Figura 4:** Distribuição de cronotipos na população geral. Existem poucas pessoas que são extremamente matutinas ou extremamente vespertinas. A seta vermelha indica onde a maior parte da população se encontra: entre os extremos e com um cronotipo intermediário. Este grupo dorme, em média, das 00:00 às 8h. Adaptado de Roenneberg et al.

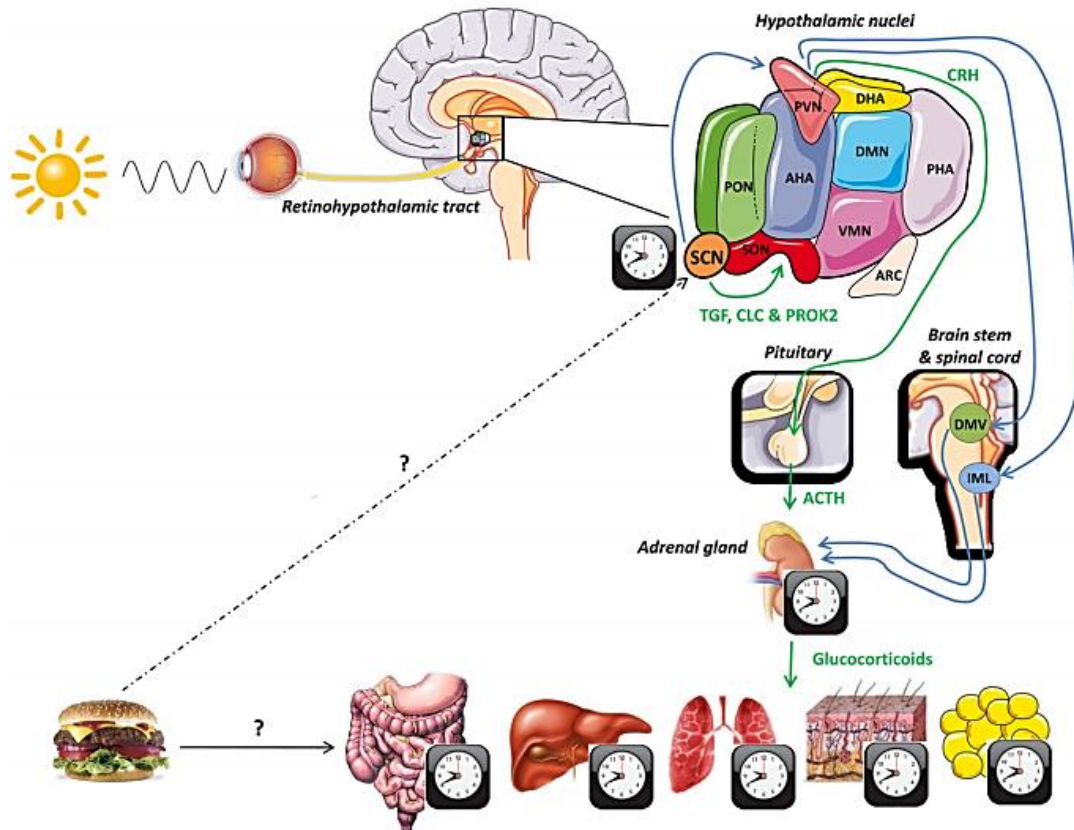
Estudos sobre os efeitos da luz no sistema circadiano de insetos, plantas e animais demonstraram que o momento de um estímulo luminoso possui uma influência importante na resposta das atividades rítmicas a este estímulo (De Coursey, 1960; Pittendrigh, 1960). A curva de fase-resposta (PRC, do inglês *phase response curve*) é uma ferramenta da Cronobiologia que descreve a magnitude e a direção das mudanças de fase dos ritmos circadianos conforme o horário de exposição a um *zeitgeber* (Pittendrigh; Daan, 1976). Por exemplo, em humanos a PRC para luz é caracterizada por avanços de fase quando a exposição a este estímulo ocorre durante a manhã, atrasos de fase quando a exposição acontece durante o fim da tarde/noite e respostas menores ou insignificantes quando a exposição é feita no meio do dia (Dumont; Carrier, 1997; Kripke et al., 2007; Revell et al., 2005) (**Figura 5**). Estas mudanças de fase também são fortemente influenciadas pela intensidade, duração e comprimento de onda da luz (Boivin et al., 1996; Dewan et al., 2011; Rüger et al., 2013).



**Figura 5:** Curva de fase-resposta (PRC) da atividade motora de um roedor. O mesmo pulso de luz administrado em 5 momentos diferentes (A, B, C, D, E) produz diferentes respostas: nenhum efeito (A), atraso de fase (B, C) e avanço de fase (D, E). Adaptado de Moore-Ede et al (1982).

Embora o núcleo supraquiasmático (NSQ) seja usualmente conhecido como “marcapasso central”, a presença de relógios funcionais em diversos tecidos do corpo é indispensável para a manutenção dos ritmos circadianos. Isso explica o fato de osciladores circadianos estarem presentes senão em todas, na maioria das células do corpo (Yoo et al., 2004). É importante considerar, no entanto, que apesar do termo “periférico”, estes relógios estão presentes em outros locais do sistema nervoso central (fora do NSQ). Em condições normais, o SCN fica no topo da hierarquia circadiana transmitindo informação temporal para todos os relógios periféricos (Buijs et al., 2013). Na ausência de NSQ, a ritmicidade das funções é perdida e os tecidos periféricos perdem a relação de fase de um em relação ao outro. No entanto, interessante, a disponibilidade restrita de alimentos em horários programados (TRE, do inglês *time restricted eating*) é capaz de organizar os ritmos circadianos na ausência de NSQ, o que indica que pistas relacionadas à comida podem ser potentes sincronizadores dos ritmos endógenos (Stephan, 2002) (**Figura 6**). Além disso, experimentos *in vitro* também demonstraram mudanças de fase

na expressão de *clock genes* no fígado, rim, pâncreas e outros tecidos em animais submetidos ao TRE (Kohsaka et al., 2007).

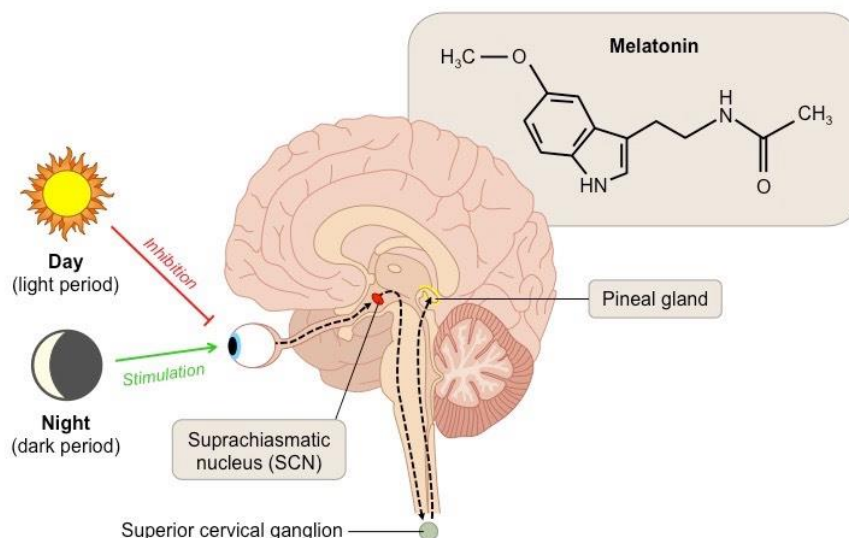


**Figura 6:** Sincronização do sistema circadiano pela luz e pela alimentação. A luz é absorvida pela retina e transmitida para o relógio central localizado no NSQ via trato retino-hipotalâmico. O NSQ sincroniza relógios periféricos via sinalização neural (flechas azuis) e hormonal (flechas verdes). Além da luz, pistas alimentares atuam como potenciais *zeitgebers* de relógios periféricos (linha preta) e, na ausência de pistas luminosas, algumas evidências sugerem que comida pode se tornar um sincronizador para o relógio central também (linha preta pontilhada). Adaptado de Laermans; Depoortere, 2016.

Além da luz e da alimentação, outros fatores externos também contribuem para a sincronização dos ritmos circadianos. O exercício físico programado vem sendo estudado como potencial pista para sustentar ritmos circadianos robustos, uma vez que o horário do exercício impacta nas vias metabólicas do músculo esquelético e na homeostase energética sistêmica (Sato et al., 2019). Além disso, camundongos e humanos apresentam uma variação diária na capacidade de se exercitar com boa performance (Ezagouri et al., 2019). Outro *zeitgeber* não-fótico é o contato social. As interações sociais são estímulos complexos que podem determinar o horário de dormir, de acordar, de comer e de se exercitar e, como consequência, afetar os ritmos circadianos. No entanto, estudos com

voluntários submetidos a condições de iluminação constantes demonstram que o contato social não é suficiente para manter seus ritmos sincronizados. Isso indica que, ao contrário das pistas fóticas, as pistas sociais são fracas e apenas a sua presença não é suficiente para causar uma sincronização (Mistlberger; Skene, 2004; Middleton et al., 1996). As pistas sociais podem, por outro lado, indiretamente influenciar a sincronização ao ambiente por modular a exposição à luz. Estímulos auditivos também já foram apontados como *zeitgebers*, principalmente estímulos com significado social, por exemplo gongos sinalizando horário das refeições, mas estes estudos são inconclusivos e com potenciais confundidores (Aschoff et al., 1975).

Um importante sinalizador gerado pelo NSQ, quando este recebe informação fótica, é a supressão da síntese de melatonina, também conhecida como “hormônio da escuridão”, essencial para o funcionamento do relógio. A síntese e secreção de melatonina pela glândula pineal é estimulada pela ausência de luz que, via ação noradrenérgica, ativa a enzima *aralkylamine N-acetyltransferase* (AANAT), que converte serotonina em *n*-acetil-serotonina (NAS, a qual é convertida em melatonina pela enzima *hydroxyindol-O-metiltransferase* (HIOMT) (Klein et al., 1992). A melatonina produzida no escuro é diretamente liberada na corrente sanguínea (onde se liga à albumina) e no líquido cefalorraquidiano, enviando a informação temporal do período noturno para todo o organismo e servindo como comando biológico para regular o horário do ciclo sono-vigília (Cippola-Neto; Amaral, 2018). O estímulo luminoso (principalmente luz azul, com comprimento de onda de 450 nm) inibe a síntese de melatonina (Lockley et al., 2003). Visto que os níveis plasmáticos deste hormônio são baixos durante o dia e altos durante a noite, a melatonina possui um papel fisiológico essencial na temporização circadiana do ciclo sono-vigília e das funções metabólicas diárias. Este sistema neural complexo é produto da seleção natural que tornou o perfil noturno de secreção de melatonina como um representante interno da noite externa (Tordjman et al., 2017) (**Figura 7**). Além do importante papel temporizador circadiano da melatonina, é bem estabelecido seu papel temporizador sazonal. A duração da secreção noturna de melatonina segue a duração da noite e, assim, varia ao longo do ano, fornecendo uma pista para a organização de funções rítmicas sazonais, como por exemplo a reprodução e o crescimento do pelo em alguns animais (Lincoln; Loudon, 2015).



**Figura 7:** Regulação da síntese de melatonina pela glândula pineal. (Adaptado de Shinari; Loius, 2009)

## 2.2 REGULAÇÃO CIRCADIANA DO SISTEMA REPRODUTIVO

Os ritmos circadianos regulam diversos aspectos da reprodução, incluindo o horário de liberação hormonal, ovulação, acasalamento. Por sua vez, os hormônios do sistema reprodutivo também influenciam o sistema circadiano. Em mamíferos, o sistema reprodutivo é controlado por hormônios do eixo hipotálamo-hipófise-gônadas (HPG). O hipotálamo libera pulsos do Hormônio Liberador de Gonadotrofinas (GnRH) que, por sua vez, estimula a liberação dos hormônios folículo estimulante (FSH) e luteinizante (LH) pela pituitária anterior. Por fim, o FSH e o LH sanguíneos estimularão um ambiente hormonal propício para a reprodução. Os hormônios esteroides produzidos pelos testículos (testosterona, di-hidrotestosterona) e pelos ovários (estradiol e progesterona) também são requeridos no hipotálamo e na pituitária para regular sua própria produção (Hagenauer; Lee, 2012).

Em fêmeas adultas, a produção hormonal varia ao longo do ciclo reprodutivo. Em roedores de laboratório (ratos e camundongos), este ciclo é referido como ciclo estral, possui uma duração curta (4-5 dias) e é dividido em quatro etapas: proestro, estro, metaestro e diestro (Cora et al., 2015). Nestas espécies, ocorre um pico de estradiol no proestro, aproximadamente 12 horas da ovulação, que é seguido por um pico de progesterona algumas horas depois. A ovulação é seguida por um período de maior



receptividade sexual (estro) e uma queda concomitante dos níveis de estradiol (Toms et al., 2018).

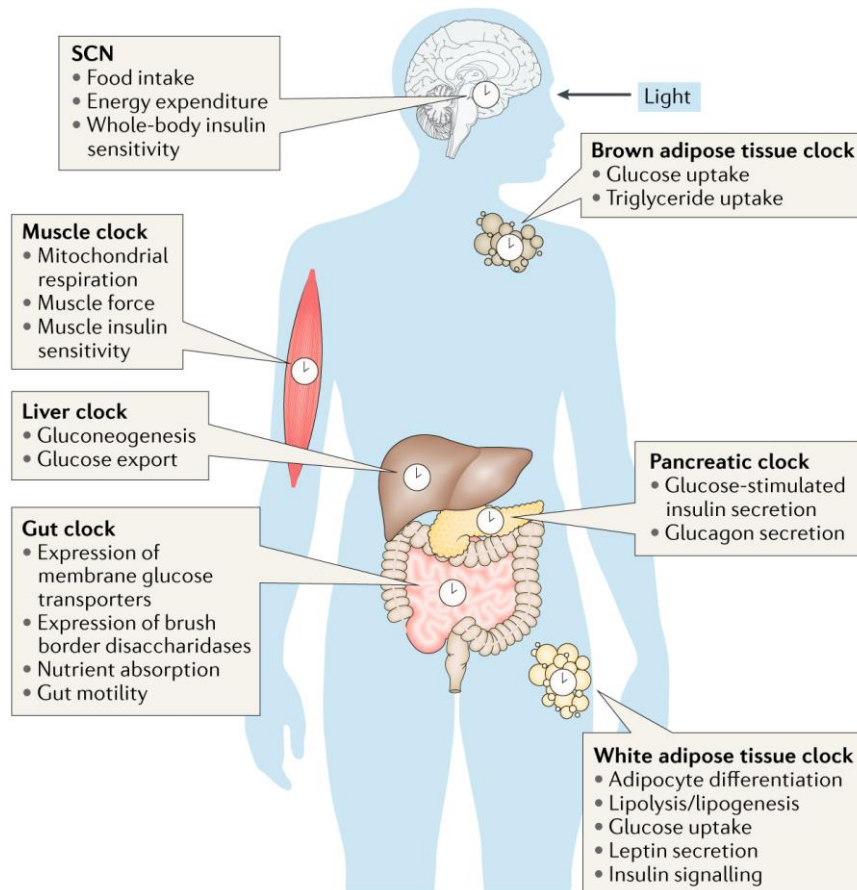
A regulação circadiana do eixo HPG é fundamental para que ocorram ciclos reprodutivos regulares. Sabe-se que lesões no NSQ eliminam a ovulação e produzem um estado de estro persistente, o que indica que os ciclos reprodutivos são vulneráveis a ruptura circadiana (Mills; Kuohung, 2019). Além disso, o NSQ envia sinais diários para neurônios do hipotálamo que produzem GnRH para conduzir o pico de GnRH/LH que leva à ovulação. Recentemente, foram identificados *clock genes* expressos em todos os níveis do eixo HPG, que parecem desempenhar um papel importante na geração de pulsos hormonais sincronizados e na preparação do ovário para a ovulação (Hagenauer et al., 2011).

Os hormônios gonadais, em contrapartida, afetam a atividade do sistema circadiano. Um dos efeitos mais conhecidos é o aumento da atividade no estro, após níveis elevados de estradiol em ratas fêmeas. Quando os níveis hormonais caem, as fêmeas atrasam o início da atividade e diminuem seus níveis de atividade (Sen; Hoffman, 2020). Hormônios gonadais também podem influenciar outros parâmetros circadianos, incluindo o período endógeno, a amplitude dos ritmos, a sincronização com pistas externas e a sensibilidade a *zeitgebers* (Crowley et al., 2015).

### 2.3 REGULAÇÃO CIRCADIANA DO METABOLISMO

O relógio circadiano regula o metabolismo e a homeostase energética em tecidos periféricos. Esta fisiologia inerentemente integrativa requer que uma rede de células especializadas, órgãos e tecidos comuniquem-se entre si via sistema nervoso e endócrino para desempenhar diferentes papéis no comportamento, consumo e armazenamento de energia, entre outros (Gerhart-Hines; Lazar, 2015). O núcleo NSQ desempenha um papel central na regulação circadiana do metabolismo ao sustentar ritmos de aproximadamente 24 horas de atividade-reposo e alimentação-jejum. Como consequência, processos metabólicos opostos (como anabolismo e catabolismo) são separados temporalmente e os ciclos recorrentes de alimentação-jejum são antecipados pelo sistema circadiano para otimizar a eficiência metabólica. Estudos pioneiros demonstraram que lesões no núcleo supraquiasmático eliminam a ritmicidade dos níveis de glicose, insulina e glucagon, indicando que esta estrutura é essencial para gerar mudanças diárias nas concentrações destas substâncias (Yamamoto et al., 1987; La Fleur et al., 1999). Assim, uma série de

processos metabólicos, incluindo sensibilidade à insulina, secreção de insulina, síntese de colesterol, oxidação de ácidos graxos, gasto energético, segue um ritmo ao longo das 24 horas do dia, modulado por um sistema de relógios presentes no marcapasso central do cérebro (NSQ) e em diversos tecidos do corpo, como fígado, músculo, tecido adiposo (Figura 8).



**Figura 8:** Relógios circadianos regulam o metabolismo de glicose, sensibilidade à insulina e secreção de insulina. Relógios centrais e periféricos são responsáveis por uma variedade de funções. SCN: núcleo supraquiasmático. Adaptado de Stenvers et al., 2018.

Como descrito anteriormente, osciladores moleculares estão presentes em todas as células do corpo e, fora do NSQ, são usualmente referidos como osciladores periféricos, uma vez que conferem ritmicidade circadiana a diversas funções específicas de cada tecido. Por exemplo, nos rins, o relógio circadiano modula o fluxo sanguíneo, a taxa de filtração glomerular e a excreção de água e íons (Solocinski; Gumz, 2015). No pâncreas, a secreção de insulina e glucagon está sob controle circadiano (Petrenko et al., 2017). No músculo, a respiração e a autofagia – processos que permitem uma produção robusta de energia e mantêm a homeostase das organelas, respectivamente – seguem uma ritmicidade circadiana (Mayeuf-Louchart et al., 2015). Igualmente importante é a síntese

e secreção rítmica de hormônios e de outras moléculas ativas em tecidos periféricos, os quais possibilitam a comunicação entre órgãos. No tecido adiposo, a mobilização de lipídeos é mediada pela ação de genes do relógio, especialmente *Clock* e *Bmal* (Shostak et al., 2013).

Um órgão central, com grande repertório de funções fisiológicas e um dos modelos mais estudados na cronobiologia é o fígado (Zwighaft et al., 2016). Para atingir o balanço entre síntese e armazenamento de lipídeos e fontes de carboidratos, o fígado recebe pistas do NSQ e do comportamento de alimentação-jejum dos animais para manter a homeostase de glicose no sangue e induzir antecipadamente o armazenamento ou depleção de glicogênio, de acordo com o consumo alimentar (Stokkan et al., 2001). O metabolismo de glicose também ilustra a intrincada regulação circadiana do metabolismo periférico. O pico de expressão dos transportadores de glicose e de receptores de glucagon acontece no início da fase ativa, o que coincide com o consumo de alimento (Panda et al., 2002). O armazenamento do excesso de carboidratos e a síntese de glicose (via gliconeogênese) são processos que ocorrem tipicamente no início da fase ativa e durante o repouso, respectivamente, e são modulados por proteínas CRY, CLOCK e pelo receptor nuclear REV-ERBA $\alpha$ , um componente do relógio responsável pela interface entre sistema circadiano e regulação metabólica no fígado (Storch et al., 2002).

A nível sistêmico, o abastecimento de células e de tecidos com oxigênio e nutrientes também exhibe flutuações diárias para que a absorção seja mais intensa durante a fase ativa e reduzida durante a fase de repouso (Rivera-Coll et al., 1994). No entanto, não só o sistema circadiano impõe ritmicidade a processos metabólicos, como sinais e estados metabólicos também retornam informações para o relógio central. O efeito do metabolismo no marcapasso central ou nos relógios periféricos se origina da alimentação, metabólitos dos alimentos ou hormônios cuja secreção é controlada pela presença ou ausência de comida. Isto explica o fato de alguns metabólitos sistêmicos atuarem como *zeitgebers* de relógios periféricos: dois nutrientes que circulam em abundância para gerar energia são o glutamato e o lactato. Ambos estão ligados a vias de sinalização de controle circadiano e apresentam seu fluxo intensificado ou atenuado de acordo com o estado metabólico (alimentado ou jejum). Diversos estudos identificaram nutrientes capazes de resetar ou alterar a fase dos ritmos circadianos, como a glicose (Hirota et al., 2002), aminoácidos (Iwanaga et al., 2005), sódio (Waddington et al., 2007), etanol (Chen et al., 2004), cafeína (Antle et al., 2001).

Além da função de sincronizar os ritmos circadianos em tecidos periféricos, a composição dos alimentos, o horário e a frequência em que são consumidos influencia o relógio circadiano. Em roedores, limitar o acesso à alimentação para um certo período do dia induz o desenvolvimento de uma atividade antecipatória que precede o evento de alimentação e se caracteriza por maior atividade locomotora, maior comportamento exploratório, pico de corticosterona e grelina e redução de insulina plasmática precedendo a refeição diária (Mistlberger, 2009; Blum et al., 2009; Díaz-Muñoz et al., 2000). Além disso, quando submetidos a dietas ricas em gordura, roedores mudam seu padrão de alimentação e passam a ingerir boa parte do seu alimento durante sua fase usual de sono/repouso (Manoogian et al., 2019). Estudos em humanos demonstraram que comer durante a noite, quando os níveis de melatonina começam a aumentar está associado a maior gordura corporal, maiores níveis de glicose no sangue e pior saúde cardiometabólica (McHill et al., 2017; Yoshida et al., 2018). Em relação à composição das refeições, sabe-se que ingerir um café da manhã rico em proteínas otimiza o controle de glicose no sangue e a perda de peso em indivíduos com Diabetes tipo II (Jakubowicz et al., 2017). Apesar de não se ter identificado uma estrutura anatômica oscilatória que seja sincronizada pela alimentação e não se saber os mecanismos por trás do efeito da composição e horário das alimentações no sistema circadiano, esclarecer isto pode ser importante para fazer ajustes que permitam melhores desfechos em saúde, dado os benefícios plausíveis de se ingerir refeições com as composições adequadas em horários apropriados.

Do ponto de vista da evolução, é benéfico para organismos multicelulares manterem uma rede de relógios organizada de forma mais complexa, ao invés de uma organização hierárquica “direta” com um regulador/marcapasso principal controlando toda a fisiologia do corpo. Uma possível explicação para isto é o fato de que os relógios celulares assumiram funções adaptativas adicionais resultando em um aumento da aptidão geral. Outra justificativa seria a maior resiliência a *zeitgebers* conflitantes e maior plasticidade em resposta a ambientes complexos e, portanto, demandas ambientais, uma vez que diferentes relógios respondem de forma diferente a certos estímulos (ex: locomoção é sincronizada principalmente pela luz). Esta organização permite que cada processo fisiológico seja sincronizado por aqueles sinais que são mais relevantes para o processo específico, resultado em uma resposta personalizada e melhor adaptada (Hushe et al., 2015).

## 2.4 CRONODISRUÇÃO E METABOLISMO

A partir de uma perspectiva evolutiva, a organização molecular e fisiológica do sistema circadiano foi estabelecida para se adaptar e prosperar sob as pressões seletivas da escassez de alimentos, mudanças sazonais na disponibilidade de luz solar e variações de temperatura. Embora este sistema ajustado tenha sido adequado por milhares de anos, foram desenvolvidas condições sociais que excedem as limitações adaptativas da nossa programação circadiana. A introdução à eletricidade, restaurantes *fast food* funcionando 24 horas por dia, refeições baratas com alto teor calórico disponíveis a qualquer horário do dia combinados com uma população crescente de trabalhadores noturnos, viagens regulares entre diferentes fusos horários, constante exposição à poluição luminosa artificial e estilo de vida sedentário criaram um ambiente substancialmente diferente da realidade que nossos ancestrais enfrentaram. Ao mesmo tempo que a tecnologia tem efeitos positivos no dia a dia, tem sido proposto que estas mudanças nos parâmetros ambientais de luz, nutrição e comportamento contribuem significativamente para o aumento da prevalência de “doenças relacionadas ao estilo de vida”, como por exemplo, doenças metabólicas, distúrbios do sono, transtornos psiquiátricos e alguns tipos de câncer (Wang et al., 2011; Sahar; Sassone-Corsi, 2009; Roenneberg et al., 2012).

Os ritmos diários da sociedade moderna interferem na coordenação temporal de processos fisiológicos do corpo. Esta perturbação do sistema circadiano é frequentemente referida como “cronodisrupção” ou “desalinhamento circadiano” ou “disrupção circadiana”. Estes termos têm sido utilizados desde os anos 80 para sugerir a falta de sincronização entre pistas ambientais externas e processos fisiológicos internos (de Castro et al., 1978). Além disso, sabe-se que a cronodisrupção pode também resultar de fatores internos, como por exemplo cegueira pré-quiásmática, tumores ou lesões que danifiquem o NSQ e variações genéticas deletérias (Rüger; Scheer, 2009). Este desalinhamento pode ainda ocorrer em diversos níveis: 1) Disrupção ambiental: ocorre quando uma pista ambiental (e.g., ciclo claro-escuro) está fora de sincronia com a fase do NSQ; 2) Disrupção comportamental: quando ciclos como alimentação-jejum ou sono-vigília estão desalinhados com a fase do NSQ; 3) Disrupção a nível celular: quando os ritmos do marcapasso central estão desalinhados aos ritmos dos relógios periféricos (Vetter, 2020). Ainda, esta condição pode ser caracterizada por uma perda total de ritmicidade, uma redução na amplitude dos ritmos ou, como descrito anteriormente, por diferenças de fase entre o relógio do NSQ e os relógios periféricos. As manifestações de desalinhamento

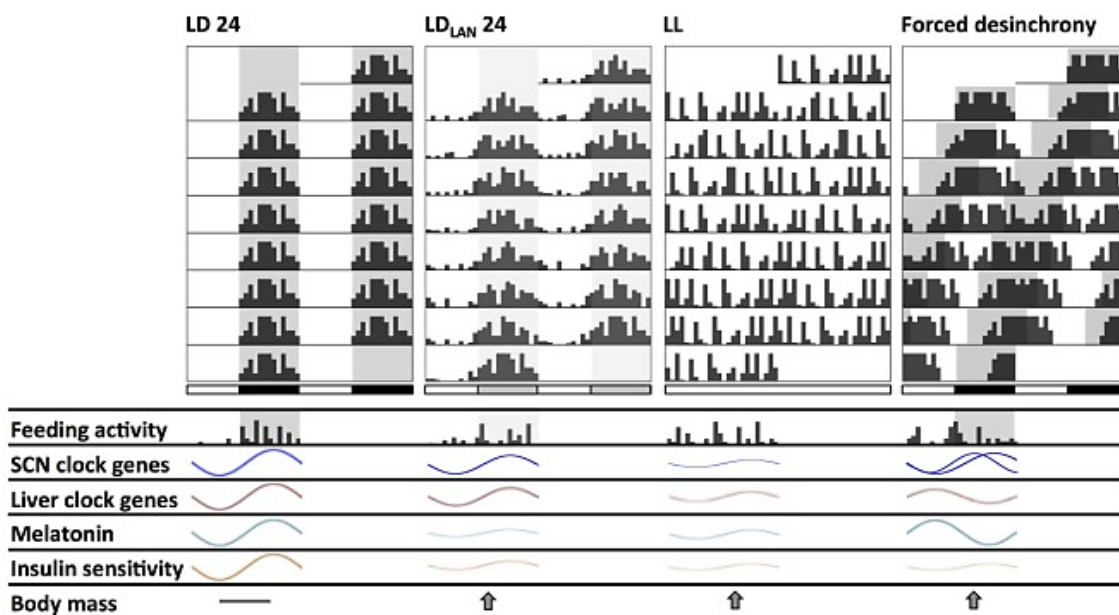
circadiano incluem sonolência diurna, distúrbios de sono, performance cognitiva reduzida, queixas gastrointestinais e sensação geral de mal-estar (Rüger; Scheer, 2009).

Uma das formas de estimar cronodisrupção é através do *jetlag social*, termo cunhado por Roenneberg et al (2006) para medir a diferença entre os horários de sono dos dias de trabalho e dias livres. Considera-se que o sono nos dias livres reflete o ciclo natural de sono, servindo como uma aproximação do tempo interno, enquanto o sono nos dias de trabalho reflete o tempo externo, social. Assim, o *jetlag social* representa a discrepância entre o tempo interno e o tempo social. Estudos epidemiológicos demonstram que o *jetlag social* está associado a maior índice de massa corporal (IMC) (Roenneberg et al., 2012; Parsons et al., 2015) e a biomarcadores de saúde cardiometabólica (Rutters et al., 2014).

Perturbações ao sistema circadiano foram citadas pela primeira vez como um fator envolvido em doenças através de estudos epidemiológicos que mostraram maior incidência de câncer em trabalhadores de turno (Tynes et al., 1996); a Organização Mundial da Saúde atualmente sugere uma série de riscos para a saúde associados ao trabalho de turno ([http://apps.who.int/iris/bitstream/10665/97940/1/9789241501729\\_eng.%20pdf?ua=1](http://apps.who.int/iris/bitstream/10665/97940/1/9789241501729_eng.%20pdf?ua=1)). No geral, humanos são ativos durante o dia e, rotinas envolvendo trabalho noturno são bastante desafiadoras para o sistema circadiano. Trabalho noturno sugere exposição à luz durante a noite, ingestão calórica em horários inapropriados, estar acordado e ativo em momentos que usualmente estaria dormindo, dormir durante o dia (o que diminui a qualidade e duração do sono) e maior ingestão de estimulantes à base de cafeína. Tudo isso causa um desalinhamento entre o ritmo endógeno e o ciclos claro-escuro. Existe uma vasta literatura com estudos epidemiológicos associando trabalho de turno com doenças cardiovasculares, diabetes e obesidade (Knutsson, 2003; Wolk et al., 2005).

Além do trabalho de turno, alguns aspectos da exposição à luz também vêm sendo associados à ruptura circadiana. Com o estilo de vida moderno, a intensidade e composição espectral da luz elétrica são frequentemente inadequados durante o dia para sincronizar o sistema circadiano e muito fortes durante a noite (Takahashi et al., 2008). Diversos estudos experimentais desenharam protocolos de exposição à luz durante a noite para simular a poluição luminosa de ambientes urbanos (**Figura 9**). Sob condições padrão de iluminação (ciclo 12:12 claro-escuro), os ritmos de atividade-reposo, bem como expressão de genes do relógio e liberação de hormônios se mantém sincronizados com o

ciclo externo. No entanto, reduzir o contraste entre claro e escuro ao se inserir iluminação fraca à noite impacta na organização dos ritmos circadianos. Os efeitos da luz à noite (LAN) incluem redução na amplitude da expressão de genes relógio, bem como mudanças na amplitude dos ciclos de melatonina e glicocorticoides (Bedrosian et al., 2013). Além disso, estudos demonstraram que a LAN promove o aumento do peso corporal, da gordura epididimal e reduz a tolerância à glicose em camundongos (Fonken et al., 2010). Já a exposição à luz constante induz um comportamento *free running* (livre-curso) dos ritmos de atividade-reposo, que se caracteriza por um ritmo não sincronizado a pistas ambientais e que oscila em um ciclo diferente de 24 horas. Além disso, os ritmos de alimentação são alterados, a amplitude da expressão de genes do relógio é reduzida e esta dessincronização também está associada a distúrbios metabólicos, como maiores níveis de tecido adiposo, alterações no gasto energético, resistência à insulina e metabolismo de glicose alterado (Sudo et al., 2003; Coomans et al., 2013; Shi et al., 2013). Em protocolos de dessincronização forçada, onde animais são submetidos a períodos diferentes de 24 horas também são observados ganho de peso, maior gordura corporal e maiores níveis de triglicérides (Casiraghi et al., 2016).

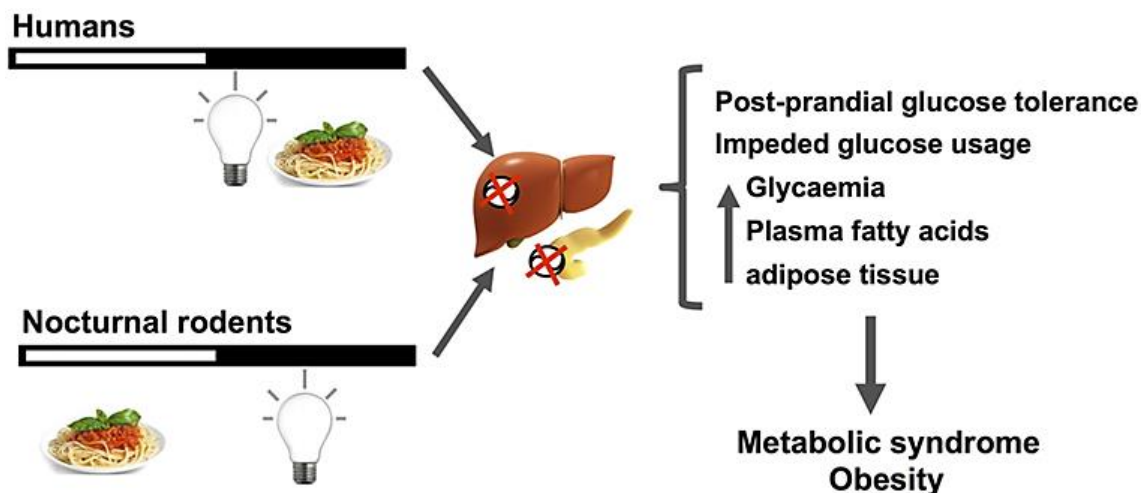


**Figura 9:** Esquema das alterações metabólicas e circadianas encontradas em roedores submetidos a diferentes protocolos de iluminação. LD 24: Condição padrão de claro-escuro; LD<sub>LAN</sub>24: Claro-escuro com luz fraca à noite; LL: Claro constante. (Adaptado de Plano et al., 2017).

As alterações de iluminação e ruptura de ritmos não são os únicos fatores capazes de causar alterações metabólicas e obesidade (**Figura 10**). O desenvolvimento de grandes centros urbanos e o estilo de vida moderno também englobam hábitos alimentares que afetam a saúde e os ritmos. Dentro destes hábitos, alguns aspectos devem ser considerados: irregularidade (inconsistências nas rotinas alimentares), frequência, horário e composição da alimentação (Pot, 2017). Um estudo epidemiológico demonstrou que pessoas com rotinas de refeições mais irregulares apresentam maior risco para obesidade mesmo consumindo menos calorias do que pessoas com rotinas de alimentação mais regulares (Pot et al., 2014). Além disso, sabe-se que a glicemia pós-prandial está sob controle circadiano. Em humanos, há uma redução na tolerância à glicose à noite, como resultado da diminuição na secreção de insulina. Portanto, a ingestão calórica em horários em que o corpo não está organizado para armazenar/mobilizar estes nutrientes pode levar à resistência a insulina e obesidade (Bo et al., 2014).

Dietas ricas em gordura também são uma característica comum da sociedade moderna. Estudos experimentais mostram que alimentar ratos com dietas altas em gordura leva à redução nos níveis de insulina, leptina, adiponectina e interleucina-1 e o padrão diário de concentração plasmática destes fatores fica alterado. (Cano et al., 2009). Resultados similares são obtidos para insulina e leptina de humanos após um dia com três refeições ricas em gordura (Havel et al., 1999).





**Figura 10:** Representação do impacto da luz à noite e de padrões alterados de alimentação nos ritmos circadianos e metabolismo de roedores noturnos e humanos. Quando roedores e humanos recebem pistas alimentares e luminosas em horários inadequados, ocorre uma dessincronização nas funções de órgãos que regulam o balanço energético, causando uma redução na tolerância à glicose pós-prandial e diminuindo a utilização de glicose. Os principais desfechos destas alterações são aumento da glicemia basal, dos ácidos graxos plasmáticos e do tecido adiposo. Quando isto é estabelecido de forma crônica, aumentam os riscos para desenvolver obesidade e doenças metabólicas. Adaptado de Plano et al., 2017.

Os mecanismos precisos que explicam a associação entre cronodisrupção e alterações metabólicas ainda não são bem conhecidos, no entanto algumas hipóteses foram propostas. Um estudo mostrou que a melatonina reduziu a obesidade e melhorou o perfil metabólico em uma linhagem de ratos com Diabetes tipo II, sem afetar a ingestão alimentar e a atividade (Jiménez-Aranda et al., 2013). Isso indica que a exposição à luz durante a noite pode estar associada a maior ganho de peso devido a maior supressão de melatonina. Outra hipótese está relacionada às vias neurais conectando o sistema nervoso autônomo (SNA) aos órgãos periféricos. Sabe-se que os neurônios do SNA se projetam tanto para os compartimentos intra-abdominais quanto subcutâneos do corpo e, estas sinalizações podem variar dependendo do horário do dia. Assim, em condições de cronodisrupção, órgãos intra-abdominais, como gordura visceral, fígado e pâncreas, podem receber estímulos diferentes em horários inapropriados (Perez-Tilve et al., 2006). Da mesma forma, porém relacionado a via pineal-hipotálamo-adipócito, Scott e Grant (2006) propuseram uma hipótese com base no fato de que animais que hibernam acumulam gordura seguindo um ritmo sazonal, sincronizado pela luz ambiental. Assim, foi hipotetizado que o homem moderno ocidental está sempre se preparando para um

estado de privação de comida (inverno) que nunca chega. No entanto, a grande maioria das hipóteses presentes na literatura para explicar a relação entre obesidade e cronobiologia apontam para uma dessincronização entre os diferentes ritmos circadianos envolvidos no metabolismo. Hábitos atuais como alta frequência de alimentação, redução na duração de sono, pouca exposição à luz natural durante o dia e exposição à luz artificial à noite enfraquecem o contraste em ritmos internos e externos, levando a distúrbios metabólicos, incluindo obesidade.

Tendo em vista os efeitos das perturbações do sistema circadiano sobre o metabolismo, com este estudo pretendemos investigar a influência de diferentes padrões de iluminação sobre a fisiologia e perfil metabólico de ratas Wistar. Além disso, considerando os desafios ocasionados pelo avanço da urbanização e industrialização, pretendemos avaliar a relação entre parâmetros circadianos e de exposição à luz com o índice de massa corporal (IMC) de comunidades rurais e urbanas.

### 3. JUSTIFICATIVA

Diante da importância da luz na regulação fisiológica dos organismos vivos e tendo em vista o crescente aumento no tempo de exposição à iluminação e a qualidade desta, faz-se necessário ampliar os estudos que busquem entender sua influência no metabolismo e no ganho de peso.

Tendo em vista os desafios que a vida moderna impõe ao sistema circadiano, os efeitos da cronodisrupção sobre a fisiologia e o comportamento devem ser investigados.

As taxas de obesidade vêm crescendo nas últimas décadas, tornando esta doença uma epidemia global. Estudos que avaliem parâmetros circadianos associados a esta condição ainda são inconclusivos, tornando necessária a investigação de características ambientais e endógenas envolvidas na patofisiologia da obesidade.

Estudos que avaliem o comportamento de atividade-reposo e exposição à luz tanto em populações rurais quanto em populações urbanas são escassos. Avaliar populações em diferentes níveis de urbanização contribui para esclarecer questões relacionadas ao impacto da urbanização nos ritmos biológicos e no metabolismo.

Pelas razões apresentadas, justifica-se a realização do presente estudo com o propósito de investigar diferentes padrões de iluminação na fisiologia e no metabolismo de ratas, bem como investigar características dos ritmos circadianos que possam estar associadas a um maior índice de massa corporal (IMC) em populações quilombolas que vivem em diferentes níveis de urbanização.

#### **4. HIPÓTESE**

Hipotetizamos que um atraso de fase na sincronização circadiana, exposição irregular à luz e viver em ambientes com maiores níveis de urbanização estão associados a sobrepeso e obesidade.

## 5. OBJETIVOS

### 5.1 OBJETIVOS GERAIS

Investigar o efeito de diferentes padrões de iluminação na fisiologia de ratas Wistar e avaliar a associação entre IMC, exposição à luz e ritmos de atividade-reposo em comunidades rurais e urbanas.

### 5.2 OBJETIVOS ESPECÍFICOS – ARTIGO 1

- Avaliar o efeito de diferentes padrões de iluminação sobre desfechos relacionados à maturação sexual: o tempo e abertura vaginal, primeiro ciclo estral completo, quantidade de ciclos completos consecutivos, tempo para a primeira fase de estro de ratas Wistar;
- Avaliar o efeito de diferentes padrões de iluminação sobre ganho de peso, peso de gordura visceral, e níveis séricos de glicemia, triglicerídeos, HDL e colesterol total de ratas Wistar.

### 5.3 OBJETIVOS ESPECÍFICOS – ARTIGO 2

- Avaliar a exposição à luz e potencial associação com maior índice de massa corporal em comunidades rurais e urbanas;
- Investigar a associação entre cronotipo e jetlag social com índice de massa corporal;
- Avaliar se há correlação entre maiores níveis de urbanização e obesidade em comunidades quilombolas;
- Examinar a associação entre parâmetros derivados de actimetria com maiores chances de ter sobrepeso/obesidade.

## 6. ARTIGO 1

### EFFECTS OF LIGHTING PATTERNS IN PUBERTAL DEVELOPMENT AND METABOLISM OF FEMALE WISTAR RATS

(Artigo submetido na revista *Physiology and Behavior* em 09/08/2021)

**Authors:** Débora Barroggi Constantino<sup>1,2</sup>, André Comiran Tonon<sup>1,2</sup>, Melissa Alves Braga de Oliveira<sup>1,2</sup>, Guilherme Rodriguez Amando<sup>1,2</sup>, Juliana Jury Freitas<sup>1,2</sup>, Nicoli Bertuol Xavier<sup>1,2</sup>, Rafael Júnior Ribeiro<sup>4</sup>, Marco Idiart<sup>3</sup>, Maria Paz Loayza Hidalgo<sup>1,2</sup>

#### **Affiliations:**

<sup>1</sup>Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento da Universidade Federal do Rio Grande de Sul - HCPA/UFRGS

<sup>2</sup>Laboratório de Cronobiologia e Sono – HCPA/UFRGS

<sup>3</sup>Departamento de Física – UFRGS

<sup>4</sup>Instituto de Informática – UFRGS

#### **ABSTRACT**

Modern life style is characterized by constant exposure to artificial light, which is associated with alterations in biological rhythms, abnormalities to reproductive cycles and metabolic changes. In this study, we aimed to evaluate the effects of four different lighting patterns on puberty timing and on possible metabolic changes in female Wistar rats. Additionally, we developed a machine learning algorithm to automatically classify the stages of the estrous cycle. Adult Wistar rats mated during a week at a photoperiod station where they were exposed to combined red-green-blue lights (RGB) during the photoperiod that varied its spectral composition (i.e., variable color temperature) during the day (RGB-v; N = 14), RGB during the photoperiod with a fixed light color temperature (RGB-f; N = 13) during the whole photoperiod; constant darkness (DD; N = 13) and constant fixed light (LL; N = 15). Experiments were performed only on female litters from postnatal day (PND) 22 to 50. Body weight, puberty onset, estrous cyclicity and serum metabolic parameters were measured. We also collected pictures of vaginal smears to create a dataset of 15936 images to construct an automatic classifier based on convolutional neural networks. No significant differences were found in the age of vaginal opening, however the RGB-v group showed a significantly lower number of

complete and consecutive cycles. Also, the RGB-f group showed the first complete estrous cycle significantly earlier than the RGB-v group. Female rats housed in LL condition presented significantly lower mean body weight from PND 33 to PND 47 compared to the other groups. Furthermore, higher levels of plasma triglycerides were found in the DD group compared to RGB-f and RGB-v. HDL levels were significantly lower in RGB-v compared to RGB-f and LL groups. Total cholesterol was significantly lower in RGB-v compared to all groups. Visceral fat was significantly higher in RGB-f compared to the LL group. These results suggest that both changes in photoperiod and lighting quality affect pubertal development and alter lipid profiles and visceral fat accumulation.

**Key words:** Estrous cycle, puberty, circadian rhythms, metabolism, light exposure, machine learning.

## INTRODUCTION

Puberty is the period of profound hormonal, behavioral and physical changes by which an individual acquires reproductive competence (Foster et al., 2006). This transition is driven by complex neuroendocrine mechanisms involving the activation of the hypothalamic-pituitary-gonadal (HPG) axis. The onset of HPG axis activity depends on the activation of specialized neurons that secrete pulses of gonadotropin releasing hormone (GnRH), which controls the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Sato et al., 2008).

The age of puberty has been changing and there is growing concerns about sexual precocity, considering its association with higher risk of reproductive tract cancers, obesity, depression, and psychosocial difficulties (Prentice et al., 2013; Ong et al. 2012; Charalampopoulos et al., 2014). In mammals, puberty onset may be influenced by both genetic and environmental conditions, such as nutrition, chronic diseases, geographic location, stressful events, pollution (Parent et al. 2005). Further, one of these conditions is light exposure and, nowadays, it has been widely investigated as a factor contributing to this variability in the timing of puberty. (Gamble et al., 2017; Crowley et al., 2015).

Light-dark cycles are the most important signal (*zeitgeber*) in charge of synchronizing daily variations in mammalian physiology, including factors that regulate the reproductive system (Evans et al., 2018). In the absence of light, the suprachiasmatic nucleus (SCN), a biological pacemaker that receives photic information, drives melatonin

secretion by the pineal gland (Zisapel et al., 2018). This hormone provides an extremely robust endocrine signal of day-length and is considered an important factor for the control of reproduction, since it has been described to induce anti-gonadotropic effects. Furthermore, short-days or exogenous melatonin treatment seem to inhibit ovulation (Wurtman et al., 1963; Ying et al., 1973; Kasahara et al., 2010).

In female rats, vaginal opening is an anatomic landmark in development of the reproductive system, starting the reproductive cycle, called the estrous cycle (Cora et al., 2015). The detection of estrous cycle phases is a vital factor to optimize mating, predict gestation time, maintain reproductive performance in laboratory animal science and register the stage as a variable that may affect research. The most common method used to detect estrous cycle phases is collecting vaginal smears and placing it on a clean microscope slide (Bayers et al., 2012). The stages of the estrous cycle are determined according to the predominant cell type present (Cora et al., 2015). It requires at least two previous trained evaluators and it is a process that can lead to misinterpretations. Therefore, automated estrous detection technologies, such as machine learning techniques, are an alternative potential method to replace visual estrous cycle detection.

Beyond changes in puberty timing, disruption in natural light-dark cycles can derange metabolism, a major physiological process under circadian control. Animal studies have established strong relationships between abnormal light exposure and dysregulated glucose, weight gain and metabolic disorders (Fonken et al., 2013; Nelson et al., 2018; Coomans et al., 2013). Many of these metabolic effects have been attributed to circadian disruption, with rodent models of obesity reporting alterations in clock genes expression inducing obesity (Eckel-Mahan et al., 2009; Pendergast et al., 2013). Epidemiological studies with night shift workers also demonstrate an increased risk to be overweight or obese, suggesting that working on night shifts and being exposed to light at night can disrupt circadian signals, which may dysregulate metabolism (Ritonja et al., 2019; Haus et al., 2016).

Modern lifestyle is characterized by constant exposure to artificial light and disruption of internal circadian rhythms has become quite common (Touitou et al., 2017). To date, very few studies have investigated how light exposure might be a trigger that pushes females into early puberty and metabolic alterations. In the present study we aimed to evaluate the influence of four different lighting patterns on puberty timing, serum metabolic markers and visceral fat accumulation in female Wistar rats. Additionally, we



developed a machine learning algorithm to classify images of the estrous cycle in the right stage of the cycle.

## **METHODS**

### **Animals**

Adult Wistar rats were obtained from the Animal Experimentation Unit (UEA) of the Hospital de Clínicas de Porto Alegre (HCPA) and were mated during a week at a photoperiod station. This station is equipped with different light patterns in four unique environments. Animals were housed with food and water ad libitum in transparent acrylic home-cages (Panlab Harvard Apparatus; 25 x 15 x 25 cm) and controlled temperature (20°C). Animals were maintained on the photoperiod station while males were euthanized. Females were allowed to raise their litters until the pups completed 21 days (Postnatal day, PND 21) of life and, thereafter, were weaned. Experiments were performed only on female litters (N = 55) from PND 22 to 50. Female genitors were euthanized after weaning. Pups were weighed every three days, between 5 and 6 p.m.

Animals were divided into four groups, according to the lighting: combined red-green-blue lights (RGB) during the photoperiod that varied its spectral composition during the day (RGB-v; N = 14), to simulate daily changes in color temperature of the natural sunlight; RGB during the photoperiod with a fixed light color temperature (RGB-f; N = 13) during the whole photoperiod; constant darkness (DD; N = 13) and constant light (LL; N = 15). The RGB-v group was exposed to a light pattern whereby a gradual change in color temperature occurred. Reddish wavelengths (2700 K) initiate the cycle from the moment the lights are on, transitioning through a gradual warming of the color temperature until reaching the maximum heating peak, 6 hours after the start (simulating noon of the light in a natural environment of blueish color, 400K). Subsequently, the light gradually becomes colder until the end of the light period, 16 hours after the lights are on (simulating the sunset in a natural environment). At the end of 16 hours of light, the lights were completely off during the 8 hours of darkness. The RGB-f group maintained the color temperature throughout the light phase. The LL group kept the entire protocol in constant fixed light, while the DD group was kept in constant darkness. More details about the spectral composition of the lighting system were previously reported by de Oliveira et al (2019).

## **Assessment of Estrous Cycle**

Vaginal opening occurs between postnatal days (PND) 32 – 34, starting the reproductive cycle. The estrous cycle in rats averages 4-5 days and is generally divided in four stages: proestrus, estrus, metestrus and diestrus. To investigate the timing of puberty, all litters were examined daily for vaginal opening, starting from PND 28. Once vaginal opening occurred, vaginal smears were taken daily, between 5 and 6 p.m., for vaginal cytology until the end of the experiment. Vaginal smears were prepared by introducing a drop of saline solution (NaCl 0.9%) into the vagina, collecting back and placing it on a clean microscope slide. Under a light microscope at 40x of magnification, the stages of the estrous cycle were determined according to the predominant cell type present in the vaginal smears. Two independent evaluators analyze the microscope slice to ensure consistency of stage interpretation. The experiment continued until PND 50 (**Figure 1**).

The determination of estrous cyclicity was made following previous reports. Complete cycles were counted considering the length of the four stages, which can vary from 6 to 72 hours. Proestrus (average length of 14h in rats) could be absent and should not be present for more than two vaginal cytologies. Estrus' duration ranges from 24 to 48h. Therefore, it should not be absent. Metestrus is a short stage of 6-8h and could also be missed. Diestrus is the longest stage with an average duration of 48-72h, thus it should not be absent and should be present for at least two consecutive days. Cycles were also dichotomized as regular, defined as 4 or 5-day cycle (with two days of estrus or 2 or 3 consecutive days of diestrus), and irregular, defined as a 6 or 7-day cycles with those exhibiting a persistent estrus or diestrus

## **Development of the Machine Learning Algorithm to Assess Estrous Cycle**

After optical microscopic initial evaluation of vaginal smears, pictures were taken using a mobile phone and the estrous phase cycle was classified by human experts as the gold standard method. We developed an algorithm in two steps: the supervised approach and the unsupervised approach. The dataset of images (N=15936) and their classifications were used to train and test an automatic supervised algorithm based on convolutional neural networks (CNN), implemented by the Python package Keras. In the second moment, we performed an unsupervised algorithm also based on CNN. All images were

cutted using 800x800 pixels as standard. More information about the algorithm development is available in the supplementary material.

### **Blood Sampling**

Blood samples were collected at euthanasia by cardiac puncture after anesthesia with isoflurane 5%. Female rats were fasted for 4 hours prior to testing. Samples were centrifuged at 4000 rpm for 15 min and analyzed for the serum levels of glucose, triglycerides, HDL and total cholesterol.

### **Statistical Analysis**

Data are presented as mean  $\pm$  standard deviation (SD) or median and interquartile range (IQR), when appropriate. Statistical comparisons between groups were evaluated by two-way ANOVA and repeated measures ANOVA, followed by post hoc tests (Tukey). The Kruskal-Wallis test was used to compare non-parametric data. Statistical significance was accepted as  $p < 0.05$ . Analyses were performed using SPSS software version 18.

## **RESULTS**

### *Timing of Puberty and Estrous Cyclicity*

There was no difference between groups in the age of vaginal opening (DD:  $35.23 \pm 2.65$ ; LL:  $34.13 \pm 2.38$ ; RGB-f:  $35.23 \pm 2.24$ ; RGB-v:  $34.71 \pm 1.06$ ;  $p = 0.487$ ). However, significant differences related to the PND of the first complete estrous cycle were found between RGB-f and RGB-v (**Figure 2a**). The age of the first estrus was similar among groups ( $p = 0.904$ ). We also observed differences in the number of complete and consecutive cycles between DD and RGB-v (**Figure 2b, 2c**).

We observed that 91.66% of the rats exposed to LL presented regular cycles, which was significantly higher compared to RGB-v where 68.75% of the rats showed regular cycles ( $p < 0.05$ ). There was no significant difference in the proportion of samples spent in proestrus, estrus, metestrus and diestrus. However, when we subtracted the estrus proportion by the diestrus proportion of each group, we observed that there was a trend ( $p > 0.05$ ;  $p < 0.1$ ) of the RGB-f group to have more estrus, since the difference was positive.

### *Weight gain and metabolic changes*

Body weight was differentially affected by light conditions over experimental weeks. Significantly lower average body weight was noted in the LL group from PND 33 to PND 47 compared to the other groups ( $p < 0.05$ ). We also observed significant differences in body weight between RGB-v and RGB-f from PND 30 to PND 39 and in PND 24 and 47 ( $p < 0.05$ ) (**Figure 3**).

Serum levels of glucose, triglycerides, HDL, total cholesterol and visceral fat are shown in **figure 4** as means  $\pm$  SD. No significant differences were found in levels of glucose between groups ( $p = 0.118$ ). Higher levels of plasma triglycerides were found in the DD group compared to RGB-f ( $p = 0.001$ ) and RGB-v ( $p = 0.003$ ). HDL levels were significantly lower in RGB-v compared to RGB-f ( $p = 0.002$ ) and LL ( $p = 0.002$ ) groups. Total cholesterol was significantly lower in RGB-v compared to all groups ( $p < 0.05$ ). Visceral fat was significantly higher in RGB-f compared to the LL group ( $p = 0.02$ ) (**Figure 5**).

## **DISCUSSION**

We reported here the novel finding that different lighting patterns influence estrous cycling in female Wistar rats and the effects of these patterns in lipid profiles, body weight and visceral fat accumulation. Additionally, we proposed an automatic algorithm based on convolutional neural network analysis to classify estrous cycle images.

Many animal studies have reported that exposure to constant light accelerates sexual maturation, as indicated by the day of vaginal opening (Puig-Domingo et al., 1992; Waldhauser et al., 1991; Bohlen et al., 2018). However, our results did not indicate differences in the age of vaginal opening. In this study, estrous cyclicity was the main endpoint affected by light conditions. The neuroendocrine mechanisms by which variations in light exposure modulate the HPG axis are not fully understood. Nevertheless, the effects of the lighting patterns on reproductive cycles might be attributed to melatonin secretion. In rats, melatonin acts on the hypothalamic functions by inhibiting the gonadotropin-releasing hormone (GnRH) and, consequently, inhibiting gonadal development. Besides, as demonstrated by Crowley et al (2015) pre- and mid-pubertal adolescents showed greater melatonin suppression during evening light exposure, indicating an increased sensitivity of the circadian system to light in early and

mid puberty. In line with these observations, and considering the quality of light exposure, the LL (constant light) group presented a higher percentage of regular estrous cycle, which might indicate that they reached sexual maturity more rapidly. Furthermore, we showed that there was a trend of the RGB-f (fixed spectral composition) to have a higher proportion of estrus. The estrus phase has an important role in fertility and sexual maturity as ovulation and hormonal changes arise within this phase, whereas diestrus is characterized by hormonal and behavioral changes not favorable to reproduction (Smith et al., 2009). This result might indicate an increased fertility potential, which means that the RGB-f group may also have reached sexual maturity earlier. Thus, further studies are needed to highlight the importance of stable circadian rhythms and melatonin cycles to optimize female reproductive physiology, as well as the consequences of circadian disruption on puberty timing.

Few studies have investigated the influence of different lighting patterns, including changes in color temperature, on metabolic responses of rats. Daily interactions between suprachiasmatic nucleus (NSQ) and peripheral clocks regulate physiology and metabolism to adjust temporal variations in homeostatic regulation (Mohawk et al., 2012). Several studies demonstrate that when the circadian system is disrupted, the desynchrony between the central and peripheral clocks leads to an increased metabolic risk (Potter et al., 2016; Qian et al., 2016; Maury et al., 2014). Constant light (LL) is a condition that generates an absence of rhythmicity with loss of feeding/fasting rhythm (Rumanova et al., 2020). This internal desynchronization due to LL is associated with several metabolic alterations: high levels of body mass, alterations in levels of cholesterol and glucose (Abulmeaty et al., 2021; Kolbe et al., 2019; Plano et al., 2017). However, in our study the LL group presented significantly lower body weight in almost all PNDs. This can be due to a reduced food intake, which has already been described as a consequence of exposure to constant light (Fonken et al., 2010). The DD (constant darkness) group showed higher levels of triglycerides. These results show that these aberrant photoperiod changes induce alterations in metabolic measures. The RGB-f group presented significantly higher levels of total cholesterol compared to RGB-v (varied spectral composition), although these differences seem to be of no biological relevance. The next necessary step for this area of study is to further explore the mechanisms that lead the distinct light regimens to alter serum parameters linked to metabolism. Also, it

is highly necessary to elucidate the healthy characteristics of a rodent under development to identify which conditions are harmful or not to its growth.

The most interesting and challenging characteristic of sexual maturation is the heterogeneity of patterns, so it is necessary to find points of convergence to better understand how to characterize this period. A limitation of this study is that the protocol duration was not long enough to determine when all female rats had regularized cycles and reached sexual maturation. Another limitation of our study is that we analyzed vaginal cytology once a day, missing some short phases of the estrous cycle. This misstep was corrected by considering the duration of each phase and standardizing a rule for complete cycles based on literature (Cora et al., 2015; Goldman et al., 2007). Lastly, since rats were housed in groups of 3 animals per cage, we were not able to measure dietary intake, a variable that might also be associated with weight gain.

In summary, our results demonstrate that exposure to a light pattern whereby a gradual change in color temperature occurs, appears to promote a change in puberty timing, whereas lighting with fixed color temperature appears to accelerate reproductive development. Also, we suggest that both changes in photoperiod and lighting quality alter the lipid profile and the pattern of visceral fat accumulation. However, it is difficult to state, from the metabolic and reproductive point of view, which is the ideal lighting condition, since there are biological variations among animals. Therefore, we believe it is crucial to expand studies in order to clarify the mechanisms underlying the relationship of light exposure with sexual maturation and metabolism.

## **ACKNOWLEDGMENTS**

The authors acknowledge the Animal Experimentation Unit team (UEA) of the Hospital de Clínicas de Porto Alegre (HCPA) for technical and logistical support.

## **DECLARATION OF INTEREST**

The work was supported by the Incentive Fund to Research and Events (FIPE – HCPA) and the National Counsel of Technological and Scientific Development (CNPq – UFRGS). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

## REFERENCES

- Abulmeaty, M. M. A., Almajwal, A. M., Alnumair, K. S., Razak, S., Hasan, M. M., Fawzy, A., ... & Aljuraiban, G. S. (2021). Effect of Long-Term Continuous Light Exposure and Western Diet on Adropin Expression, Lipid Metabolism, and Energy Homeostasis in Rats. *Biology*, 10(5), 413.
- Bohlen, T. M., Silveira, M. A., Buonfiglio, D. D. C., Ferreira-Neto, H. C., Cipolla-Neto, J., Donato Jr, J., & Frazao, R. (2018). A short-day photoperiod delays the timing of puberty in female mice via changes in the kisspeptin system. *Frontiers in endocrinology*, 9, 44.
- Byers, S. L., Wiles, M. V., Dunn, S. L., & Taft, R. A. (2012). Mouse estrous cycle identification tool and images. *PloS one*, 7(4), e35538.
- Charalampopoulos D, McLoughlin A, Elks CE, Ong KK. Age at menarche and risks of all-cause and cardiovascular death: a systematic review and meta-analysis. *Am J Epidemiol*. 2014;180(1):29-40. doi:10.1093/aje/kwu113
- Coomans, C. P., van den Berg, S. A., Houben, T., van Klinken, J. B., van den Berg, R., Pronk, A. C., ... & Meijer, J. H. (2013). Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity. *The FASEB Journal*, 27(4), 1721-1732.
- Cora, M. C., Kooistra, L., & Travlos, G. (2015). Vaginal cytology of the laboratory rat and mouse: review and criteria for the staging of the estrous cycle using stained vaginal smears. *Toxicologic pathology*, 43(6), 776-793.
- Crowley, S. J., Cain, S. W., Burns, A. C., Acebo, C., & Carskadon, M. A. (2015). Increased sensitivity of the circadian system to light in early/mid-puberty. *The Journal of Clinical Endocrinology & Metabolism*, 100(11), 4067-4073.
- de Oliveira, M. A., Scop, M., Abreu, A. C. O., Sanches, P. R. S., Rossi, A. C., Díez-Noguera, A., ... & Hidalgo, M. P. (2019). Entraining effects of variations in light spectral composition on the rest-activity rhythm of a nocturnal rodent. *Chronobiology international*, 36(7), 934-944.

Evans, M. C., & Anderson, G. M. (2018). Integration of circadian and metabolic control of reproductive function. *Endocrinology*, 159(11), 3661-3673.

Fonken, L. K., Workman, J. L., Walton, J. C., Weil, Z. M., Morris, J. S., Haim, A., & Nelson, R. J. (2010). Light at night increases body mass by shifting the time of food intake. *Proceedings of the National Academy of Sciences*, 107(43), 18664-18669.

Fonken, L. K., Aubrecht, T. G., Meléndez-Fernández, O. H., Weil, Z. M., & Nelson, R. J. (2013). Dim light at night disrupts molecular circadian rhythms and increases body weight. *Journal of biological rhythms*, 28(4), 262-271.

Foster DL, Jackson LM, Padmanabhan V. Programming of GnRH feedback controls timing puberty and adult reproductive activity. *Mol Cell Endocrinol* 2006; 254–255:109–119.

Gamble, J. Early starters. *Nature* 550, S10–S11 (2017). <https://doi.org/10.1038/550S10a>

Goldman, J. M., Murr, A. S., & Cooper, R. L. (2007). The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*, 80(2), 84-97.

Haus, E., Reinberg, A., Mauvieux, B., Le Floc'h, N., Sackett-Lundeen, L., & Touitou, Y. (2016). Risk of obesity in male shift workers: a chronophysiological approach. *Chronobiology international*, 33(8), 1018-1036.

Kasahara T, Abe K, Mekada K, Yoshiki A, Kato T. Genetic variation of melatonin productivity in laboratory mice under domestication. *Proc Natl Acad Sci U S A* (2010) 107(14):6412–7. doi:10.1073/pnas.0914399107

Kolbe, I., Leinweber, B., Brandenburger, M., & Oster, H. (2019). Circadian clock network desynchrony promotes weight gain and alters glucose homeostasis in mice. *Molecular metabolism*, 30, 140-151.

Maury, E., Hong, H. K., & Bass, J. (2014). Circadian disruption in the pathogenesis of metabolic syndrome. *Diabetes & metabolism*, 40(5), 338-346.

Mohawk, J. A., Green, C. B., & Takahashi, J. S. (2012). Central and peripheral circadian clocks in mammals. *Annual review of neuroscience*, 35, 445-462.



Nelson, R. J., & Chbeir, S. (2018). Dark matters: effects of light at night on metabolism. *Proceedings of the Nutrition Society*, 77(3), 223-229.

Ong KK, Bann D, Wills AK, et al; National Survey of Health and Development Scientific and Data Collection Team. Timing of voice breaking in males associated with growth and weight gain across the life course. *J Clin Endocrinol Metab*. 2012;97(8):2844-2852. doi:10.1210/jc.2011-3445

Parent AS, Rasier G, Gerard A, et al. Early onset of puberty: tracking genetic and environmental factors. *Horm Res* 2005; 64 (Suppl 2):41–47.

Plano, S. A., Casiraghi, L. P., García Moro, P., Paladino, N., Golombek, D. A., & Chiesa, J. J. (2017). Circadian and metabolic effects of light: implications in weight homeostasis and health. *Frontiers in neurology*, 8, 558.

Potter, G. D., Skene, D. J., Arendt, J., Cade, J. E., Grant, P. J., & Hardie, L. J. (2016). Circadian rhythm and sleep disruption: causes, metabolic consequences, and countermeasures. *Endocrine reviews*, 37(6), 584-608.

Prentice P, Viner RM. Pubertal timing and adult obesity and cardiometabolic risk in women and men: a systematic review and meta-analysis. *Int J Obes (Lond)*. 2013;37(8):1036-1043. doi:10.1038/ijo.2012.177

Puig-Domingo, M., Webb, S. M., Serrano, J., Peinado, M. A., Corcoy, R., Ruscalleda, J., ... & De Leiva, A. (1992). Melatonin-related hypogonadotropic hypogonadism. *New England Journal of Medicine*, 327(19), 1356-1359.

Qian, J., & Scheer, F. A. (2016). Circadian system and glucose metabolism: implications for physiology and disease. *Trends in Endocrinology & Metabolism*, 27(5), 282-293.

Ritonja, J., Tranmer, J., & Aronson, K. J. (2019). The relationship between night work, chronotype, and cardiometabolic risk factors in female hospital employees. *Chronobiology international*, 36(5), 616-628.

Rumanova, V. S., Okuliarova, M., & Zeman, M. (2020). Differential effects of constant light and dim light at night on the circadian control of metabolism and behavior. *International Journal of Molecular Sciences*, 21(15), 5478.

Sato SM, Schulz KM, Sisk CL, Wood RI. Adolescents and androgens, receptors and rewards. *Horm Behav.* 2008 May;53(5):647–658. PMID: PMC2435368

Smith, M. S. (2009). Estrus and menstrual cycles: neuroendocrine control. In *Encyclopedia of Neuroscience* (pp. 1-5). Elsevier Ltd.

Touitou, Y., Reinberg, A., & Touitou, D. (2017). Association between light at night, melatonin secretion, sleep deprivation, and the internal clock: Health impacts and mechanisms of circadian disruption. *Life sciences*, 173, 94-106.

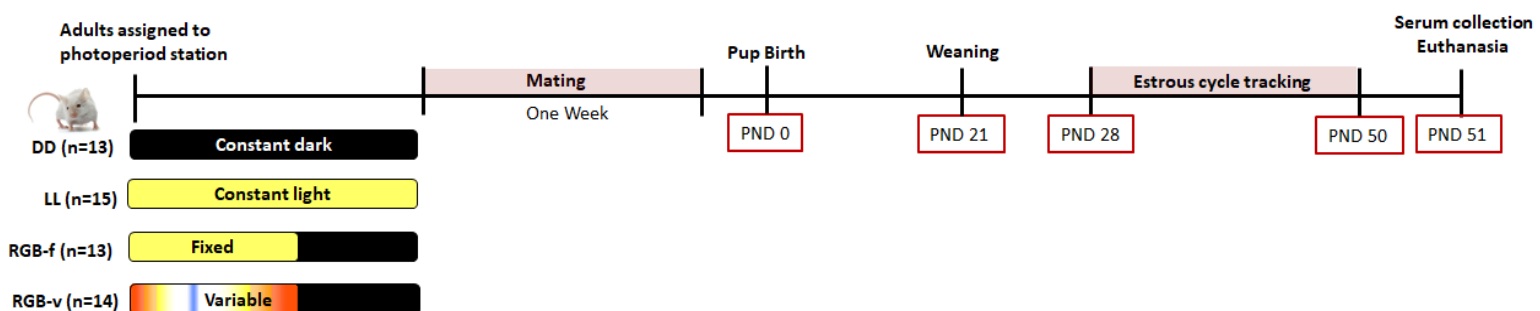
Waldhauser, F., Boepple, P. A., Schemper, M., Mansfield, M. J., & Crowley Jr, W. F. (1991). Serum melatonin in central precocious puberty is lower than in age-matched prepubertal children. *The Journal of Clinical Endocrinology & Metabolism*, 73(4), 793-796.

Wurtman, R. J., Axelrod, J., & Chu, E. W. (1963). Melatonin, a pineal substance: effect on the rat ovary. *Science*, 141(3577), 277-278.

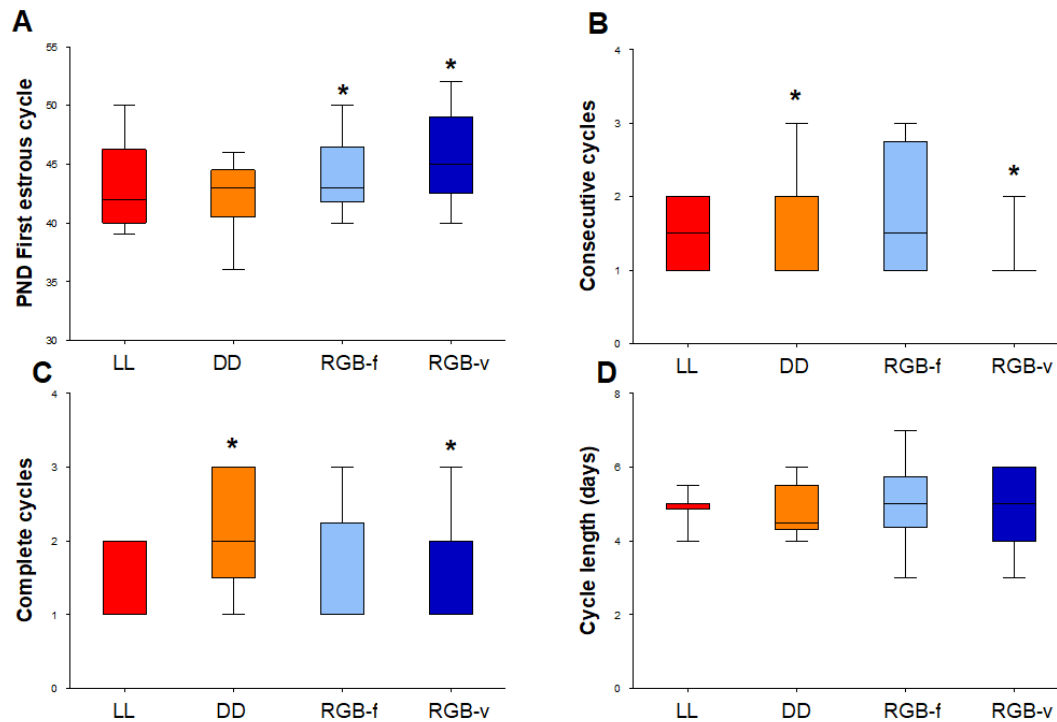
Ying SY, Greep RO. Inhibition of ovulation by melatonin in the cyclic rat. *Endocrinology* (1973) 92(1):333–5. doi:10.1210/endo-92-1-333

Zisapel, N. (2018). New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. *British journal of pharmacology*, 175(16), 3190-3199.

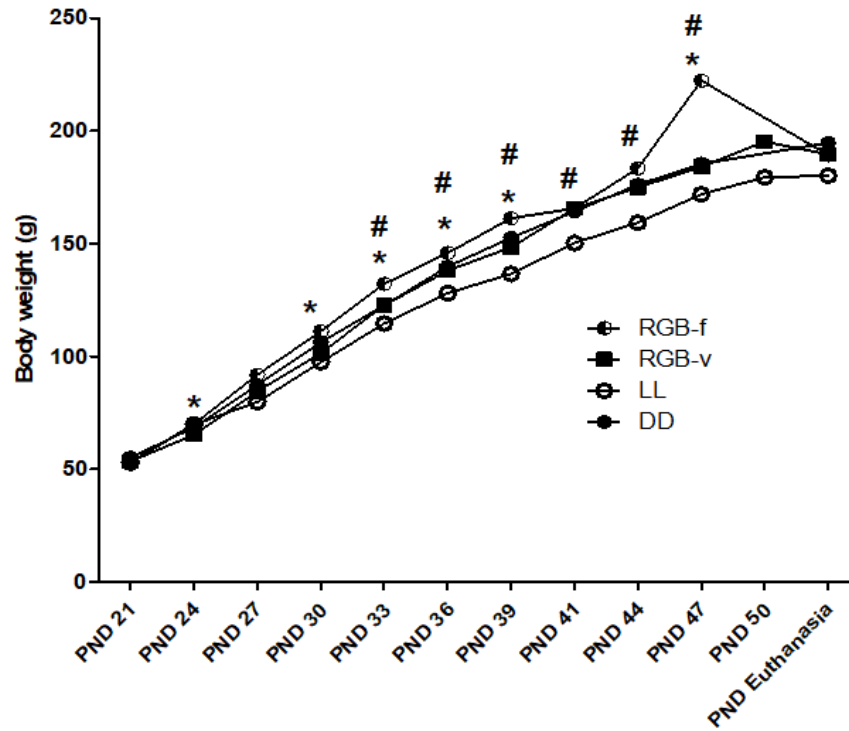
## FIGURES



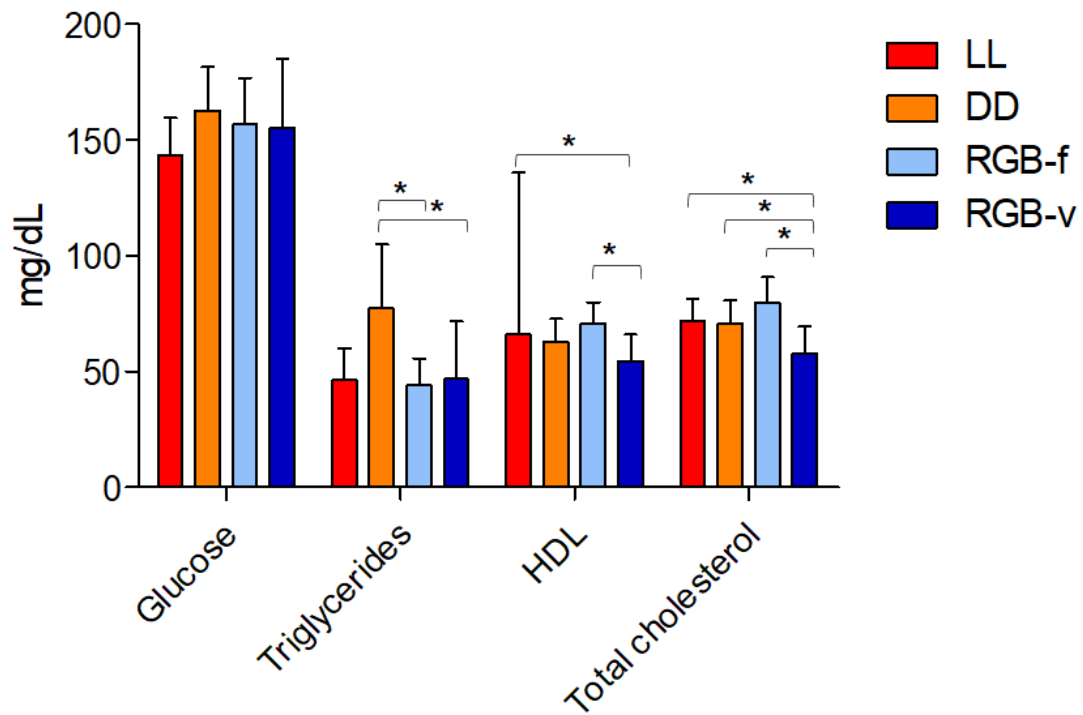
**Figure 1.** Representation of the experimental timeline. Abbreviations: PND = postnatal day.



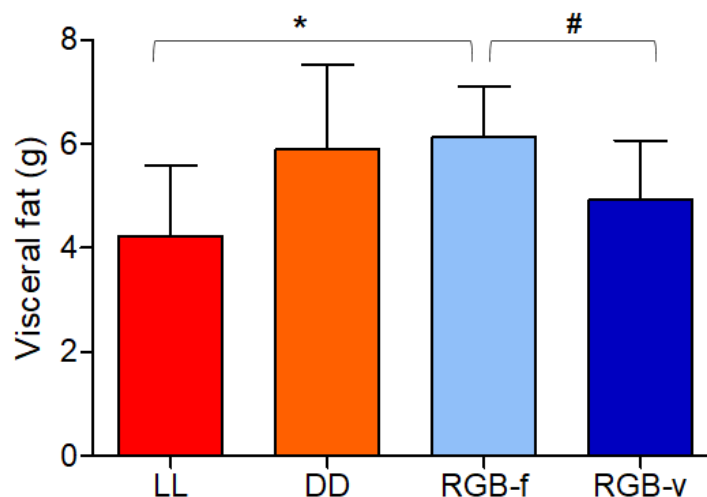
**Figure 2:** (A) Mean of postnatal days (PND) of first complete estrous cycle; groups were compared with two-way ANOVA. (B) Median of complete and consecutive (C) cycles. Median length of cycles in days of female pups. Groups were compared using Kruskal-wallis followed by Dunn. Constant darkness (DD), n=15; Constant light (LL), n= 13; Fixed spectral composition (RGB-f), n= 13; Variation on spectral composition during the day (RGB-v), n=14. (\*p<0.05).



**Figure 3:** Body weight changes during the experimental protocol. Groups were compared using Kruskal-Wallis followed by Dunn. DD, n=15; LL, n= 13; RGB-f, n= 13; RGB-v, n=14. (\* Statistical difference between RGB-f and RGB-v; # Statistical difference between LL and all groups) ( $p < 0.05$ ).



**Figure 4:** Multiple comparisons between metabolic parameters of groups. Significant differences when  $p < 0.05$ . Groups were compared using Kruskal-wallis followed by Dunn. Constant darkness (DD),  $n=15$ ; Constant light (LL),  $n=13$ ; Fixed spectral composition (RGB-f),  $n=13$ ; Variation on spectral composition during the day (RGB-v),  $n=14$ .



**Figure 5:** Average visceral fat among groups. Groups were compared using Kruskal-wallis followed by Dunn. Constant darkness (DD),  $n=15$ ; Constant light (LL),  $n=13$ ;

Fixed spectral composition (RGB-f), n= 13; Variation on spectral composition during the day (RGB-v), n=14. \* $p < 0.05$ ; # trend ( $p < 0.1$ ;  $p > 0.05$ ).

## **SUPPLEMENTARY MATERIAL**

### **Development of the Machine Learning Algorithm to Detect Estrous Cycle Phases**

#### **Data Collection**

All pictures were collected in 2018 at the animal research unit of Hospital de Clínicas de Porto Alegre (Ethical consent GPPG 2016-0378). Vaginal smear cytology was collected by trained researchers of Laboratório de Cronobiologia e Sono every day from vaginal opening until postnatal day 50 at the same hour (5 p.m.). Immediately after observation in a microscopic lens, pictures were taken using a mobile phone directly from an optical microscopic lens (with 40x magnification) available at the laboratory. First, 80 images were selected, 80% of the pictures were used for training and 20% for testing. Second, it was used a larger database with 500 new images, to improve previous results. Finally, we selected 400 new images (100 for each estrous cycle phase) to enhance the accuracy of the algorithm recognition. The database was organized according to the collection day. There is not enough evidence to support the number of pictures necessary to develop a machine learning algorithm to classify images. Thus, for this project, 498 pictures were selected for testing, according to image quality and classified by proportion and distribution of cells in each image as proestrus, estrus, metestrus or diestrus (Cora et al., 2015). Images were classified by three researchers according to the predominant cell type present. After the classification process, image sizes were cut to 800x800 pixels and pictures were named after their number and phase (Ex.: 30\_3, indicates that the 30th image is of the metaestrus (3) phase.).

#### **Development of the Algorithm**

- Preprocessing:

Images were converted to a grey scale. After that, each image went through an adaptive histogram equalization process. In the data augmentation process, the images were cut in 4 quadrants generating smaller images of 400x400 pixels. We then generated new images by rotating them each 0, 90, 180, and 270 degrees, and for each rotation the

images were further reflected horizontally. Therefore, our final dataset had  $498 \times 4 \times 4 \times 2 = 15936$  images.

- Template model:

Using Python 3.8 with Keras package, a multilayer neural network was created. In the first 4 layers there was a 2D convolution  $3 \times 3$  with padding of type “same” -> 2D max pooling  $3 \times 3$  -> batch normalization -> activation. The last two layers are fully connected layers with batch normalization -> activation -> dropout. The output layer has 4 neurons and a softmax activation function. Adam optimizer was used in the gradient descent and, in the calculation of the loss, categorical cross entropy was used with categorical accuracy as metric.

- Hyperparameter Tuning

Using a random search approach, the following parameters were optimized: number of filters in each layer, learning rate, dropout of the 2 fully connected layers, batch size and activation function. In 50 iterations of the search, the best hyperparameters found were:

layer 1 #filters: 16

layer 2 #filters: 32

layer 3 #filters: 128

layer 4 #filters: 128

layer 5 #filters: 32

layer 6 #filters: 8

learning rate: 0.0005

Dropout FCC 1: 0.499

Dropout FCC 2: 0.299

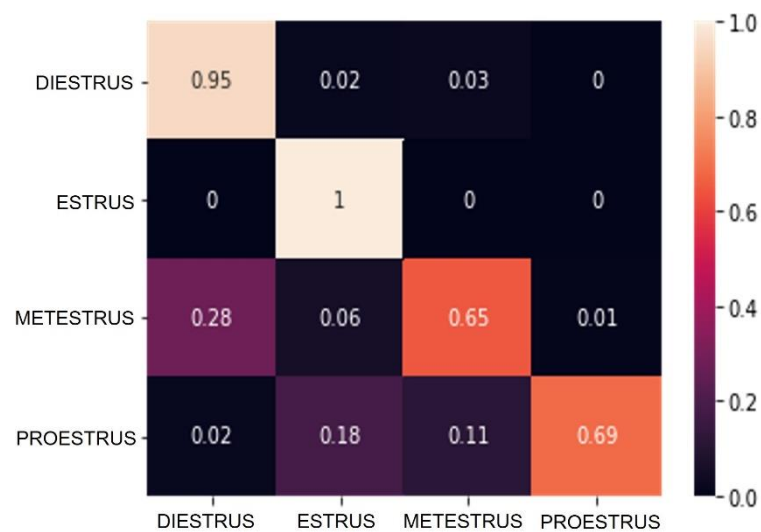
Batch size: 32

- Validation:

We used stratified k-fold cross-validation with 30 splits in the dataset to validate the model and prevent overfitting.

## Metrics to Evaluate the Algorithm and Main Results

First, we used the Classification Accuracy (i.e. the ratio of number of correct predictions to the total number of input images). For the training dataset, with 14336 images, the accuracy was 93%, and for the testing dataset, with 1600 images, the accuracy was 79%. The confusion matrix below describes the complete performance of the model. The cases in which the algorithm incorrectly predicted the phases were more prevalent in the metaestrus phase.



**Figure S1:** Confusion matrix plot used to evaluate the quality of the model output.

We also implemented a not supervised classification, based on clusters, in which the model finds a pattern in a collection of uncategorized data and then identifies natural clusters. With this approach, the model obtained a similar performance compared with the supervised approach.



## 7. ARTIGO 2

### **Relationships Between Circadian Strain, Light Exposure and Body Mass Index in Rural and Urban Quilombolas Communities**

(A ser submetido para a revista *Frontiers in Physiology | Chronobiology Section*)

**Authors:** Débora Barrogi Constantino<sup>1,2</sup>, Nicoli Bertuol Xavier<sup>1,2</sup>, Rosa Levandovski<sup>3</sup>, Till Roenneberg<sup>4</sup>, Maria Paz Hidalgo<sup>1,2,\*</sup>, Luísa K. Pilz<sup>1,2,\*</sup>

#### **Affiliations:**

<sup>1</sup> Laboratório de Cronobiologia e Sono, HCPA/UFRGS. Porto Alegre, RS - Brazil.

<sup>2</sup> PPG em Psiquiatria e Ciências do Comportamento, UFRGS. Porto Alegre, RS - Brazil.

<sup>3</sup> PPG Avaliação e Produção de Tecnologias para o SUS, GHC; PPG Saúde Coletiva, UFRGS. Porto Alegre, RS – Brazil

<sup>4</sup> Institute of Medical Psychology – LMU, Munich - Germany.

\* Contributed equally as senior authors.

#### **Corresponding author:**

Luísa K. Pilz

Universidade Federal do Rio Grande do Sul

Porto Alegre, Rio Grande do Sul, Brasil

luisa.pilz@ufrgs.br

## ABSTRACT

Industrialization have greatly changed human lifestyle; work and leisure activities have been moved indoors, and we artificially illuminate the night. As cyclic environmental cues such as light and feeding become weak and/or irregular, endogenous circadian systems are increasingly being disrupted. These disruptions are associated with metabolic dysfunction, possibly contributing to increased rates of overweight and obesity worldwide. Here, we aimed to investigate how activity-rest rhythms, patterns of light exposure and levels of urbanization may be associated with body mass index in a sample of rural and urban Quilombolas communities, which were classified into five groups according to their stage of urbanization: from rural areas with no access to electricity to highly urbanized communities. We collected anthropometric data to calculate body mass index, which was categorized as:  $\geq 18.5 \text{ kg/m}^2$  to  $< 25 \text{ kg/m}^2$  = normal weight;  $\geq 25 \text{ kg/m}^2$  to  $< 30 \text{ kg/m}^2$  = overweight;  $\geq 30 \text{ kg/m}^2$  = obese. Subjects were asked about their sleep routines and light exposure on workdays and work-free days using the Munich Chronotype Questionnaire (N = 244 included). In addition, we analysed actimetry data from 121 participants with seven consecutive days of recordings. Living in more urbanized areas and higher intradaily variability of activity-rest rhythms were associated with an increased risk of obesity, when controlling for age and sex. This result indicates that higher rhythm fragmentation might characterize metabolic pathologies. These findings are consistent with preclinical data and point to potential strategies in obesity prevention and promotion of healthy metabolic profiles.

**Key words:** Circadian rhythms, light exposure, body mass index, actimetry, obesity, intradaily variability, chronobiology

## INTRODUCTION

Worldwide, the prevalence of obesity has tripled since 1975 and constitutes a major challenge for the medical and scientific communities (Abarca-Gómez et al., 2017). In 2016, more than 1.2 billion adults were overweight and nearly 650 million obese (WHO, 2020). Increased body mass index (BMI) is associated with many comorbidities, such as cardiovascular diseases, diabetes, osteoarthritis, and some forms of cancers (Haslam and James, 2005; Wolin et al., 2010). Weight gain results from multiple conditions and complex interactions between factors that still need better characterization (Blüher, 2019). Disruption of circadian rhythms, which is mostly driven by societal constraints (McHill and Wright, 2017), has been described as one factor contributing to weight gain (Roenneberg et al., 2012).

To synchronize (entrain) to the rhythmic environment, the central circadian clock in the suprachiasmatic nucleus (SCN) predominantly uses light and darkness as *zeitgeber* (environmental signals specific for entrainment) (Roenneberg and Merrow, 2007; LeGates et al., 2014). The SCN provides an internal rhythmic milieu that is used by the cellular clocks in tissues and organs to entrain to the light-dark cycle of the environment (via the SCN), as well as to other clocks of the peripheral circadian system. This system modulates all aspects of physiology (e.g., body temperature, hormone secretion, feeding behavior, alertness, cognitive function; Mohawk et al., 2013; Astiz et al., 2019) thereby also coordinating metabolism on a daily basis (Reinke and Asher, 2019; Marcheva et al., 2013). Notably, peripheral clocks, e.g., in the liver, can also be entrained by food intake.

Our circadian organization is affected by current lifestyles and behaviors in modern societies, including weak *zeitgeber* signals (due to less time spent outdoors and artificial light after dusk) (Roenneberg and Merrow, 2016) – even self-selected light-dark cycles, or sleep curtailment and frequent snacking. Timing, duration and intensity of light exposure have strong effects on health and behavioral outcomes (Rumanova et al., 2020; Gonzalez, 2018); exposure to weak (low-amplitude) light-dark cycles, for example, is associated with metabolic changes and weight gain (Borniger et al., 2014). Weak or irregular light exposure is often associated with circadian misalignment (e.g., in shift workers or simply early work start times that lead to using alarm clocks), which itself is linked to obesity (Roenneberg et al., 2012; Liu et al., 2018). Disturbances of daily rhythmicity have been related to cardiovascular disease, diabetes, aging and obesity (Sletten et al., 2020; Sohail et al., 2015). When rodents are put in forced desynchrony (modelling circadian disruption), glucose metabolism and insulin sensitivity become

impaired (de Oliveira et al., 2019; Karatsoreos et al., 2011). However, the precise mechanisms linking circadian disruption with adverse metabolic consequences are not fully understood.

Few studies have evaluated the relationship between circadian rhythms, light exposure and body weight in clinical, epidemiological or field studies. Yet, identifying modifiable behaviors/aspects promoting weight gain is essential to understand the underlying mechanisms and to devise effective interventions. Here we report the results of studying the association between BMI, light exposure, and activity-rest rhythms in Quilombola communities in the South of Brazil that live under different levels of urbanization. We hypothesized that later phases of entrainment, irregular light exposure and higher levels of urbanization are associated with overweight/obesity.

## **METHODS**

### **Study population**

Participants were recruited between March 2012 and November 2019 from rural and urban Quilombolas communities. These are characterized as remaining social groups established in the past to escape or resist slavery (and slavery remnants) in Brazil with an established ethnic identity and culture. These communities, already described in a previous study (Pilz et al., 2018), have varied histories of access to electricity and live at different stages of urbanization and, therefore, represent a unique opportunity to study the association between circadian misalignment and metabolic factors under the conditions brought by modern industrialized societies. Both rural and urban communities were located in the South Brazil. 320 participants (60% women) of rural and urban quilombolas communities aging from 16 years old to 92 yrs were enrolled in this cross-sectional study. Sociodemographic data, anthropometry, actimetry and sleep variables were collected. Exclusion criteria included not being able to provide necessary information for the development of the study, having done night shift work in the last 6 months before data collection or having used an alarm clock on work-free days (see Figure 1; since only 2 subjects with questionnaires' valid data were underweight, they were also excluded. 244 subjects were included in questionnaire analyses and 121 in actimetry data analyses.

We used a set of previously validated questionnaires and interviewers were trained to adapt questions to the level of the participants' understanding. Additionally, height and weight were measured with a tape measure and a digital scale. BMI was calculated as

weight(kg)/height(m)<sup>2</sup> and categorized as follows:  $\geq 18.5$  kg/m<sup>2</sup> to  $< 25$  kg/m<sup>2</sup> = normal weight;  $\geq 25$  kg/m<sup>2</sup> to  $< 30$  kg/m<sup>2</sup> = overweight;  $\geq 30$  kg/m<sup>2</sup> = obese, according to the recommendation of the World Health Organization. On the few occasions in which data could not be directly collected, self-reported values were used instead.

## **Ethics**

The study was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre (project numbers 2019-0218, 2015-0568, and 2011-0502). All participants provided written informed consent and all study procedures were conducted according to the Declaration of Helsinki. In cases of illiterate participants, an informed consent witnessed by another family member, or the community leader was collected. When participants were younger than 18 years old, parents gave their consent.

## **Assessment of Daily Rhythmicity**

Participants were asked to wear an actimeter (ActTrust Condor™, Actiwatch-2 Philips Respironics) on their non-dominant wrist for at least 30 consecutive days to characterize their activity-rest rhythms and light exposure. Data were binned in 10-minute epochs for analysis. For this study we included actimetry records of 7 consecutive days (N= 121) with a maximum of 4h of missing data per day. Off-wrist periods (nonwear) were detected using ChronoSapiens software (Roenneberg et al., 2015). These were identified as stretches of at least 10 consecutive zeros, confirmed by visual inspection and set to NA (not available). Activity and light recordings collected using ActTrust were normalized to be comparable to Actiwatch-2. Supplementary material includes plots of data with no transformation. Considered the smaller sample size, we did not run comparison analyses.

To characterize rhythms of activity and light we used *Cosinor* analysis (Díez-Noguera et al., 2013). This test provides the following parameters: *mesor* (Midline Estimating Statistic of a Rhythm), *amplitude* (difference between the maximum and minimum value of a rhythmic variable) and *acrophase* (time at which a variable reaches its peak; i.e., peak of the fitted curve). We computed *Cosinor* tests using the R package “psych” (Revelle, 2019).

Daily rhythms were also analyzed by nonparametric circadian rhythm analyses (NPCRA) (Van Someren et al., 1999), including:

- a) Interdaily stability (IS): indicates how constant or stable the 24h-rhythmic pattern is across days. The range varies from 0 to 1 and higher values indicate more stable rhythms;
- b) Intradaily variability (IV): estimates rhythm fragmentation by reflecting transitions between rest and activity or light and dark. The range varies from 0 to 1 and higher values indicate more fragmented rhythms;
- c) M10: mean of measurements over the 10 consecutive hours with the highest values of a daily profile (24h mean wave);
- d) L5: average of measurements over the 5 consecutive hours with the lowest values of a daily profile (24h mean wave).
- e) Relative amplitude (RA): difference between M10 and L5, divided by their sum.

We also computed and plotted the median and mean daily profiles of light exposure and activity-rest by BMI categories. These were calculated at the individual level, and median group profiles were then computed from the individual daily profiles.

#### Self-reported sleep variables

The Brazilian Portuguese Version of the Munich Chronotype Questionnaire (MCTQ, Roenneberg et al., 2003) was used to assess sleep-wake patterns and natural light exposure on work and work-free days. The following variables were computed from the MCTQ data: midpoint of sleep on free days (MSF; as a marker of chronotype), sleep duration on workdays and work-free days, social jetlag (measured by subtracting the midpoint of sleep on work-free days from midpoint of sleep on workdays) and the amount of time spent outdoors on workdays and free days.

#### Statistical Analyses

Data are reported as means and standard deviations or median and interquartile range, when appropriated. Normality was tested using Shapiro-Wilk and visual inspection of histograms. Since they were not normally distributed, we used Kruskal-Wallis followed by Dunn (Sidak correction) to compare parameters derived from the MCTQ or from the actimeter recordings between BMI categories (normal weight, overweight and obese). Effect sizes and confidence intervals were computed as epsilon-squared (Kelley, 1935) using the R package '*rcompanion*' (Mangiafico, 2021), and as eta-squared (Tomczak et al., 2014) using the R package '*rstatix*' (Kassambara, 2020). Data were plotted using

'*ggplot2*' (Wickham, 2016), and the package '*jtools*' for assessing Poisson results (Long, 2019).

Significant relationships between actimetry- and MCTQ-derived variables and BMI (dependent variable) were further investigated using Poisson regression, as well as the association between levels of urbanization and BMI. Since our sample has different sizes for subjective and objective data, we used one model for parameters derived from MCTQ and one model for actimetry data. We also analyzed the association between data derived from the actimeters and BMI in two models: one for activity- and the other for light-variables. The variables were selected based on both the results from Kruskal-Wallis tests (difference between normal-BMI and overweight) and effect sizes (moderate). For the final actimetry model, we selected the variables that were significant in the previous models. Models were controlled for age and sex, used a robust estimator (HC0) and were tested for dispersion. We assessed multicollinearity using Variance Inflation Factor (VIF) test and excluded variables when collinearity was severe ( $VIF > 5$  for IV and RA; in our models  $VIF < 2$  for all independent variables) (Kutner et al., 2004). Lastly, we tested the linearity of parameters included in the models using the Wald test. Since social jetlag did not meet the linearity assumption, we chose to categorize this variable.

## RESULTS

### Characteristics of the study population

General characteristics of the sample are shown in **Table 1**. In the MCTQ dataset, the median of age was 45 years [Q<sub>1</sub>-Q<sub>3</sub>: 28-58] and 144 (59%) participants were women. In the actimetry dataset, the median of age was 49 years [Q<sub>1</sub>-Q<sub>3</sub>: 29-61] and 77 (64%) were women. Most participants had only completed primary school. 32.1% of participants were overweight (BMI  $\geq 25$  kg/m<sup>2</sup> to  $< 30$  kg/m<sup>2</sup>) and 29.6% were obese (BMI  $> 30$  kg/m<sup>2</sup>).

### *MCTQ variables and level of urbanization according to BMI groups*

Comparisons of MCTQ variables between BMI groups are provided in supplementary material (**Table 2**). Overall, we did not find significant differences.

To examine the association of MCTQ variables and levels of urbanization with the odds of being overweight/obese we also used a Poisson regression model adjusted for age and sex. To that end, we divided subjects into two groups, normal ( $\text{BMI} \geq 18.5 \text{ kg/m}^2$  to  $< 25 \text{ kg/m}^2$ ) and overweight/obese ( $\text{BMI} > 25 \text{ kg/m}^2$ ). In model 1 (see table 2), living in highly urbanized communities was associated with increased odds of being overweight/obese (PR: 1.63 [1.05 – 2.53]). Social jetlag was not associated with the outcomes in this sample.

#### *Rest-activity and light exposure patterns according to BMI groups*

The mean group profiles of light exposure and activity by BMI are shown in **Figure 4**. Median profiles are shown in supplementary material (figure 1S).

As shown in **figure 3**, the intradaily variability derived from actimetry was different between groups (IV, KW:  $\chi^2 = 13.49$ ,  $p < 0.01$ ), being higher in both the overweight and the obese group, compared to the normal-weight group. There was no difference between groups in interdaily stability derived from actimetry (IS, KW:  $\chi^2 = 2.67$ ,  $p = 0.26$ ).

Regarding light exposure, the IV was different between groups (KW:  $\chi^2 = 16.16$ ,  $p < 0.001$ ), being higher in the overweight and obese group as compared to the normal group. Light IS was not significantly different between groups (KW:  $\chi^2 = 5.48$ ,  $p = 0.06$ ).

**Figure 4** represents M10, L5 and relative amplitude (RA) of activity and light. Differences were detected in M10 activity between groups (KW:  $\chi^2 = 9.20$ ,  $p < 0.05$ ): the overweight group had lower values compared to the normal weight. Although there was no difference in activity L5 between groups (KW:  $\chi^2 = 2.47$ ,  $p = 0.29$ ), differences were detected in activity relative amplitude (RA; KW:  $\chi^2 = 8.78$ ,  $p < 0.05$ ): the overweight and obese groups showed lower values.

Regarding light recordings, both M10 (KW:  $\chi^2 = 12.30$ ,  $p < 0.01$ ) and L5 (KW:  $\chi^2 = 9.29$ ,  $p < 0.01$ ) were different between groups: light M10 was significantly higher and light L5 was significantly lower in the normal weight group compared to the obese and overweight, respectively. This indicates higher exposure to light during the day and lower during the night in the normal group compared to the other groups.

**Figures S2 and S3** show NPRCA data faceted by actimeter brand (Actiwatch 2 vs. ActTrust). We did not run comparison tests considered the smaller sample size. Group mean profiles are shown in **figures S4 and S5**. Since light RA had a narrow range due to



the often low values of light L5, we also plotted light RA as  $(\log_{10}(M10+10) - \log_{10}(L5+10)) / (\log_{10}(M10+10) + \log_{10}(L5+10))$ , which is shown in **figure S8**.

There were also significant differences between groups in the MESOR of activity and light (see more details in supplementary material).

Effect sizes and their confidence intervals (by bootstrap) for all Kruskal-Wallis tests, expressed as epsilon-squared ( $\epsilon^2$ ) and eta-squared ( $\eta^2$ ), are shown in Table S3. They were interpreted as being small to moderate.

To examine the association of variables derived from actimetry with the odds of being overweight/obese, we also used Poisson regressions adjusted for age and sex. Among the variables derived from actimetry tested in model 2 (see table 3), we found that higher IV of activity is associated with higher odds of belonging to the overweight/obese group (PR: 3.04 [1.43 – 6.47]). As in model 1 (table 2), the probability of being in the overweight/obese group was significantly higher for women. The separate models with activity and light variables are available in the supplementary material (see table S4 and S5). The final model additionally adjusted for actimeter brand (ActTrust vs. Actiwatch2) is also available in the supplementary material (see table S6).

## DISCUSSION

Our study investigated the association of daily behavior and levels of urbanization with body mass index (BMI). The results show that both activity and light are more fragmented (i.e., higher values of intradaily variability) in the overweight/obesity group than in the normal-weight participants. Night-time light exposure was positively and day-time light exposure was negatively associated with having higher than normal BMI. Furthermore, living in areas that are more urbanized was associated with an increased risk of belonging to the overweight/obese group.

Contrary to previous findings of studies in other populations (McMahon et al., 2019; Parsons et al., 2015; Roenneberg et al., 2012; Patterson et al., 2018), social jetlag (SjL) and chronotype were not associated with BMI in our sample: here, we used MSF (midpoint of sleep on free days) as a marker of chronotype and we did not detect differences in phase of entrainment or social jetlag between BMI groups either, meaning we could not reject the null hypothesis. The lack of a significant difference in this instance may be related to the low levels of social jetlag in our rural sample, which may not be sufficient to impose a risk. Additionally, SJL reflects circadian misalignment in the context of work structures normally seen in urban or industrialized societies (workdays

vs. free days). Other metrics of circadian strain could better show how challenges to the system may be a risk factor to obesity.

In fact, we did see differences in IV and RA between BMI groups. The intradaily variability (IV) of activity and light was significantly higher in the overweight and obese groups, compared to the normal weight group, which indicates that higher fragmentation of daily rhythms associates with overweight and obesity. This result supports those of Garaulet and colleagues, who found highly fragmented activity rhythms associated with obesity and central adiposity in a sample of adolescents (Garaulet et al., 2017) as well as with an earlier study that suggested higher fragmentation as a predictor of lower weight loss in overweight and obese women undergoing a weight-reduction program (Bandin et al., 2014). Other large epidemiological studies have shown a positive association between obesity, diabetes, metabolic risk and fragmentation of activity rhythms in urban populations (Sohail et al., 2015; Luik et al., 2013; Van den Berg et al., 2008). Our study adds to the mounting evidence by showing that activity IV also correlates with BMI in a sample comprised of subjects at different urbanization stages. The higher activity and light variability do not seem to be an artifact produced by different actimeter brands (see figure S2). Even adjusting model 2 to actimeter brand, activity was still significantly associated to being overweight/obese.

Few studies have investigated the associations of stability in activity rhythms (measured by interdaily stability - IS) with BMI; some of them found significant associations (Sohail et al., 2015; Luik et al., 2013), whereas other did not (Cespedes Feliciano et al., 2017). In our sample, we did not find any significant differences in the IS between groups.

In addition to the higher fragmentation of light exposure and activity rhythms, in our study, lower relative amplitude (RA) of activity was related to overweight/. RA is calculated as the difference between M10 and L5, normalized by their sum (Van Someren et al., 1999). Lower values indicate a less robust 24-hour activity-rest or light-dark pattern, reflecting both lower activity and light during the wake periods and/or higher activity and light during the rest phase. According to the existing literature, this flattening of the rest-activity rhythm, indicates that the circadian pacemaker is less entrained to its zeitgebers, correlating with metabolic disruption (Wright Jr et al., 2013; Hsieh et al., 2010). As expected, the overweight group was less active during the day (i.e., had a lower M10), even though the L5 of activity was the same across groups. Among NPRCA estimates, RA has been widely investigated as a factor inversely correlated with

conditions such as psychiatric disorders, cognitive malfunctioning, and metabolic dysfunction (Ng et al., 2015; Cespedes Feliciano et al., 2017; Lyall et al., 2018). Although we observed the inverse association of RA (of both activity and light) with BMI, such relationship did not persist in the multivariate model, possibly due to the low sample size (data not shown). Furthermore, descriptives suggest similar patterns across groups regardless of the actimeter brand except for M10 and RA of activity. M10 and RA of activity seem to be lower in obese compared to the normal group only among those wearing ActTrust, and similar in those wearing the Actiwatch 2. ActTrust is more sensitive to movement, but these differences may also reflect the characteristics of both samples collected with each actimeter, since ActTrust's sample was larger and more varied in terms of urbanization (and therefore in variability measures): no-electricity and urban groups data were collected using actTrust. Even if causal relationships underlying these associations are still not clear, there is growing evidence that simple environmental interventions, such as keeping regular light exposure, feeding and physical activity times, may improve metabolic health outcomes (Dowling et al., 2005; Van Someren and Riemersma-Van Der Lek, 2007).

The NPRCA estimates for light exposure indicate that a weaker signal and irregular patterns may be related with adverse metabolic consequences and are consistent with recent findings in the literature (Wyse et al., 2014; McFadden et al., 2014, Gerhart-Hines, Lazar, 2015). In mammals, light is the main cue for entraining internal circadian rhythms to environmental cycles. The lack of a strong zeitgeber contrast between day and night leads to advancing extreme early types and delaying all other chronotypes (Papatsimpa et al., 2021). The resulting interaction with local time leads to sleeping and eating at the wrong circadian times, constituting potential mediators linking light exposure to BMI, as shown in both humans and rodents (Fonken et al., 2013; Shi et al 2013; Plano et al., 2017). Several other studies report that daytime bright light therapy improves glycemic control in diabetics patients (Versteeg et al., 2017), reduce insulin resistance, body weight and fat mass (Sene-Fiorese et al., 2015) and improves carbohydrate metabolism (Dunai et al., 2007; Danilenko et al., 2013). Our results support the potential of strategies aimed at adjusting light exposure and promoting circadian organization for preventing obesity.

Another important aspect to be discussed regarding the development of obesity is the changes in lifestyle brought by industrialization that may impact health and increase risk for metabolic diseases. The urban environment comprises factors that affect circadian rhythms and health, such as working in night shifts, prolonged exposure to artificial light,

stress, pollution, social jetlag, poor diets (Benedito-Silva et al., 2020; Wyse et al., 2014; Pot et al., 2018). One study comparing Brazilian communities with different levels of urbanization demonstrated that urban dwellers had higher BMI and higher levels of insulin, fasting glucose and insulin resistance (Martins et al., 2020). Here we found an association between higher levels of urbanization and increased risk of being overweight/obese. One of the factors explaining these results may be the occupations in rural settings. Since their economy is mostly based on agricultural jobs, they have higher levels of activity during the day. Future studies are needed to investigate how levels of physical activity during work could be a factor contributing to the differences in BMI. Another contributing factor could be the difference in light exposure between some of these communities (Pilz et al., 2018). Reduced natural daylight exposure and increased levels of nocturnal light are common features of urbanized societies and may disrupt circadian rhythmicity, contributing to the association between global urbanization and obesity (Rybnikova et al., 2016). Since we observed a significant association of light at night /low light exposure during the day with overweight /obesity, this may be considered as one of the driving forces behind metabolic diseases. Finally, eating habits were greatly changed by urban settings in many aspects, such as timing of eating, frequency of meals and diet quality. All these factors may be associated with body weight as well (Pot et al., 2018).

The main strengths of the current work include the use of objective measures to assess daily behavior. Furthermore, we considered some important covariates and potential confounders such as age, sex, and use of alarm clocks, which may influence the calculation of MSF and social jetlag. Lastly, we could compare many communities that differ in access to electricity and urbanization, which represents a unique opportunity to study the impacts of modern lifestyles on human biological rhythms and metabolism. Conversely, there are some limitations to be considered when interpreting our findings. Firstly, the limited generalizability of the results, considering the study sample size. Furthermore, its cross-sectional nature precludes inferring causality and direction of the associations. Secondly, due to our sample size, we may have not had enough statistical power to rule out confounders and detect the effects of some variables on the multivariate models; the wide confidence intervals for effect sizes suggest that further information is needed for greater precision. Thirdly, as a methodological limitation, BMI is a populational measure that does not cover individual characteristics of body composition, including body fat and lean mass percentage, although it is the standard clinical measure

of obesity. Future work should consider the use of other estimates of adiposity, such as waist circumference, body fat and direct measurements of metabolic parameters that were not assessed in this study due to logistics difficulties that prevented collection, storage and transportation of biological samples. Fourth, when we were unable to measure height and weight or there was any discomfort from participants, some of the BMI measures were self-reported. Thus, we chose to categorize this variable into three groups for it to be more reliable. Finally, it was not possible to assess food intake, a factor that might also be associated with obesity.

In summary, results from this study show a link between more fragmented rhythms of activity and exposure to light and higher BMI. Also, higher levels of urbanization and lower amplitude of light exposure were associated with increased risk of being overweight/obese. The present findings support the hypothesis that disturbed circadian rhythms and irregular light exposure might be associated with increased risk for obesity, which warrants further investigation, considering the strong potential of preventive measures.

## **ACKNOWLEDGMENTS**

We are very thankful to the residents and leaders of the Quilombolas communities for participating in our study and helping in its preparation. We also thank Rogerio Boff Borges for the support and assistance with statistical analysis. This work was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) and FIPE-HCPA (#2019-0218, #2015-0568).

## **DECLARATION OF INTEREST**

The authors have no conflict of interest.

## **REFERENCES**

Abarca-Gómez, L., Abdeen, Z. A., Hamid, Z. A., Abu-Rmeileh, N. M., Acosta-Cazares, B., Acuin, C., ... & Cho, Y. (2017). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128· 9 million children, adolescents, and adults. *The Lancet*, *390*(10113), 2627-2642. doi: 10.1016/S0140-6736(17)32129-3

- Astiz, M., Heyde, I., & Oster, H. (2019). Mechanisms of communication in the mammalian circadian timing system. *International journal of molecular sciences*, 20(2), 343. doi:10.3390/ijms20020343
- Bandin, C., Martinez-Nicolas, A., Ordovas, J. M., Madrid, J. A., & Garaulet, M. (2014). Circadian rhythmicity as a predictor of weight-loss effectiveness. *International journal of obesity*, 38(8), 1083-1088. doi: 10.1038/ijo.2013.211
- Bayon, V., Leger, D., Gomez-Merino, D., Vecchierini, M. F., & Chennaoui, M. (2014). Sleep debt and obesity. *Annals of medicine*, 46(5), 264-272. doi: **10.3109/07853890.2014.931103**
- Benedito-Silva, A. A., Evans, S., Viana Mendes, J., Castro, J., Gonçalves, B. D. S. B., Ruiz, F. S., ... & Pedrazzoli, M. (2020). Association between light exposure and metabolic syndrome in a rural Brazilian town. *Plos one*, 15(9), e0238772. doi: 10.1371/journal.pone.0238772
- Blüher, M. (2019). Obesity: global epidemiology and pathogenesis. *Nature Reviews Endocrinology*, 15(5), 288-298. doi:10.1038/s41574-019-0176-8
- Borniger, J. C., Maurya, S. K., Periasamy, M., & Nelson, R. J. (2014). Acute dim light at night increases body mass, alters metabolism, and shifts core body temperature circadian rhythms. *Chronobiology international*, 31(8), 917-925. doi: 10.3109/07420528.2014.926911
- Branecky, K. L., Niswender, K. D., & Pendergast, J. S. (2015). Disruption of daily rhythms by high-fat diet is reversible. *PloS one*, 10(9), e0137970. doi: 10.1371/journal.pone.0137970
- Cedernaes, J., Osler, M. E., Voisin, S., Broman, J. E., Vogel, H., Dickson, S. L., ... & Benedict, C. (2015). Acute sleep loss induces tissue-specific epigenetic and transcriptional alterations to circadian clock genes in men. *The Journal of Clinical Endocrinology & Metabolism*, 100(9), E1255-E1261. doi: 10.1210/JC.2015-2284
- Cespedes Feliciano, E. M., Quante, M., Weng, J., Mitchell, J. A., James, P., Marinac, C. R., ... & Hipp, J. A. (2017). Actigraphy-derived daily rest-activity patterns and body mass index in community-dwelling adults. *Sleep*, 40(12), zsx168. doi: 10.1093/sleep/zsx168
- Danilenko, K. V., Mustafina, S. V., & Pechenkina, E. A. (2013). Bright light for weight loss: results of a controlled crossover trial. *Obesity facts*, 6(1), 28-38. doi: 10.1159/000348549
- de Oliveira, I. G. B., Junior, M. D. F., Lopes, P. R., Campos, D. B. T., Ferreira-Neto, M. L., Santos, E. H. R., ... & Rosa, D. A. (2019). Forced internal desynchrony induces cardiometabolic alterations in adult rats. *Journal of Endocrinology*, 242(2), 25-36. doi: 10.1530/JOE-19-0026.

- Díez-Noguera, A. (2013). Methods for serial analysis of long time series in the study of biological rhythms. *Journal of circadian rhythms*, *11*(1), 1-19. doi: 10.1186/1740-3391-11-7
- Dowling, G. A., Hubbard, E. M., Mastick, J., Luxenberg, J. S., Burr, R. L., & Van Someren, E. J. (2005). Effect of morning bright light treatment for rest–activity disruption in institutionalized patients with severe Alzheimer’s disease. *International psychogeriatrics/IPA*, *17*(2), 221. doi: 10.1017/s1041610205001584
- Dunai, A., Novak, M., Chung, S. A., Kayumov, L., Keszei, A., Levitan, R., & Shapiro, C. M. (2007). Moderate exercise and bright light treatment in overweight and obese individuals. *Obesity*, *15*(7), 1749-1757. doi: 10.1038/oby.2007.208
- Fonken, L. K., Aubrecht, T. G., Meléndez-Fernández, O. H., Weil, Z. M., & Nelson, R. J. (2013). Dim light at night disrupts molecular circadian rhythms and increases body weight. *Journal of biological rhythms*, *28*(4), 262-271. doi: 10.1177/0748730413493862
- Garaulet, M., Martinez-Nicolas, A., Ruiz, J. R., Konstabel, K., Labayen, I., González-Gross, M., ... & HELENA study group. (2017). Fragmentation of daily rhythms associates with obesity and cardiorespiratory fitness in adolescents: The HELENA study. *Clinical nutrition*, *36*(6), 1558-1566. doi: 10.1016/j.clnu.2016.09.026
- Gerhart-Hines, Z., & Lazar, M. A. (2015). Circadian metabolism in the light of evolution. *Endocrine reviews*, *36*(3), 289-304. doi: **10.1210/er.2015-1007**
- Gil-Lozano, M., Hunter, P. M., Behan, L. A., Gladanac, B., Casper, R. F., & Brubaker, P. L. (2016). Short-term sleep deprivation with nocturnal light exposure alters time-dependent glucagon-like peptide-1 and insulin secretion in male volunteers. *American Journal of Physiology-Endocrinology and Metabolism*, *310*(1), E41-E50. doi: 10.1152/ajpendo.00298.2015
- Gonzalez, M. (2018). Dim light at night and constant darkness: Two frequently used lighting conditions that jeopardize the health and well-being of laboratory rodents. *Frontiers in neurology*, *9*, 609. doi: 10.3389/fneur.2018.00609
- Haslam, D. W., & James, W. P. T. (2005). Obesity. *Lancet (London, England)*, *366*(9492), 1197–1209. doi: 10.1016/S0140-6736(05)67483-1.
- Hsieh, M. C., Yang, S. C., Tseng, H. L., Hwang, L. L., Chen, C. T., & Shieh, K. R. (2010). Abnormal expressions of circadian-clock and circadian clock-controlled genes in the livers and kidneys of long-term, high-fat-diet-treated mice. *International Journal of Obesity*, *34*(2), 227-239. doi: 10.1038/ijo.2009.228

- Karatsoreos, I. N., Bhagat, S., Bloss, E. B., Morrison, J. H., & McEwen, B. S. (2011). Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proceedings of the national Academy of Sciences*, *108*(4), 1657-1662. doi: 10.1073/pnas.1018375108
- Kassambara, A. (2020). rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.6.0. <https://CRAN.R-project.org/package=rstatix>
- Kelley, T. (1935). An unbiased correlation ratio measure. *Proceedings of the National Academy of Sciences*, *21*(9), 554-559. doi: 10.1073/pnas.21.9.554
- Kim, C. E., Shin, S., Lee, H. W., Lim, J., Lee, J. K., Shin, A., & Kang, D. (2018). Association between sleep duration and metabolic syndrome: a cross-sectional study. *BMC public health*, *18*(1), 1-8. doi: 10.1186/s12889-018-5557-8
- Kohsaka, A., Laposky, A. D., Ramsey, K. M., Estrada, C., Joshu, C., Kobayashi, Y., ... & Bass, J. (2007). High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell metabolism*, *6*(5), 414-421. doi: 10.1016/j.cmet.2007.09.006
- Kutner, M.; Nachtsheim, C.; Neter, J. *Applied Linear Statistical Models*. 4th. McGraw-Hill; Irwin; 2004.
- LeGates, T. A., Fernandez, D. C., & Hattar, S. (2014). Light as a central modulator of circadian rhythms, sleep and affect. *Nature Reviews Neuroscience*, *15*(7), 443-454. doi: 10.1038/nrn3743
- Liu, Q., Shi, J., Duan, P., Liu, B., Li, T., Wang, C., ... & Lu, Z. (2018). Is shift work associated with a higher risk of overweight or obesity? A systematic review of observational studies with meta-analysis. *International journal of epidemiology*, *47*(6), 1956-1971. doi: 10.1093/ije/dyy079
- Long J.A. (2019) jtools: Analysis and Presentation of Social Scientific Data. <https://CRAN.R-project.org/package=jtools>.
- Luik, A. I., Zuurbier, L. A., Hofman, A., Van Someren, E. J., & Tiemeier, H. (2013). Stability and fragmentation of the activity rhythm across the sleep-wake cycle: the importance of age, lifestyle, and mental health. *Chronobiology international*, *30*(10), 1223-1230. doi: 10.3109/07420528.2013.813528
- Lyall, L. M., Wyse, C. A., Graham, N., Ferguson, A., Lyall, D. M., Cullen, B., ... & Smith, D. J. (2018). Association of disrupted circadian rhythmicity with mood disorders, subjective wellbeing, and cognitive function: a cross-sectional study of 91 105 participants from the UK Biobank. *The Lancet Psychiatry*, *5*(6), 507-514. doi: 10.1016/S2215-0366(18)30139-1



- Mangiafico S. (2021). rcompanion: Functions to Support Extension Education Program Evaluation. R package version 2.3.27. <https://CRAN.R-project.org/package=rcompanion>
- Marcheva, B., Ramsey, K. M., Peek, C. B., Affinati, A., Maury, E., & Bass, J. (2013). Circadian clocks and metabolism. *Circadian clocks*, 127-155. doi: 10.1007/978-3-642-25950-0\_6
- Martins, A. J., Isherwood, C. M., Vasconcelos, S. P., Lowden, A., Skene, D. J., & Moreno, C. R. (2020). The effect of urbanization on sleep, sleep/wake routine, and metabolic health of residents in the Amazon region of Brazil. *Chronobiology International*, 37(9-10), 1335-1343. doi: 10.1080/07420528.2020.1802287
- McFadden, E., Jones, M. E., Schoemaker, M. J., Ashworth, A., & Swerdlow, A. J. (2014). The relationship between obesity and exposure to light at night: cross-sectional analyses of over 100,000 women in the Breakthrough Generations Study. *American journal of epidemiology*, 180(3), 245-250. doi: 10.1093/aje/kwu117
- McHill, A. W., & Wright Jr, K. P. (2017). Role of sleep and circadian disruption on energy expenditure and in metabolic predisposition to human obesity and metabolic disease. *Obesity reviews*, 18, 15-24. doi: 10.1111/obr.12503
- McMahon, D. M., Burch, J. B., Youngstedt, S. D., Wirth, M. D., Hardin, J. W., Hurley, T. G., ... & Hebert, J. R. (2019). Relationships between chronotype, social jetlag, sleep, obesity and blood pressure in healthy young adults. *Chronobiology international*, 36(4), 493-509. doi: 10.1080/07420528.2018.1563094
- Mohawk, J. A., Green, C. B., & Takahashi, J. S. (2012). Central and peripheral circadian clocks in mammals. *Annual review of neuroscience*, 35, 445-462. doi: 10.1146/annurev-neuro-060909-153128
- Nedeltsheva, A. V., & Scheer, F. A. (2014). Metabolic effects of sleep disruption, links to obesity and diabetes. *Current opinion in endocrinology, diabetes, and obesity*, 21(4), 293. doi: 10.1097/MED.0000000000000082
- Ng, T. H., Chung, K. F., Ho, F. Y. Y., Yeung, W. F., Yung, K. P., & Lam, T. H. (2015). Sleep-wake disturbance in interepisode bipolar disorder and high-risk individuals: A systematic review and meta-analysis. *Sleep medicine reviews*, 20, 46-58. doi: 10.1016/j.smrv.2014.06.006
- Papatsimpa, C., Schlangen, L. J. M., Smolders, K. C. H. J., Linnartz, J. P., & de Kort, Y. A. W. (2021). The interindividual variability of sleep timing and circadian phase in humans is influenced

by daytime and evening light conditions. *Scientific Reports*, 11(1), 1-14. doi: 10.1038/s41598-021-92863-z

Parsons, M. J., Moffitt, T. E., Gregory, A. M., Goldman-Mellor, S., Nolan, P. M., Poulton, R., & Caspi, A. (2015). Social jetlag, obesity and metabolic disorder: investigation in a cohort study. *International Journal of Obesity*, 39(5), 842-848. doi: 10.1038/ijo.2014.201

Patterson, F., Malone, S. K., Grandner, M. A., Lozano, A., Perrett, M., & Hanlon, A. (2018). Interactive effects of sleep duration and morning/evening preference on cardiovascular risk factors. *The European Journal of Public Health*, 28(1), 155-161. doi: 10.1093/eurpub/ckx029.

Pilz, L. K., Levandovski, R., Oliveira, M. A., Hidalgo, M. P., & Roenneberg, T. (2018). Sleep and light exposure across different levels of urbanisation in Brazilian communities. *Scientific reports*, 8(1), 1-11. doi: 10.1038/s41598-018-29494-4

Plano, S. A., Casiraghi, L. P., García Moro, P., Paladino, N., Golombek, D. A., & Chiesa, J. J. (2017). Circadian and metabolic effects of light: implications in weight homeostasis and health. *Frontiers in neurology*, 8, 558. doi: 10.3389/fneur.2017.00558

Pot, G. K. (2018). Sleep and dietary habits in the urban environment: the role of chrono-nutrition. *Proceedings of the Nutrition Society*, 77(3), 189-198. doi: 10.1017/S0029665117003974

Reinke, H., & Asher, G. (2019). Crosstalk between metabolism and circadian clocks. *Nature Reviews Molecular Cell Biology*, 20(4), 227-241. doi: **10.1038/s41580-018-0096-9**

Roenneberg, T., Allebrandt, K. V., Merrow, M., & Vetter, C. (2012). Social jetlag and obesity. *Current Biology*, 22(10), 939-943. doi:10.1016/j.cub.2012.03.038

Roenneberg, T., & Merrow, M. (2007, January). Entrainment of the human circadian clock. In *Cold Spring Harbor symposia on quantitative biology* (Vol. 72, pp. 293-299). Cold Spring Harbor Laboratory Press. doi: 10.1101/sqb.2007.72.043

Roenneberg, T., & Merrow, M. (2016). The circadian clock and human health. *Current biology*, 26(10), R432-R443. doi: 10.1016/j.cub.2016.04.011

Rumanova, V. S., Okuliarova, M., & Zeman, M. (2020). Differential effects of constant light and dim light at night on the circadian control of metabolism and behavior. *International Journal of Molecular Sciences*, 21(15), 5478. doi: 10.3390/ijms21155478

Rybnikova, N. A., Haim, A., & Portnov, B. A. (2016). Does artificial light-at-night exposure contribute to the worldwide obesity pandemic?. *International Journal of Obesity*, *40*(5), 815-823. doi: 10.1038/ijo.2015.255

Sene-Fiorese, M., Duarte, F. O., de Aquino Junior, A. E., Campos, R. M. D. S., Masquio, D. C. L., Tock, L., ... & Bagnato, V. S. (2015). The potential of phototherapy to reduce body fat, insulin resistance and “metabolic inflexibility” related to obesity in women undergoing weight loss treatment. *Lasers in surgery and medicine*, *47*(8), 634-642. doi: 10.1002/lsm.22395

Seo, S. H., & Shim, Y. S. (2019). Association of sleep duration with obesity and cardiometabolic risk factors in children and adolescents: a population-based study. *Scientific reports*, *9*(1), 1-10. doi: 10.1038/s41598-019-45951-0

Shi, S. Q., Ansari, T. S., McGuinness, O. P., Wasserman, D. H., & Johnson, C. H. (2013). Circadian disruption leads to insulin resistance and obesity. *Current Biology*, *23*(5), 372-381. doi: 10.1016/j.cub.2013.01.048

Sletten, T. L., Cappuccio, F. P., Davidson, A. J., Van Cauter, E., Rajaratnam, S. M., & Scheer, F. A. (2020). Health consequences of circadian disruption. *Sleep*, *43*(1), zsz194. doi: 10.1093/sleep/zsz194

Sohail, S., Yu, L., Bennett, D. A., Buchman, A. S., & Lim, A. S. (2015). Irregular 24-hour activity rhythms and the metabolic syndrome in older adults. *Chronobiology international*, *32*(6), 802-813. doi: 10.3109/07420528.2015.1041597

Spaeth, A. M., Dinges, D. F., & Goel, N. (2013). Effects of experimental sleep restriction on weight gain, caloric intake, and meal timing in healthy adults. *Sleep*, *36*(7), 981-990. doi: 10.5665/sleep.2792

Van den Berg, J. F., Neven, A. K., Tulen, J. H. M., Hofman, A., Witteman, J. C. M., Miedema, H. M. E., & Tiemeier, H. (2008). Actigraphic sleep duration and fragmentation are related to obesity in the elderly: the Rotterdam Study. *International journal of obesity*, *32*(7), 1083-1090. doi: 10.1038/ijo.2008.57

Van Someren, E. J., & Riemersma-Van Der Lek, R. F. (2007). Live to the rhythm, slave to the rhythm. *Sleep medicine reviews*, *11*(6), 465-484. doi: 10.1016/j.smr.2007.07.003

Van Someren, E. J., Swaab, D. F., Colenda, C. C., Cohen, W., McCall, W. V., & Rosenquist, P. B. (1999). Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in

Alzheimer patients by application of nonparametric methods. *Chronobiology international*, 16(4), 505-518. doi: 10.3109/07420529908998724.

Versteeg, R. I., Stenvers, D. J., Visintainer, D., Linnenbank, A., Tanck, M. W., Zwanenburg, G., ... & Bisschop, P. H. (2017). Acute effects of morning light on plasma glucose and triglycerides in healthy men and men with type 2 diabetes. *Journal of biological rhythms*, 32(2), 130-142. doi: 10.1177/0748730417693480

Wickham H. (2016). ggplot2: Elegant Graphics for Data Analysis. *Springer-Verlag New York*. ISBN 978-3-319-24277-4, <https://ggplot2.tidyverse.org>.

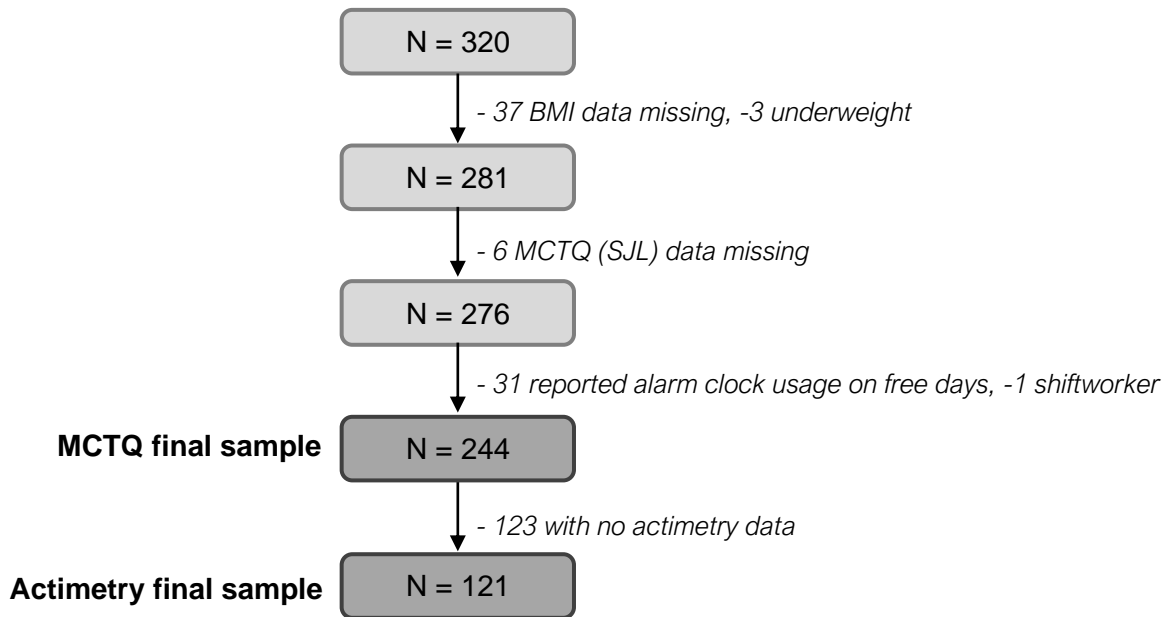
Wolin, K. Y., Carson, K., & Colditz, G. A. (2010). Obesity and cancer. *The oncologist*, 15(6), 556.

World Health Organization. (2020). <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight> [Accessed March 31, 2021].

Wright Jr, K. P., McHill, A. W., Birks, B. R., Griffin, B. R., Rusterholz, T., & Chinoy, E. D. (2013). Entrainment of the human circadian clock to the natural light-dark cycle. *Current Biology*, 23(16), 1554-1558. doi: 10.1016/j.cub.2013.06.039

Wyse, C. A., Biello, S. M., & Gill, J. M. (2014). The bright-nights and dim-days of the urban photoperiod: implications for circadian rhythmicity, metabolism and obesity. *Annals of medicine*, 46(5), 253-263. doi: 10.3109/07853890.2014.913422

## FIGURES AND TABLES



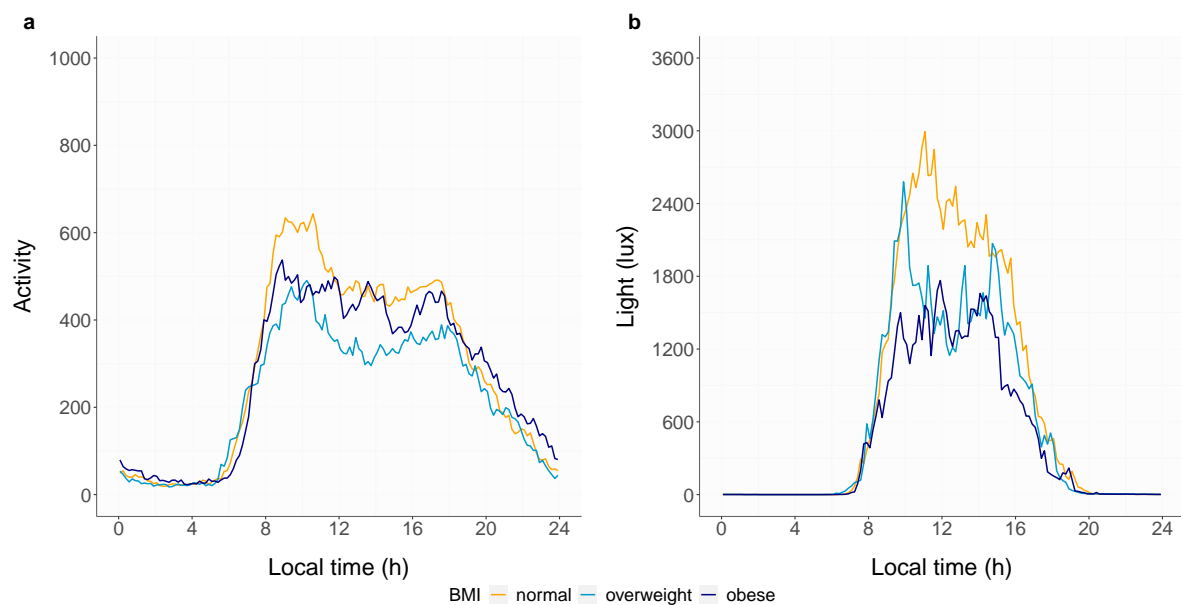
**Figure 1.** Flowchart of the participants recruitment and selection process. White squares represent final sample size.

TABLE 1: Sample characteristics

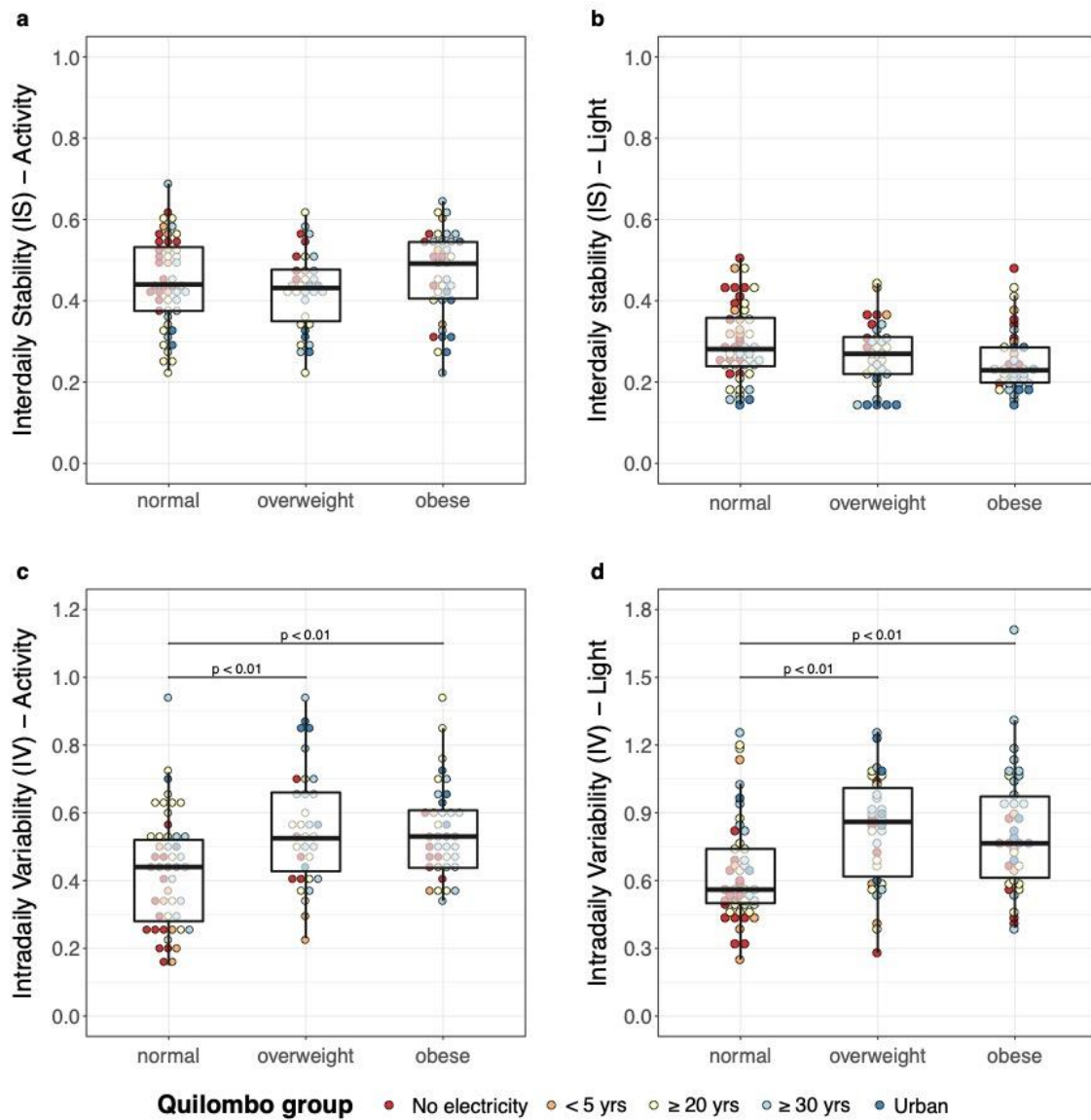
	<b>MCTQ (N = 244)</b>		<b>Actimetry (N = 121)</b>	
	<b>n</b>	<b>%</b>	<b>n</b>	<b>%</b>
<b>No electricity n (%)</b>	29	11.7	28	23.1
<b>&lt;5 yrs n (%)</b>	14	5.7	11	9.0
<b>&gt;20 yrs n (%)</b>	82	33.3	34	28.0
<b>≥30 yrs n (%)</b>	98	39.8	35	29.9
<b>Urban n (%)</b>	21	8.5	13	10.7
<b>Schooling: n (%)</b>				
Illiterate	31	12.7	18	14.8
Primary School incomplete (1 <sup>st</sup> – 4 <sup>th</sup> grade)	97	39.8	47	19.1
Primary School incomplete (5 <sup>th</sup> – 7 <sup>th</sup> grade)	39	16.0	18	14.9
Primary School complete	17	7.0	6	4.9
High School incomplete	12	4.9	6	4.9
High School complete	12	4.9	6	4.9
Undergraduate incomplete /Graduate degree	2	0.01	0	0.0
Not reported	33	13.5	20	16.5
<b>Body Mass Index (BMI) status</b>				
Normal weight: n (%)	92	37.3	49	40.4
Overweight: n (%)	79	32.1	34	28.0
Obese: n (%)	73	29.6	38	31.4
	27	[23 –		
Median BMI [Q <sub>1</sub> – Q <sub>3</sub> ]*	30]		26	[23 – 31]

TABLE 2: Poisson regression (Model1): variables derived from questionnaires associated to overweight/obesity (N = 247)

	PR	95% CI	p-value
Intercept	0.311	0.199 – 0.487	0.000
Age	1.004	0.998 – 1.010	0.194
Sex (F)	1.545	1.231 – 1.938	<b>&lt;0.001</b>
No electricity	1.000	0.535 – 1.871	0.999
< 5 yrs electricity	1.000	0.534 – 1.870	0.999
> 15 yrs electricity	1.171	0.776 – 1.769	0.452
≥ 30 yrs electricity	1.413	0.927 – 2.155	0.108
Urban	1.625	1.045 – 2.527	<b>0.031</b>
Social jetlag (>1h)	0.886	0.669 – 1.173	0.398

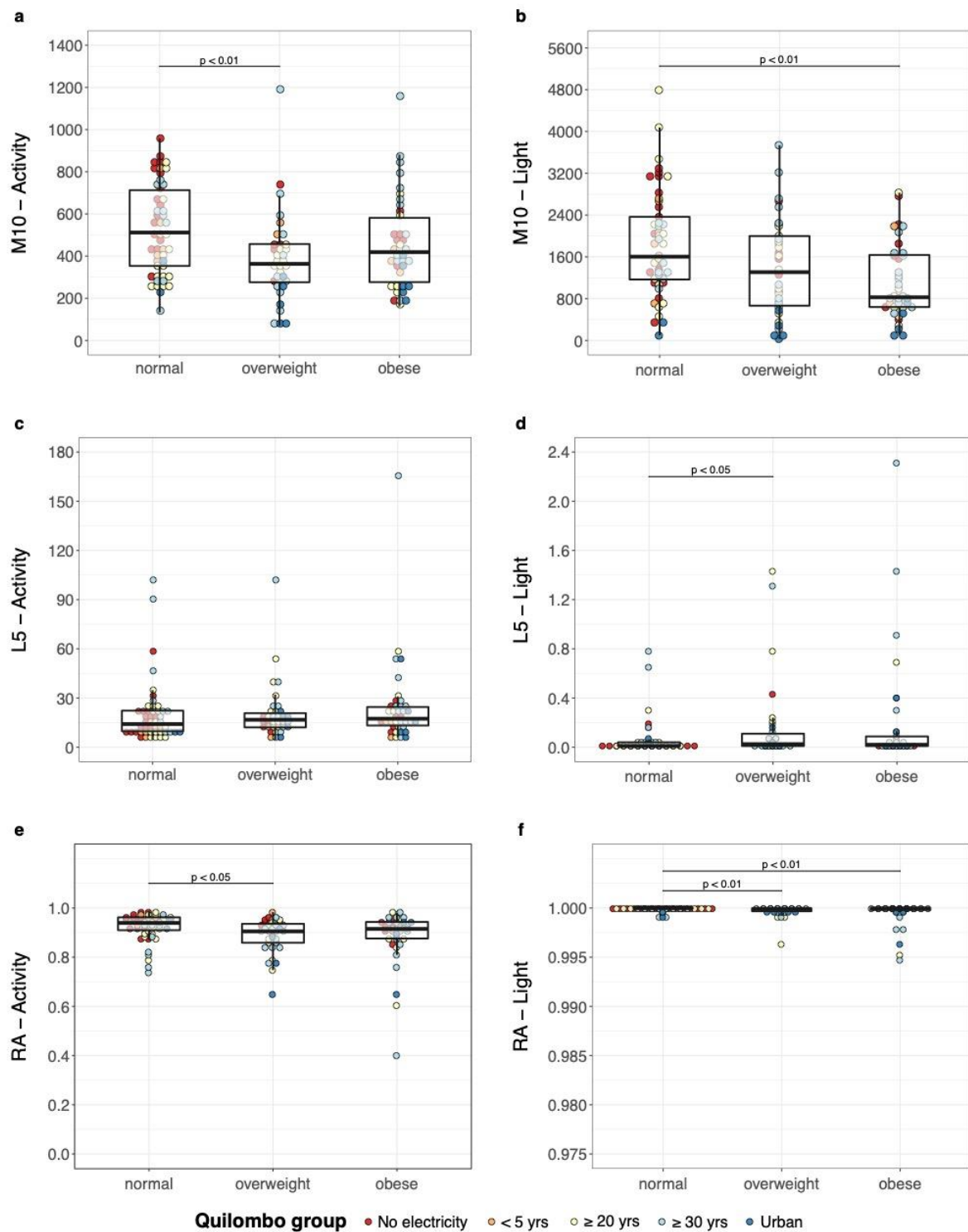


**Figure 2.** Mean activity and light profiles of each group. The means (bold line) and standard error (shadow) are presented for the normal weight group (orange), overweight group (light blue) and obese group (dark blue).



**Figure 3.** Activity IS (a), activity IV (c), light IS (b) and light IV (d) in normal, overweight group and obese group. Analyses were performed using the Kruskal-Wallis test followed by Dunn's. IS: Interdaily Stability; IV: Intradaily Variability. p-values according to Dunn's test for multiple-comparisons, adjusted with Sidak method.





**Figure 4.** Activity M10 (a), activity L5 (c), activity RA (e), light M10 (b), light L5 (d) and light RA (f) in normal, overweight and obese groups. Analyses were performed using the Kruskal-Wallis test followed by Dunn's. M10: mean activity/light exposure of the 10 consecutive hours with the highest values of a daily profile; L5: average activity/light exposure of the 5 consecutive hours with the lowest values of a daily profile; RA: relative

amplitude. p-values according to Dunn's test for multiple-comparisons, adjusted with Sidak method.

TABLE 3: Poisson regression: variables derived from actimetry associated to overweight/obesity (N= 121)

	PR	95% CI	p-value
Intercept	0.125	0.064 - 0.244	0.000
Age	1.005	0.997 - 1.012	0.227
Sex (F)	1.687	1.139 - 2.497	<b>0.009</b>
IV (Light)	1.632	0.990 – 2.690	0.055
IV (Activity)	3.041	1.429 – 6.472	<b>0.004</b>

## SUPPLEMENTARY MATERIAL

TABLE S1: Sample characteristics of each community

	<b>MCTQ</b> (N = 244)	<b>Actimetry</b> (N = 121)
<b>No electricity (N = 29)</b>		
Bombas n (%)	29 (11.9)	28 (23.1)
<b>&lt;5 yrs (N=19)</b>		
Areia Branca n (%)	12 (4.9)	11 (9.0)
São João n (%)	2 (0.8)	0
<b>&gt;15 yrs (N = 91)</b>		
Córrego do Franco n (%)	13 (5.3)	6 (4.9)
Mamãs n (%)	24 (9.8)	13 (10.7)
Serra do Apon n (%)	20 (8.2)	1 (0.8)
São Roque n (%)	19 (7.8)	10 (8.2)
Faxinal n (%)	6 (2.5)	4 (3.3)
<b>≥30 yrs (N = 110)</b>		
Cantão das Lombas n (%)	17 (7.0)	6 (4.9)
Morro do Fortunato n (%)	44 (18.0)	12 (9.9)
Peixoto dos Botinhas n (%)	37 (15.2)	17 (14)
<b>Urban (N = 27)</b>		
Areal da Baronesa n (%)	14 (5.7)	8 (6.6)
Quilombo Fidélis n (%)	6 (2.5)	4 (3.3)
Quilombo Machado n (%)	1 (0.4)	1 (0.4)

TABLE S2: Bivariate analysis of MCTQ variables, activity, light and temperature Cosinor parameters and BMI categories

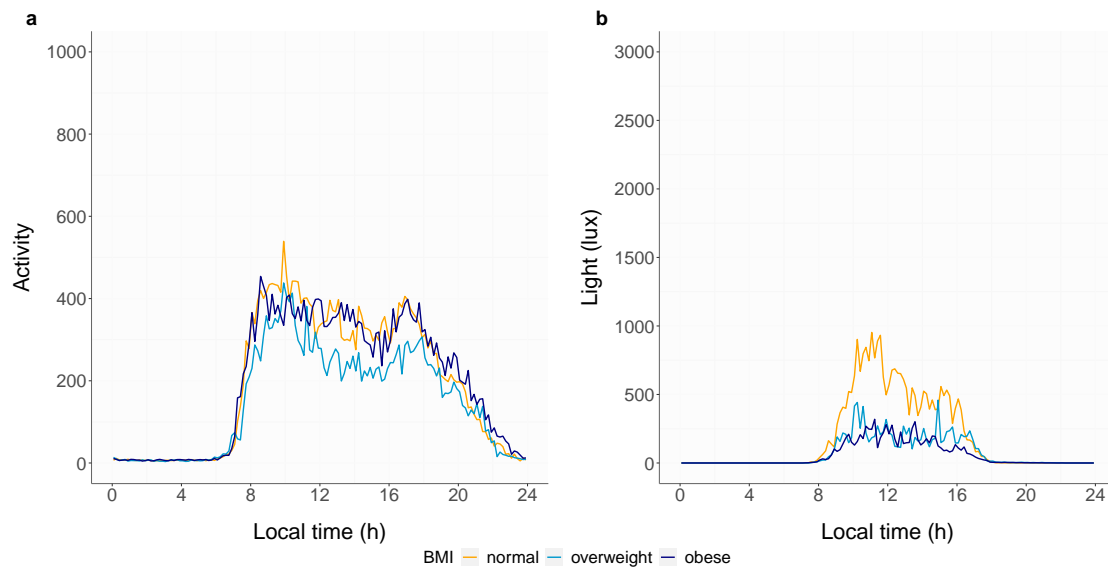
	<b>Normal Weight</b> N = 92 MCTQ N = 49 actimetry	<b>Overweight</b> N = 79 MCTQ N = 34 actimetry	<b>Obesity</b> N = 73 MCTQ N = 38 actimetry	<b>p-value</b> (Normal x Overweight )	<b>p-value</b> (Normal x Obese)	<b>p-value</b> (Overweight x Obese)
<b>MCTQ variables: median [Q<sub>1</sub> – Q<sub>3</sub>]</b>						
MSW (hh:mm)	2:32 [1:49 - 3:15]	2:49 [2:00 - 3:25]	2:34 [1:47 - 3:19]	n.s.	n.s.	n.s.
MSF (hh:mm)	2:56 [2:18 - 4:00]	3:10 [2:02 - 4:15]	2:52 [2:15 - 4:00]	n.s.	n.s.	n.s.
SD on workdays (h)	7.96 [6.92 - 8.85]	7.83 [6.54 - 8.83]	7.5 [6.00 - 8.67]	n.s.	n.s.	n.s.
SD on free days (h)	8.41 [7.25 - 9.33]	8.33 [7.38 - 9.5]	8 [6.83 - 9]	n.s.	n.s.	n.s.
Time spent outdoors on workdays (h)	6 [3.25 - 8]	6 [3 - 9]	6 [2.75 - 9.12]	n.s.	n.s.	n.s.
Time spent outdoors on work-free days (h)	5.0 [2.0 - 8.0]	5.0 [2.0 - 8.0]	5.0 [2.0 - 8.0]	n.s.	n.s.	n.s.
Social jetlag (SjL; h)	0.25 [0 - 1.06]	0.40 [0 - 1]	0.00 [0 - 0.75]	n.s.	n.s.	n.s.
<b>Activity</b>						
MESOR*	283 [190 - 369]	217 [165 - 245]	244 [188 - 328]	<b>0.02</b>	0.76	0.23
Acrophase	13.4 [12.6 - 14.2]	13.5 [12.9 - 14.6]	13.7 [13.2-14.7]	n.s.	n.s.	n.s.
<b>Light</b>						
MESOR*	670 [487-1070]	546 [279 - 846]	349 [265 - 693]	0.13	<b>0.002</b>	0.48
Acrophase	12.7 [12.1 - 13.3]	12.6 [12.3 - 13.4]	12.7 [12.0 - 13.3]	n.s.	n.s.	n.s.

\*p < .05 in Kruskal Wallis/ p-values of Dunn test (with Šidák correction).

**Table S3: Kruskal-Wallis effect sizes (epsilon- and eta-squared)**

	$\epsilon^2$ [bootstrapped 95% CI]	$\eta^2$ [bootstrapped 95% CI]	Interpretation*
<b>Activity</b>			
IV	0.112 [0.033 – 0.256]	0.097 [0.01 – 0.23]	moderate
IS	0.022 [0.002 – 0.118]	0.006 [-0.01 – 0.1]	small
M10	0.077 [0.017 – 0.200]	0.061 [0.00 – 0.19]	moderate
L5	0.021 [0.001 – 0.111]	0.004 [-0.02 – 0.1]	small
RA	0.073 [0.015 – 0.190]	0.057 [0.00 – 0.18]	small
<b>Light</b>			
IV	0.135 [0.043 – 0.278]	0.120 [0.03 – 0.27]	moderate
IS	0.046 [0.006 – 0.160]	0.029 [-0.01 – 0.15]	small
M10	0.103 [0.029 – 0.241]	0.087 [0.01 – 0.23]	moderate
L5	0.077 [0.015 – 0.210]	0.062 [0.00 – 0.20]	moderate
RA	0.129 [0.042 – 0.281]	0.115 [0.03 – 0.27]	moderate

\*Based on rule of thumb for  $\eta^2$ : small 0.01; medium 0.06; large 0.14 (Cohen, 1988).



**Figure S1.** Median activity and light profiles of each group. The medians (bold line) and standard error (shadow) are presented for the normal weight group (orange), overweight group (light blue) and obese group (dark blue).

TABLE S4: Poisson regression: activity variables derived from actimetry associated to overweight/obesity (N= 121)

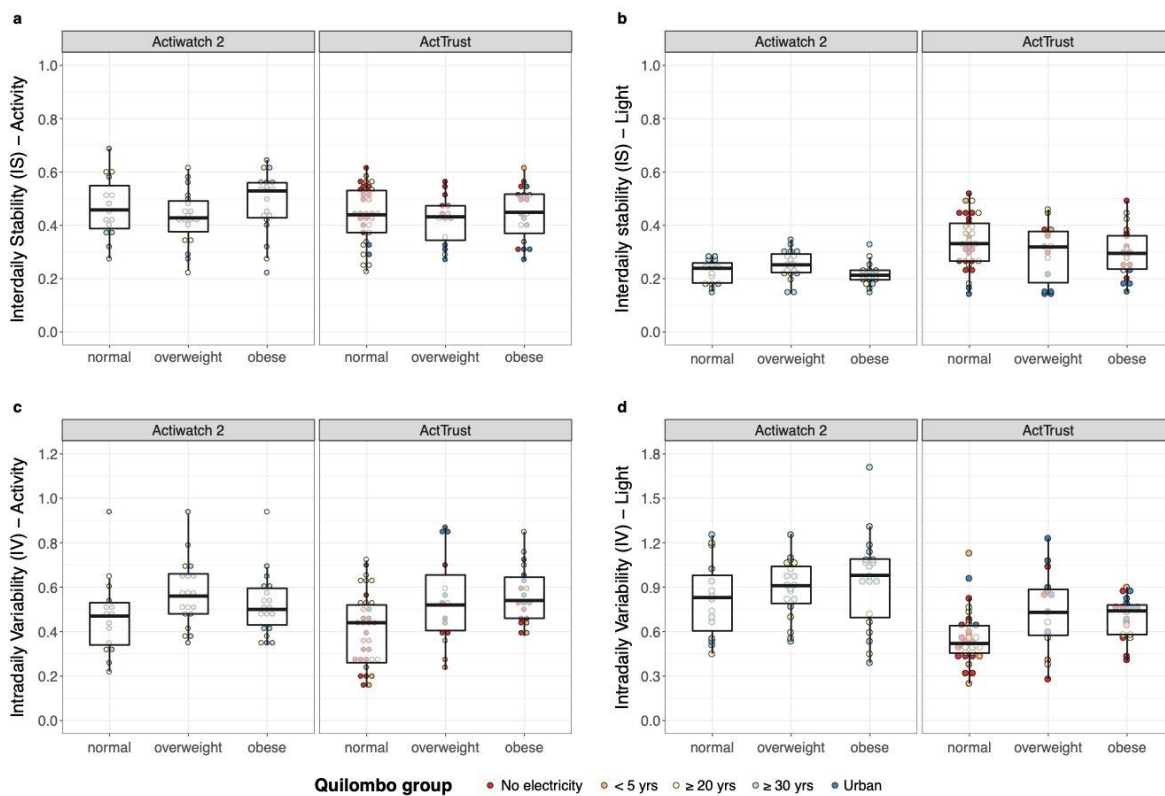
	PR	95% CI	p-value
Intercept	0.129	0.042 - 0.402	0.000
Age	1.007	1.000 - 1.014	0.067
Sex (F)	1.818	1.211 - 2.728	<b>0.004</b>
IV (Activity)	3.846	1.218 - 12.147	<b>0.022</b>
M10 (Activity)	1.000	0.999 - 1.001	0.717

TABLE S5: Poisson regression: light variables derived from actimetry associated to overweight/obesity (N= 121)

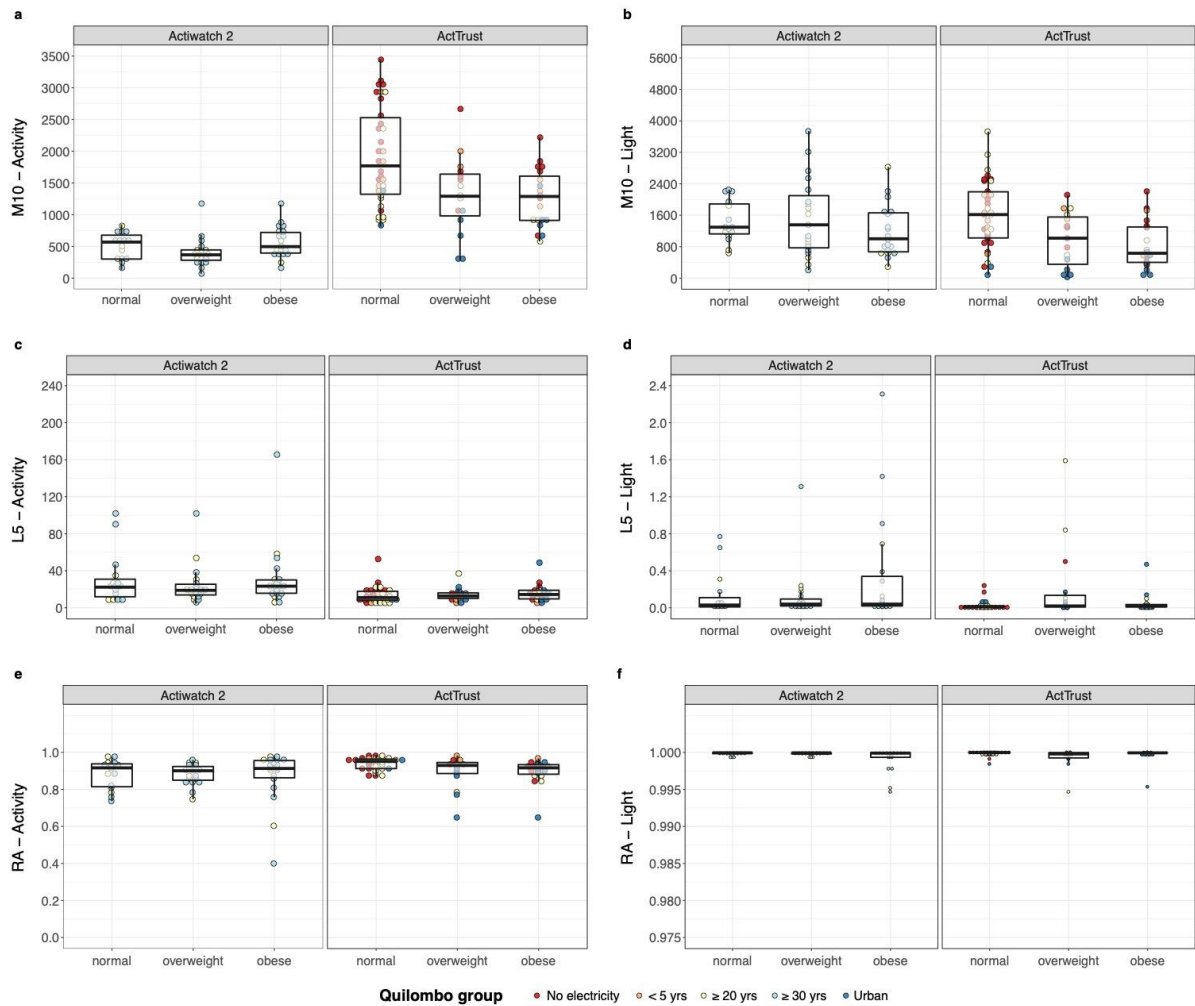
	PR	95% CI	p-value
Intercept	0.190	0.109 - 0.331	0.000
Age	1.006	0.998 - 1.013	0.143
Sex (F)	1.781	1.186 - 2.674	<b>0.005</b>
IV (Light)	1.720	1.006 – 2.940	<b>0.047</b>
L5 (Light)	1.260	0.950 - 1.673	0.109

TABLE S6: Poisson regression: variables derived from actimetry (ActTrust) associated to overweight/obesity (N= 121)

	PR	95% CI	p-value
Intercept	0.147	0.0649- 0.313	0.000
Age	1.004	0.996 - 1.011	0.349
Sex (F)	1.684	1.1397- 2.495	<b>0.009</b>
Actimeter (ActTrust)	0.887	0.649 - 1.212	0.451
IV (Light)	1.494	0.870 – 2.565	0.145
IV (Activity)	3.132	1.444 – 6.797	<b>0.004</b>

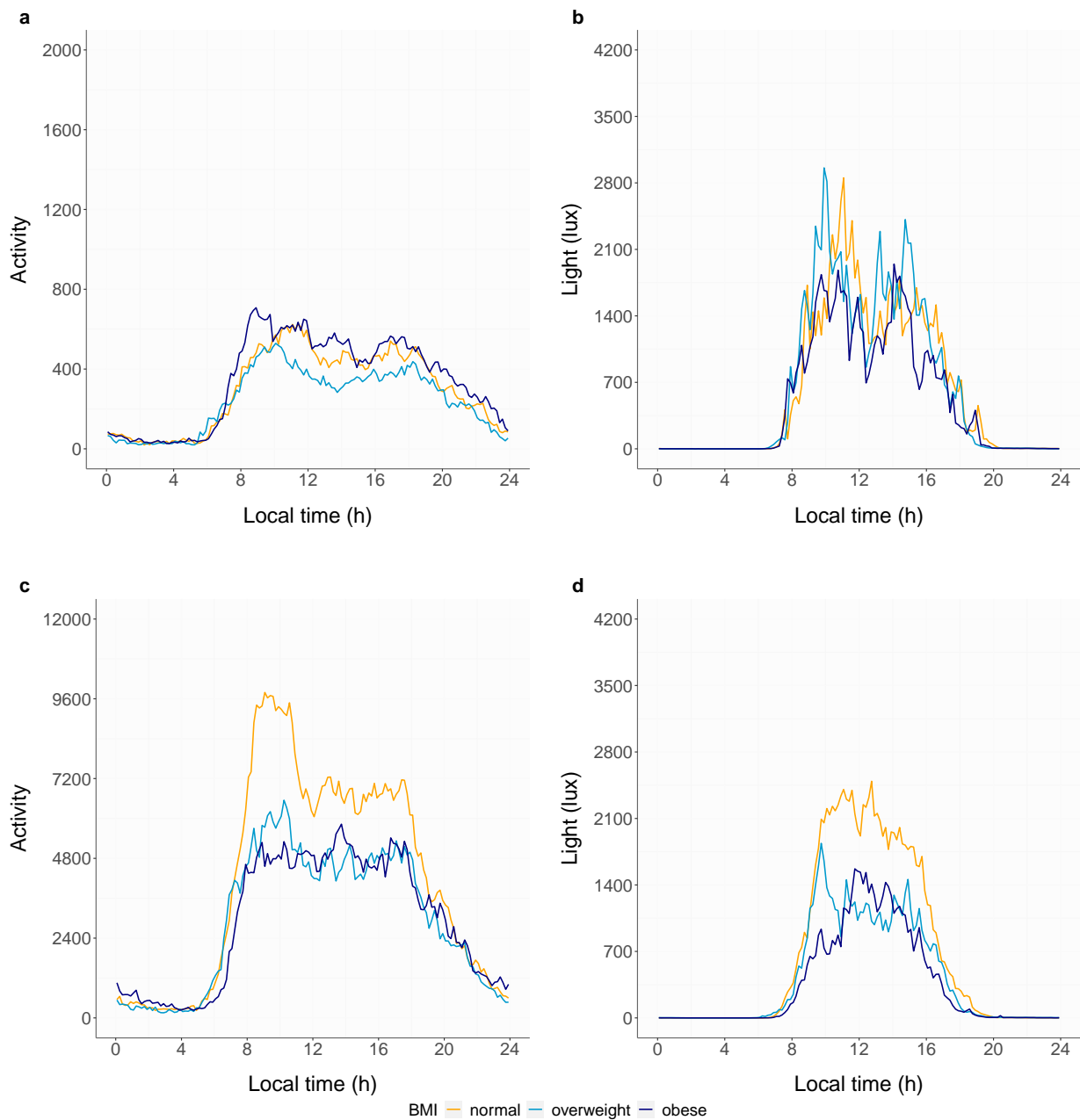


**Figure S2.** Activity IS (a), activity IV (c), light IS (b) and light IV (d) in normal, overweight group and obese group by actimeter brand. IS: Interdaily Stability; IV: Intradaily Variability.

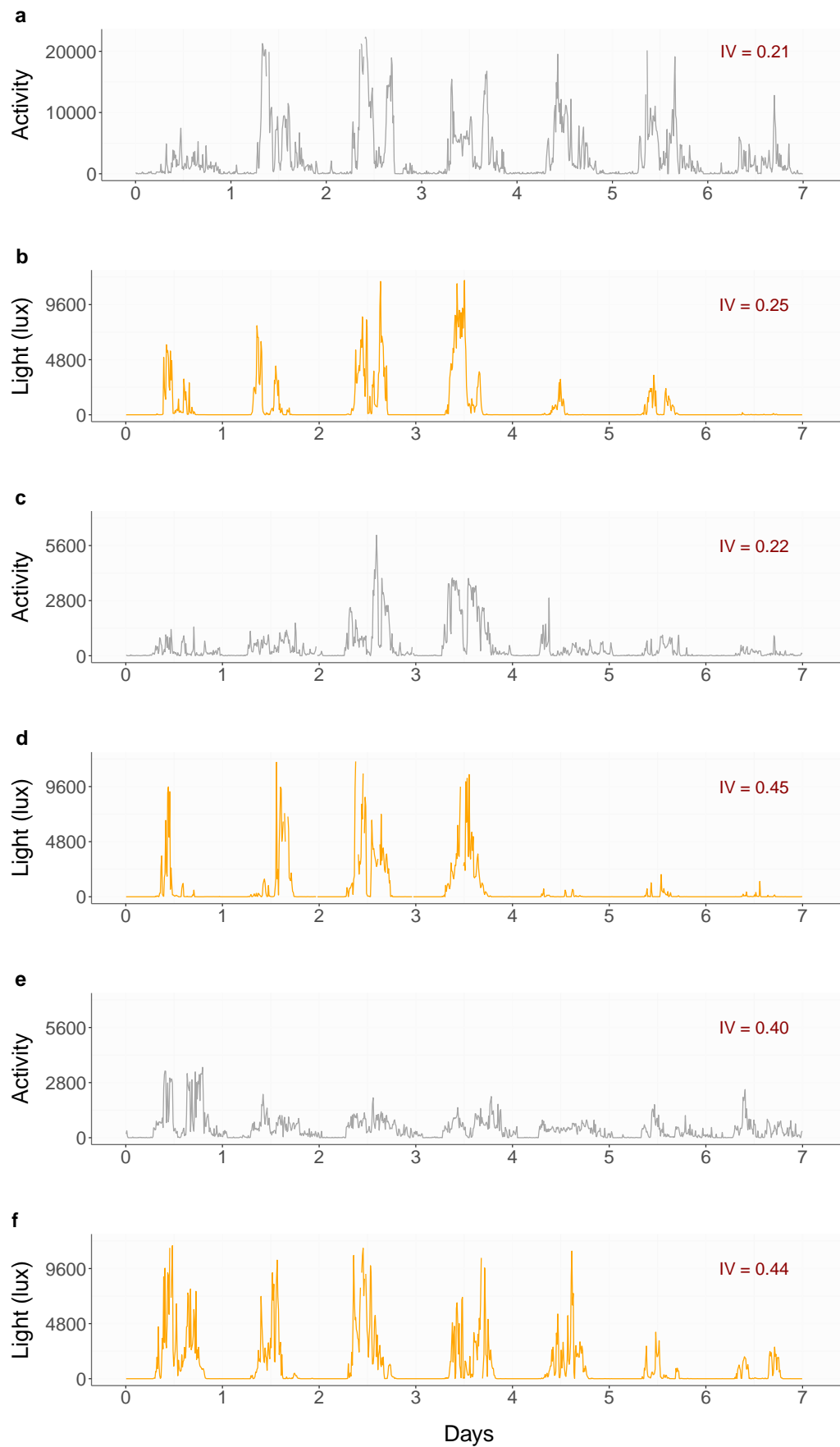


**Figure S3.** Activity M10 (a), activity L5 (c), activity RA (e), light M10 (b), light L5 (d) and light RA (f) in normal, overweight, and obese groups by actimeter brand. M10: mean activity/light exposure of the 10 consecutive hours with the highest values of a daily profile; L5: average activity/light exposure of the 5 consecutive hours with the lowest values of a daily profile; RA: relative amplitude. \* ActTrust M10 and L5 were multiplied by 0.25 for scaling the plots.

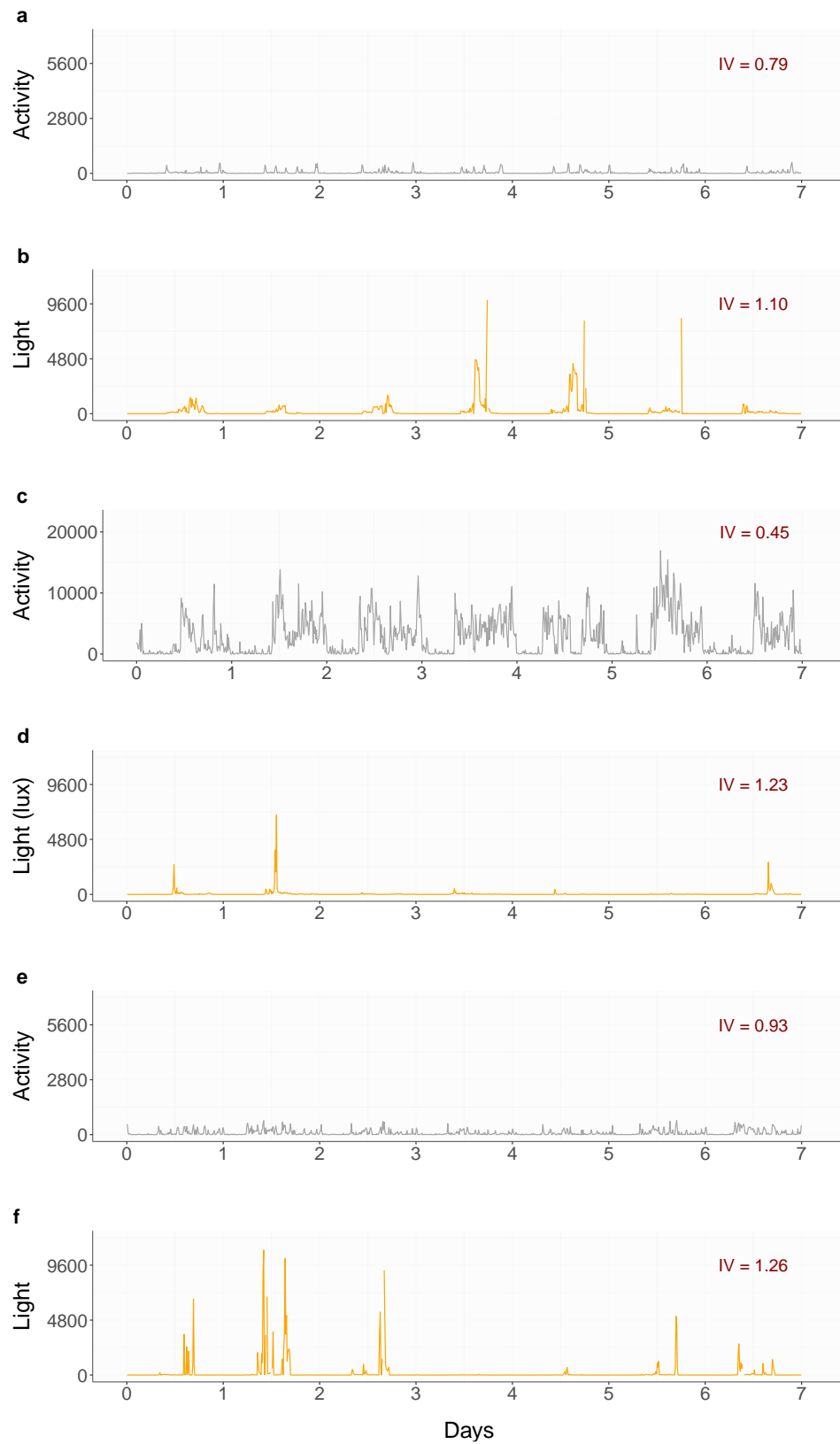




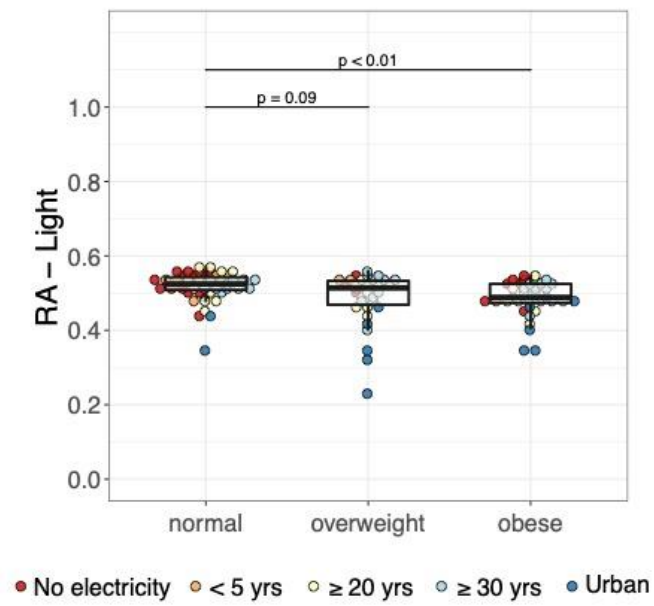
**Figure S4.** Median activity and light profiles of each group in subjects wearing Actiwatch 2 (a, b) and ActTrust (c, d). The medians (bold line) and standard error (shadow) are presented for the normal weight group (orange), overweight group (light blue) and obese group (dark blue).



**Figure S5.** Examples of series with low intra-daily variability (IV).



**Figure S6.** Examples of series with high intra-daily variability (IV).



**Figure S7.** RA of light computed as  $(\log_{10}(M10+10) - \log_{10}(L5+10)) / (\log_{10}(M10+10) + \log_{10}(L5+10))$ . Kruskal-Wallis. P-values according to Dunn-test with Sidak correction.

## 8. CONCLUSÕES E CONSIDERAÇÕES FINAIS

Destacamos as seguintes conclusões dos estudos realizados para esta dissertação:

- Em relação ao estudo experimental, observamos que, tanto a qualidade da iluminação, quanto o fotoperíodo parecem afetar a fisiologia e o metabolismo, uma vez que os animais expostos à iluminação com padrão espectral fixo tiveram maior acúmulo de gordura visceral e perfil lipídico alterado;
- Em relação ao estudo transversal, encontramos uma associação entre maior variabilidade dos ritmos de atividade-reposo e de exposição à luz com maior índice de massa corporal. Além disso, maiores níveis de urbanização e menor amplitude de exposição à luz estiveram associados a um maior risco para desenvolver sobrepeso/obesidade.

Desta forma, as evidências relacionadas ao maior acúmulo de gordura visceral sugerem que a exposição à luz tem um impacto importante no tecido adiposo, um dos principais órgãos reguladores do metabolismo. Considerando a disponibilidade de luz artificial que temos hoje, se faz necessário investigar os mecanismos por trás da associação entre exposição à luz e adiposidade.

Além disso, algumas características dos ritmos de atividade-reposo e de exposição à luz, como maior fragmentação e menor amplitude, parecem ser importantes na patofisiologia da obesidade. Assim, além das tradicionais métricas utilizadas na cronobiologia para estudar ritmos circadianos, investigar parâmetros como regularidade e amplitude de ritmos pode trazer novas possibilidades no estudo dos ritmos biológicos em sobrepeso/obesidade.

Por fim, manter uma organização circadiana ótima é essencial para a saúde e bem-estar. Portanto, é necessário repensar de que forma as estruturas sociais, isto é, horários de trabalho, comportamento alimentar e exposição à luz, intervêm na sincronização dos ritmos biológicos a fim de reduzir transtornos associados à cronodisrupção.

Portanto, as nossas perspectivas são:

- Desenvolver um desenho experimental para investigar parâmetros neuroendócrinos e moleculares da gordura periférica de animais submetidos à cronodisrupção e/ou a diferentes padrões de iluminação;
- Coletar dados de hábitos alimentares em comunidades urbanas, bem como amostras biológicas para analisar possíveis biomarcadores metabólicos da cronodisrupção;
- Investigar a associação de aspectos psicológicos e obesidade/cronobiologia, uma vez que diversas alterações psicológicas associadas a variações no sistema circadiano são acompanhadas de alterações nos padrões alimentares e maior tendência a acumular gordura visceral;
- Propor estratégias baseadas em cronobiologia para o manejo da obesidade, visto que a sincronização apropriada do sistema circadiano tem o potencial de reduzir sua prevalência.

## REFERÊNCIAS DA REVISÃO DA LITERATURA

- A Functional analysis of circadian pacemakers in nocturnal rodents* | SpringerLink. ([s.d.]). Recuperado 28 de julho de 2021, de <https://link.springer.com/article/10.1007/BF01417857>
- Albrecht, U., & Ripperger, J. A. (2018). Circadian Clocks and Sleep: Impact of Rhythmic Metabolism and Waste Clearance on the Brain. *Trends in Neurosciences*, 41(10), 677–688. <https://doi.org/10.1016/j.tins.2018.07.007>
- Antle, M. C., Steen, N. M., & Mistlberger, R. E. (2001). Adenosine and caffeine modulate circadian rhythms in the Syrian hamster. *Neuroreport*, 12(13), 2901–2905. <https://doi.org/10.1097/00001756-200109170-00029>
- Aschoff, J. (1981). A Survey on Biological Rhythms. In J. Aschoff (Org.), *Biological Rhythms* (p. 3–10). Springer US. [https://doi.org/10.1007/978-1-4615-6552-9\\_1](https://doi.org/10.1007/978-1-4615-6552-9_1)
- Aschoff, J., Hoffmann, K., Pohl, H., & Wever, R. (1975). Re-entrainment of circadian rhythms after phase-shifts of the Zeitgeber. *Chronobiologia*, 2(1), 23–78.
- Balsalobre, A., Damiola, F., & Schibler, U. (1998). A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell*, 93(6), 929–937. [https://doi.org/10.1016/s0092-8674\(00\)81199-x](https://doi.org/10.1016/s0092-8674(00)81199-x)
- Bedrosian, T. A., Aubrecht, T. G., Kaugars, K. E., Weil, Z. M., & Nelson, R. J. (2013). Artificial light at night alters delayed-type hypersensitivity reaction in response to acute stress in Siberian hamsters. *Brain, Behavior, and Immunity*, 34, 39–42. <https://doi.org/10.1016/j.bbi.2013.05.009>
- Bell-Pedersen, D., Cassone, V. M., Earnest, D. J., Golden, S. S., Hardin, P. E., Thomas, T. L., & Zoran, M. J. (2005). Circadian rhythms from multiple oscillators: Lessons from diverse organisms. *Nature Reviews Genetics*, 6(7), 544–556. <https://doi.org/10.1038/nrg1633>
- Berson, D. M., Dunn, F. A., & Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science (New York, N.Y.)*, 295(5557), 1070–1073. <https://doi.org/10.1126/science.1067262>
- Blum, I. D., Patterson, Z., Khazall, R., Lamont, E. W., Sleeman, M. W., Horvath, T. L., & Abizaid, A. (2009). Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. *Neuroscience*, 164(2), 351–359. <https://doi.org/10.1016/j.neuroscience.2009.08.009>
- Bo, S., Musso, G., Beccuti, G., Fadda, M., Fedele, D., Gambino, R., Gentile, L., Durazzo, M., Ghigo, E., & Cassader, M. (2014). Consuming more of daily caloric intake at dinner predisposes to obesity. A 6-year population-based prospective cohort study. *PloS One*, 9(9), e108467. <https://doi.org/10.1371/journal.pone.0108467>
- Boivin, D. B., Duffy, J. F., Kronauer, R. E., & Czeisler, C. A. (1996). Dose-response relationships for resetting of human circadian clock by light. *Nature*, 379(6565), 540–542. <https://doi.org/10.1038/379540a0>
- Brancaccio, M., Enoki, R., Mazuski, C. N., Jones, J., Evans, J. A., & Azzi, A. (2014). Network-mediated encoding of circadian time: The suprachiasmatic nucleus (SCN) from genes to neurons to circuits, and back. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 34(46), 15192–15199. <https://doi.org/10.1523/JNEUROSCI.3233-14.2014>
- Buijs, R. M., Eden, C. van, Goncharuk, V. D., & Kalsbeek, A. (2003). The biological clock tunes the organs of the body: Timing by hormones and the autonomic nervous system. *Journal of Endocrinology*, 177(1), 17–26. <https://doi.org/10.1677/joe.0.1770017>

- Buijs, R., Salgado, R., Sabath, E., & Escobar, C. (2013). Peripheral circadian oscillators: Time and food. *Progress in Molecular Biology and Translational Science*, 119, 83–103. <https://doi.org/10.1016/B978-0-12-396971-2.00004-X>
- Cano, P., Cardinali, D. P., Ríos-Lugo, M. J., Fernández-Mateos, M. P., Reyes Toso, C. F., & Esquifino, A. I. (2009). Effect of a high-fat diet on 24-hour pattern of circulating adipocytokines in rats. *Obesity (Silver Spring, Md.)*, 17(10), 1866–1871. <https://doi.org/10.1038/oby.2009.200>
- Casiraghi, L. P., Alzamendi, A., Giovambattista, A., Chiesa, J. J., & Golombek, D. A. (2016). Effects of chronic forced circadian desynchronization on body weight and metabolism in male mice. *Physiological Reports*, 4(8), e12743. <https://doi.org/10.14814/phy2.12743>
- Chen, C. P., Kuhn, P., Advis, J. P., & Sarkar, D. K. (2004). Chronic ethanol consumption impairs the circadian rhythm of pro-opiomelanocortin and period genes mRNA expression in the hypothalamus of the male rat. *Journal of Neurochemistry*, 88(6), 1547–1554. <https://doi.org/10.1046/j.1471-4159.2003.02300.x>
- Cipolla-Neto, J., & Amaral, F. G. do. (2018). Melatonin as a Hormone: New Physiological and Clinical Insights. *Endocrine Reviews*, 39(6), 990–1028. <https://doi.org/10.1210/er.2018-00084>
- Coomans, C. P., van den Berg, S. A. A., Houben, T., van Klinken, J.-B., van den Berg, R., Pronk, A. C. M., Havekes, L. M., Romijn, J. A., van Dijk, K. W., Biermasz, N. R., & Meijer, J. H. (2013). Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, 27(4), 1721–1732. <https://doi.org/10.1096/fj.12-210898>
- Cora, M. C., Kooistra, L., & Travlos, G. (2015). Vaginal Cytology of the Laboratory Rat and Mouse: Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears. *Toxicologic Pathology*, 43(6), 776–793. <https://doi.org/10.1177/0192623315570339>
- Crowley, S. J., Cain, S. W., Burns, A. C., Acebo, C., & Carskadon, M. A. (2015). Increased Sensitivity of the Circadian System to Light in Early/Mid-Puberty. *The Journal of Clinical Endocrinology and Metabolism*, 100(11), 4067–4073. <https://doi.org/10.1210/jc.2015-2775>
- Czeisler, C. A., Duffy, J. F., Shanahan, T. L., Brown, E. N., Mitchell, J. F., Rimmer, D. W., Ronda, J. M., Silva, E. J., Allan, J. S., Emens, J. S., Dijk, D. J., & Kronauer, R. E. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science (New York, N.Y.)*, 284(5423), 2177–2181. <https://doi.org/10.1126/science.284.5423.2177>
- de Castro, J. M., Stoerzinger, A., Barkmeier, D., & Ellen, P. (1978). Medial septal lesions: Disruptions of microregulatory patterns and circadian rhythmicity in rats. *Journal of Comparative and Physiological Psychology*, 92(1), 71–84. <https://doi.org/10.1037/h0077443>
- De Coursey, P. J. (1960). Daily light sensitivity rhythm in a rodent. *Science (New York, N.Y.)*, 131(3392), 33–35. <https://doi.org/10.1126/science.131.3392.33>
- Dewan, K., Benloucif, S., Reid, K., Wolfe, L. F., & Zee, P. C. (2011). Light-induced changes of the circadian clock of humans: Increasing duration is more effective than increasing light intensity. *Sleep*, 34(5), 593–599. <https://doi.org/10.1093/sleep/34.5.593>
- Díaz-Muñoz, M., Vázquez-Martínez, O., Aguilar-Roblero, R., & Escobar, C. (2000). Anticipatory changes in liver metabolism and entrainment of insulin, glucagon, and corticosterone in food-restricted rats. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 279(6), R2048-2056. <https://doi.org/10.1152/ajpregu.2000.279.6.R2048>
- Dibner, C., Schibler, U., & Albrecht, U. (2010). The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. *Annual Review of Physiology*, 72, 517–549. <https://doi.org/10.1146/annurev-physiol-021909-135821>



- Duffy, J. F., & Czeisler, C. A. (2009). Effect of Light on Human Circadian Physiology. *Sleep medicine clinics*, 4(2), 165–177. <https://doi.org/10.1016/j.jsmc.2009.01.004>
- Dumont, M., & Carrier, J. (1997). Daytime sleep propensity after moderate circadian phase shifts induced with bright light exposure. *Sleep*, 20(1), 11–17. <https://doi.org/10.1093/sleep/20.1.11>
- Dyar, K. A., Lutter, D., Artati, A., Ceglia, N. J., Liu, Y., Armenta, D., Jastroch, M., Schneider, S., de Mateo, S., Cervantes, M., Abbondante, S., Tognini, P., Orozco-Solis, R., Kinouchi, K., Wang, C., Swerdloff, R., Nadeef, S., Masri, S., Magistretti, P., ... Sassone-Corsi, P. (2018). Atlas of Circadian Metabolism Reveals System-wide Coordination and Communication between Clocks. *Cell*, 174(6), 1571–1585.e11. <https://doi.org/10.1016/j.cell.2018.08.042>
- Eide, E. J., Woolf, M. F., Kang, H., Woolf, P., Hurst, W., Camacho, F., Vielhaber, E. L., Giovanni, A., & Virshup, D. M. (2005). Control of mammalian circadian rhythm by CKIepsilon-regulated proteasome-mediated PER2 degradation. *Molecular and Cellular Biology*, 25(7), 2795–2807. <https://doi.org/10.1128/MCB.25.7.2795-2807.2005>
- Ezagouri, S., Zwichaft, Z., Sobel, J., Baillieux, S., Doutreleau, S., Ladeux, B., Golik, M., Verges, S., & Asher, G. (2019). Physiological and Molecular Dissection of Daily Variance in Exercise Capacity. *Cell Metabolism*, 30(1), 78–91.e4. <https://doi.org/10.1016/j.cmet.2019.03.012>
- Fonken, L. K., Workman, J. L., Walton, J. C., Weil, Z. M., Morris, J. S., Haim, A., & Nelson, R. J. (2010). Light at night increases body mass by shifting the time of food intake. *Proceedings of the National Academy of Sciences of the United States of America*, 107(43), 18664–18669. <https://doi.org/10.1073/pnas.1008734107>
- Froy, O. (2012). Circadian Rhythms and Obesity in Mammals. *ISRN Obesity*, 2012, 437198. <https://doi.org/10.5402/2012/437198>
- Gerhart-Hines, Z., & Lazar, M. A. (2015). Circadian Metabolism in the Light of Evolution. *Endocrine Reviews*, 36(3), 289–304. <https://doi.org/10.1210/er.2015-1007>
- Hagenauer, M. H., King, A. F., Possidente, B., McGinnis, M. Y., Lumia, A. R., Peckham, E. M., & Lee, T. M. (2011). Changes in Circadian Rhythms during Puberty in *Rattus Norvegicus*: Developmental Time Course and Gonadal Dependency. *Hormones and behavior*, 60(1), 46–57. <https://doi.org/10.1016/j.yhbeh.2011.03.001>
- Hagenauer, M. H., & Lee, T. M. (2012). The Neuroendocrine Control of the Circadian System: Adolescent Chronotype. *Frontiers in neuroendocrinology*, 33(3), 211–229. <https://doi.org/10.1016/j.yfrne.2012.04.003>
- Hastings, M. H., Reddy, A. B., & Maywood, E. S. (2003). A clockwork web: Circadian timing in brain and periphery, in health and disease. *Nature Reviews Neuroscience*, 4(8), 649–661. <https://doi.org/10.1038/nrn1177>
- Havel, P. J., Townsend, R., Chaump, L., & Teff, K. (1999). High-fat meals reduce 24-h circulating leptin concentrations in women. *Diabetes*, 48(2), 334–341. <https://doi.org/10.2337/diabetes.48.2.334>
- Hirota, T., Lewis, W. G., Liu, A. C., Lee, J. W., Schultz, P. G., & Kay, S. A. (2008). A chemical biology approach reveals period shortening of the mammalian circadian clock by specific inhibition of GSK-3 $\beta$ . *Proceedings of the National Academy of Sciences of the United States of America*, 105(52), 20746–20751. <https://doi.org/10.1073/pnas.0811410106>
- Hirota, T., Okano, T., Kokame, K., Shirotani-Ikejima, H., Miyata, T., & Fukada, Y. (2002). Glucose down-regulates Per1 and Per2 mRNA levels and induces circadian gene expression in cultured Rat-1 fibroblasts. *The Journal of Biological Chemistry*, 277(46), 44244–44251. <https://doi.org/10.1074/jbc.M206233200>

- Husse, J., Eichele, G., & Oster, H. (2015). Synchronization of the mammalian circadian timing system: Light can control peripheral clocks independently of the SCN clock: alternate routes of entrainment optimize the alignment of the body's circadian clock network with external time. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, 37(10), 1119–1128. <https://doi.org/10.1002/bies.201500026>
- Iwanaga, H., Yano, M., Miki, H., Okada, K., Azama, T., Takiguchi, S., Fujiwara, Y., Yasuda, T., Nakayama, M., Kobayashi, M., Oishi, K., Ishida, N., Nagai, K., & Monden, M. (2005). Per2 gene expressions in the suprachiasmatic nucleus and liver differentially respond to nutrition factors in rats. *JPEN. Journal of parenteral and Enteral Nutrition*, 29(3), 157–161. <https://doi.org/10.1177/0148607105029003157>
- Jakubowicz, D., Wainstein, J., Landau, Z., Ahren, B., Barnea, M., Bar-Dayana, Y., & Froy, O. (2017). High-energy breakfast based on whey protein reduces body weight, postprandial glycemia and HbA1C in Type 2 diabetes. *The Journal of Nutritional Biochemistry*, 49, 1–7. <https://doi.org/10.1016/j.jnutbio.2017.07.005>
- Jiménez-Aranda, A., Fernández-Vázquez, G., Campos, D., Tassi, M., Velasco-Perez, L., Tan, D.-X., Reiter, R. J., & Agil, A. (2013). Melatonin induces browning of inguinal white adipose tissue in Zucker diabetic fatty rats. *Journal of Pineal Research*, 55(4), 416–423. <https://doi.org/10.1111/jpi.12089>
- Klein, D. C., Schaad, N. L., Namboordiri, M. A., Yu, L., & Weller, J. L. (1992). Regulation of pineal serotonin N-acetyltransferase activity. *Biochemical Society Transactions*, 20(2), 299–304. <https://doi.org/10.1042/bst0200299>
- Knutsson, A. (2003). Health disorders of shift workers. *Occupational Medicine (Oxford, England)*, 53(2), 103–108. <https://doi.org/10.1093/occmed/kqg048>
- Ko, C. H., & Takahashi, J. S. (2006). Molecular components of the mammalian circadian clock. *Human Molecular Genetics*, 15 Spec No 2, R271–277. <https://doi.org/10.1093/hmg/ddl207>
- Kohsaka, A., Laposky, A. D., Ramsey, K. M., Estrada, C., Joshu, C., Kobayashi, Y., Turek, F. W., & Bass, J. (2007). High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metabolism*, 6(5), 414–421. <https://doi.org/10.1016/j.cmet.2007.09.006>
- Kripke, D. F., Elliott, J. A., Youngstedt, S. D., & Rex, K. M. (2007). Circadian phase response curves to light in older and young women and men. *Journal of Circadian Rhythms*, 5, 4. <https://doi.org/10.1186/1740-3391-5-4>
- Kuse, Y., Ogawa, K., Tsuruma, K., Shimazawa, M., & Hara, H. (2014). Damage of photoreceptor-derived cells in culture induced by light emitting diode-derived blue light. *Scientific Reports*, 4(1), 5223. <https://doi.org/10.1038/srep05223>
- La Fleur, S. E., Kalsbeek, A., Wortel, J., & Buijs, R. M. (1999). A suprachiasmatic nucleus generated rhythm in basal glucose concentrations. *Journal of Neuroendocrinology*, 11(8), 643–652. <https://doi.org/10.1046/j.1365-2826.1999.00373.x>
- Laermans, J., & Depoortere, I. (2016). Chronobesity: Role of the circadian system in the obesity epidemic: Role of circadian clocks in obesity. *Obesity Reviews*, 17(2), 108–125. <https://doi.org/10.1111/obr.12351>
- Laje, R., Agostino, P. V., & Golombek, D. A. (2018). The Times of Our Lives: Interaction Among Different Biological Periodicities. *Frontiers in Integrative Neuroscience*, 0. <https://doi.org/10.3389/fnint.2018.00010>
- Lincoln, G., & Loudon, A. (2015). Looking inside the seasonal clock. *Journal of Neuroendocrinology*, 27(1), 76–77. <https://doi.org/10.1111/jne.12238>

- Lockley, S. W., Brainard, G. C., & Czeisler, C. A. (2003). High sensitivity of the human circadian melatonin rhythm to resetting by short wavelength light. *The Journal of Clinical Endocrinology and Metabolism*, 88(9), 4502–4505. <https://doi.org/10.1210/jc.2003-030570>
- Manoogian, E. N. C., Chaix, A., & Panda, S. (2019). When to Eat: The Importance of Eating Patterns in Health and Disease. *Journal of Biological Rhythms*, 34(6), 579–581. <https://doi.org/10.1177/0748730419892105>
- Masri, S., & Sassone-Corsi, P. (2018). The emerging link between cancer, metabolism, and circadian rhythms. *Nature Medicine*, 24(12), 1795–1803. <https://doi.org/10.1038/s41591-018-0271-8>
- Mayeuf-Louchart, A., Staels, B., & Duez, H. (2015). Skeletal muscle functions around the clock. *Diabetes, Obesity & Metabolism*, 17 Suppl 1, 39–46. <https://doi.org/10.1111/dom.12517>
- McHill, A. W., Phillips, A. J., Czeisler, C. A., Keating, L., Yee, K., Barger, L. K., Garaulet, M., Scheer, F. A., & Klerman, E. B. (2017). Later circadian timing of food intake is associated with increased body fat. *The American Journal of Clinical Nutrition*, 106(5), 1213–1219. <https://doi.org/10.3945/ajcn.117.161588>
- Merrow, M. ([s.d.]). *Life between Clocks: Daily Temporal Patterns of Human Chronotypes*. Recuperado 6 de agosto de 2021, de [https://core.ac.uk/reader/148287852?utm\\_source=linkout](https://core.ac.uk/reader/148287852?utm_source=linkout)
- Middleton, B., Arendt, J., & Stone, B. M. (1996). Human circadian rhythms in constant dim light (8 lux) with knowledge of clock time. *Journal of Sleep Research*, 5(2), 69–76. <https://doi.org/10.1046/j.1365-2869.1996.d01-67.x>
- Mills, J., & Kuohung, W. (2019). Impact of circadian rhythms on female reproduction and infertility treatment success: *Current Opinion in Endocrinology & Diabetes and Obesity*, 26(6), 317–321. <https://doi.org/10.1097/MED.0000000000000511>
- Mistberger, R. E. (2009). Food-anticipatory circadian rhythms: Concepts and methods. *The European Journal of Neuroscience*, 30(9), 1718–1729. <https://doi.org/10.1111/j.1460-9568.2009.06965.x>
- Mistberger, R. E., & Skene, D. J. (2005). Nonphotic entrainment in humans? *Journal of Biological Rhythms*, 20(4), 339–352. <https://doi.org/10.1177/0748730405277982>
- Moore-Ede, M. C., Sulzman, F. M., & Fuller, C. A. (1982). *The clocks that time us: Physiology of the circadian timing system*. Cambridge, MA: Harvard University Press.
- Moore, R. Y., & Eichler, V. B. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Research*, 42(1), 201–206. [https://doi.org/10.1016/0006-8993\(72\)90054-6](https://doi.org/10.1016/0006-8993(72)90054-6)
- Morse, D., & Sassone-Corsi, P. (2002). Time after time: Inputs to and outputs from the mammalian circadian oscillators. *Trends in Neurosciences*, 25(12), 632–637. [https://doi.org/10.1016/S0166-2236\(02\)02274-9](https://doi.org/10.1016/S0166-2236(02)02274-9)
- Panda, S., Antoch, M. P., Miller, B. H., Su, A. I., Schook, A. B., Straume, M., Schultz, P. G., Kay, S. A., Takahashi, J. S., & Hogenesch, J. B. (2002). Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell*, 109(3), 307–320. [https://doi.org/10.1016/s0092-8674\(02\)00722-5](https://doi.org/10.1016/s0092-8674(02)00722-5)
- Panda, S., Hogenesch, J. B., & Kay, S. A. (2002). Circadian rhythms from flies to human. *Nature*, 417(6886), 329–335. <https://doi.org/10.1038/417329a>

- Parsons, M. J., Moffitt, T. E., Gregory, A. M., Goldman-Mellor, S., Nolan, P. M., Poulton, R., & Caspi, A. (2015). Social jetlag, obesity and metabolic disorder: Investigation in a cohort study. *International Journal of Obesity (2005)*, *39*(5), 842–848. <https://doi.org/10.1038/ijo.2014.201>
- Perez-Tilve, D., Stern, J. E., & Tschöp, M. (2006). The Brain and the Metabolic Syndrome: Not a Wireless Connection. *Endocrinology*, *147*(3), 1136–1139. <https://doi.org/10.1210/en.2005-1586>
- Petrenko, V., Saini, C., Giovannoni, L., Gobet, C., Sage, D., Unser, M., Heddad Masson, M., Gu, G., Bosco, D., Gachon, F., Philippe, J., & Dibner, C. (2017). Pancreatic  $\alpha$ - and  $\beta$ -cellular clocks have distinct molecular properties and impact on islet hormone secretion and gene expression. *Genes & Development*, *31*(4), 383–398. <https://doi.org/10.1101/gad.290379.116>
- Pittendrigh, C. S. (1960a). Circadian rhythms and the circadian organization of living systems. *Cold Spring Harbor Symposia on Quantitative Biology*, *25*, 159–184. <https://doi.org/10.1101/sqb.1960.025.01.015>
- Pittendrigh, C. S. (1960b). Circadian rhythms and the circadian organization of living systems. *Cold Spring Harbor Symposia on Quantitative Biology*, *25*, 159–184. <https://doi.org/10.1101/sqb.1960.025.01.015>
- Plano, S. A., Casiraghi, L. P., García Moro, P., Paladino, N., Golombek, D. A., & Chiesa, J. J. (2017). Circadian and Metabolic Effects of Light: Implications in Weight Homeostasis and Health. *Frontiers in Neurology*, *8*, 558. <https://doi.org/10.3389/fneur.2017.00558>
- Pot, G. K. (2018). Sleep and dietary habits in the urban environment: The role of chrono-nutrition. *Proceedings of the Nutrition Society*, *77*(3), 189–198. <https://doi.org/10.1017/S0029665117003974>
- Pot, G. K., Hardy, R., & Stephen, A. M. (2014). Irregular consumption of energy intake in meals is associated with a higher cardiometabolic risk in adults of a British birth cohort. *International Journal of Obesity (2005)*, *38*(12), 1518–1524. <https://doi.org/10.1038/ijo.2014.51>
- Queiroz, J. do N., Macedo, R. C. O., Tinsley, G. M., & Reischak-Oliveira, A. (2020). Time-restricted eating and circadian rhythms: The biological clock is ticking. *Critical Reviews in Food Science and Nutrition*, 1–13. <https://doi.org/10.1080/10408398.2020.1789550>
- Revell, V. L., Burgess, H. J., Gazda, C. J., Smith, M. R., Fogg, L. F., & Eastman, C. I. (2006). Advancing human circadian rhythms with afternoon melatonin and morning intermittent bright light. *The Journal of Clinical Endocrinology and Metabolism*, *91*(1), 54–59. <https://doi.org/10.1210/jc.2005-1009>
- Rivera-Coll, A., Fuentes-Arderiu, X., & Díez-Noguera, A. (1994). Circadian rhythmic variations in serum concentrations of clinically important lipids. *Clinical Chemistry*, *40*(8), 1549–1553.
- Roenneberg, T., Allebrandt, K. V., Mewes, M., & Vetter, C. (2012). Social jetlag and obesity. *Current Biology: CB*, *22*(10), 939–943. <https://doi.org/10.1016/j.cub.2012.03.038>
- Roenneberg, T., & Mewes, M. (2016). The Circadian Clock and Human Health. *Current Biology*, *26*(10), R432–R443. <https://doi.org/10.1016/j.cub.2016.04.011>
- Rüger, M., & Scheer, F. A. J. L. (2009). Effects of circadian disruption on the cardiometabolic system. *Reviews in Endocrine & Metabolic Disorders*, *10*(4), 245–260. <https://doi.org/10.1007/s11154-009-9122-8>
- Rüger, M., St Hilaire, M. A., Brainard, G. C., Khalsa, S.-B. S., Kronauer, R. E., Czeisler, C. A., & Lockley, S. W. (2013). Human phase response curve to a single 6.5 h pulse of short-wavelength light. *The Journal of Physiology*, *591*(1), 353–363. <https://doi.org/10.1113/jphysiol.2012.239046>

- Rutters, F., Lemmens, S. G., Adam, T. C., Bremmer, M. A., Elders, P. J., Nijpels, G., & Dekker, J. M. (2014). Is social jetlag associated with an adverse endocrine, behavioral, and cardiovascular risk profile? *Journal of Biological Rhythms*, 29(5), 377–383. <https://doi.org/10.1177/0748730414550199>
- Rybnikova, N. A., Haim, A., & Portnov, B. A. (2016). Does artificial light-at-night exposure contribute to the worldwide obesity pandemic? *International Journal of Obesity (2005)*, 40(5), 815–823. <https://doi.org/10.1038/ijo.2015.255>
- Sahar, S., & Sassone-Corsi, P. (2009). Metabolism and cancer: The circadian clock connection. *Nature Reviews. Cancer*, 9(12), 886–896. <https://doi.org/10.1038/nrc2747>
- Sato, S., Basse, A. L., Schönke, M., Chen, S., Samad, M., Altıntaş, A., Laker, R. C., Dalbram, E., Barrès, R., Baldi, P., Treebak, J. T., Zierath, J. R., & Sassone-Corsi, P. (2019). Time of Exercise Specifies the Impact on Muscle Metabolic Pathways and Systemic Energy Homeostasis. *Cell Metabolism*, 30(1), 92–110.e4. <https://doi.org/10.1016/j.cmet.2019.03.013>
- Saunders, D. S. (2016). The temporal ‘structure’ and function of the insect photoperiodic clock: A tribute to Colin S. Pittendrigh. *Physiological Entomology*, 41(1), 1–18. <https://doi.org/10.1111/phen.12131>
- Scott, E. M., & Grant, P. J. (2006). Neel revisited: The adipocyte, seasonality and type 2 diabetes. *Diabetologia*, 49(7), 1462–1466. <https://doi.org/10.1007/s00125-006-0280-x>
- Sen, A., & Hoffmann, H. M. (2020). Role of core circadian clock genes in hormone release and target tissue sensitivity in the reproductive axis. *Molecular and cellular endocrinology*, 501, 110655. <https://doi.org/10.1016/j.mce.2019.110655>
- Shi, S., Ansari, T. S., McGuinness, O. P., Wasserman, D. H., & Johnson, C. H. (2013). Circadian disruption leads to insulin resistance and obesity. *Current Biology: CB*, 23(5), 372–381. <https://doi.org/10.1016/j.cub.2013.01.048>
- Shostak, A., Meyer-Kovac, J., & Oster, H. (2013). Circadian regulation of lipid mobilization in white adipose tissues. *Diabetes*, 62(7), 2195–2203. <https://doi.org/10.2337/db12-1449>
- Skene, D., & Arendt, J. (2006). Human circadian rhythms: Physiological and therapeutic relevance of light and melatonin. *Annals of clinical biochemistry*, 43, 344–353. <https://doi.org/10.1258/000456306778520142>
- Smith, T. J. (1988). Informed consent doctrine in dental practice: A current case review. *Journal of Law and Ethics in Dentistry*, 1(3), 159–169.
- Solocinski, K., & Gumz, M. L. (2015). The Circadian Clock in the Regulation of Renal Rhythms. *Journal of biological rhythms*, 30(6), 470–486. <https://doi.org/10.1177/0748730415610879>
- Stenvers, D. J., Scheer, F. A. J. L., Schrauwen, P., la Fleur, S. E., & Kalsbeek, A. (2019). Circadian clocks and insulin resistance. *Nature Reviews Endocrinology*, 15(2), 75–89. <https://doi.org/10.1038/s41574-018-0122-1>
- Stephan, F. K. (2002). The “Other” Circadian System: Food as a Zeitgeber. *Journal of Biological Rhythms*, 17(4), 284–292. <https://doi.org/10.1177/074873002129002591>
- Stephan, F. K., & Zucker, I. (1972). Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proceedings of the National Academy of Sciences of the United States of America*, 69(6), 1583–1586. <https://doi.org/10.1073/pnas.69.6.1583>
- Stokkan, K. A., Yamazaki, S., Tei, H., Sakaki, Y., & Menaker, M. (2001). Entrainment of the circadian clock in the liver by feeding. *Science (New York, N.Y.)*, 291(5503), 490–493. <https://doi.org/10.1126/science.291.5503.490>

Storch, K.-F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F. C., Wong, W. H., & Weitz, C. J. (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature*, *417*(6884), 78–83. <https://doi.org/10.1038/nature744>

Sudo, M., Sasahara, K., Moriya, T., Akiyama, M., Hamada, T., & Shibata, S. (2003). Constant light housing attenuates circadian rhythms of mPer2 mRNA and mPER2 protein expression in the suprachiasmatic nucleus of mice. *Neuroscience*, *121*(2), 493–499. [https://doi.org/10.1016/s0306-4522\(03\)00457-3](https://doi.org/10.1016/s0306-4522(03)00457-3)

Takahashi, J. S., Hong, H.-K., Ko, C. H., & McDearmon, E. L. (2008). The genetics of mammalian circadian order and disorder: Implications for physiology and disease. *Nature Reviews. Genetics*, *9*(10), 764–775. <https://doi.org/10.1038/nrg2430>

*The Nobel Prize in Physiology or Medicine 2017*. ([s.d.]). NobelPrize.Org. Recuperado 10 de agosto de 2021, de <https://www.nobelprize.org/prizes/medicine/2017/summary/>

*The Small Molecule Nobiletin Targets the Molecular Oscillator to Enhance Circadian Rhythms and Protect against Metabolic Syndrome | Elsevier Enhanced Reader*. ([s.d.]). <https://doi.org/10.1016/j.cmet.2016.03.007>

Toms, D., Pan, B., & Li, J. (2017). Endocrine Regulation in the Ovary by MicroRNA during the Estrous Cycle. *Frontiers in Endocrinology*, *8*, 378. <https://doi.org/10.3389/fendo.2017.00378>

Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N., & Fougerou, C. (2017). Melatonin: Pharmacology, Functions and Therapeutic Benefits. *Current Neuropharmacology*, *15*(3), 434–443. <https://doi.org/10.2174/1570159X14666161228122115>

Touitou, Y., Reinberg, A., & Touitou, D. (2017). Association between light at night, melatonin secretion, sleep deprivation, and the internal clock: Health impacts and mechanisms of circadian disruption. *Life Sciences*, *173*, 94–106. <https://doi.org/10.1016/j.lfs.2017.02.008>

Tynes, T., Hannevik, M., Andersen, A., Vistnes, A. I., & Haldorsen, T. (1996). Incidence of breast cancer in Norwegian female radio and telegraph operators. *Cancer Causes & Control: CCC*, *7*(2), 197–204. <https://doi.org/10.1007/BF00051295>

Vetter, C. (2020). Circadian disruption: What do we actually mean? *The European Journal of Neuroscience*, *51*(1), 531–550. <https://doi.org/10.1111/ejn.14255>

Waddington Lamont, E., Harbour, V. L., Barry-Shaw, J., Renteria Diaz, L., Robinson, B., Stewart, J., & Amir, S. (2007). Restricted access to food, but not sucrose, saccharine, or salt, synchronizes the expression of Period2 protein in the limbic forebrain. *Neuroscience*, *144*(2), 402–411. <https://doi.org/10.1016/j.neuroscience.2006.09.027>

Wang, X.-S., Armstrong, M. E. G., Cairns, B. J., Key, T. J., & Travis, R. C. (2011). Shift work and chronic disease: The epidemiological evidence. *Occupational Medicine (Oxford, England)*, *61*(2), 78–89. <https://doi.org/10.1093/occmed/kqr001>

Welsh, D. K., Logothetis, D. E., Meister, M., & Reppert, S. M. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron*, *14*(4), 697–706. [https://doi.org/10.1016/0896-6273\(95\)90214-7](https://doi.org/10.1016/0896-6273(95)90214-7)

Wittmann, M., Dinich, J., Meroz, M., & Roenneberg, T. (2006). Social jetlag: Misalignment of biological and social time. *Chronobiology International*, *23*(1–2), 497–509. <https://doi.org/10.1080/07420520500545979>

Wolk, R., Gami, A. S., Garcia-Touchard, A., & Somers, V. K. (2005). Sleep and cardiovascular disease. *Current Problems in Cardiology*, *30*(12), 625–662. <https://doi.org/10.1016/j.cpcardiol.2005.07.002>

Yamamoto, H., Nagai, K., & Nakagawa, H. (1987). Role of SCN in daily rhythms of plasma glucose, FFA, insulin and glucagon. *Chronobiology International*, 4(4), 483–491. <https://doi.org/10.3109/07420528709078539>

Ye, Y., Xiang, Y., Ozguc, F. M., Kim, Y., Liu, C.-J., Park, P. K., Hu, Q., Diao, L., Lou, Y., Lin, C., Guo, A.-Y., Zhou, B., Wang, L., Chen, Z., Takahashi, J. S., Mills, G. B., Yoo, S.-H., & Han, L. (2018). The genomic landscape and pharmacogenomic interactions of clock genes in cancer chronotherapy. *Cell systems*, 6(3), 314–328.e2. <https://doi.org/10.1016/j.cels.2018.01.013>

Yoo, S.-H., Yamazaki, S., Lowrey, P. L., Shimomura, K., Ko, C. H., Buhr, E. D., Siepkka, S. M., Hong, H.-K., Oh, W. J., Yoo, O. J., Menaker, M., & Takahashi, J. S. (2004). PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proceedings of the National Academy of Sciences of the United States of America*, 101(15), 5339–5346. <https://doi.org/10.1073/pnas.0308709101>

Yoshida, J., Eguchi, E., Nagaoka, K., Ito, T., & Ogino, K. (2018). Association of night eating habits with metabolic syndrome and its components: A longitudinal study. *BMC Public Health*, 18, 1366. <https://doi.org/10.1186/s12889-018-6262-3>

Zwighaft, Z., Reinke, H., & Asher, G. (2016). The Liver in the Eyes of a Chronobiologist. *Journal of Biological Rhythms*, 31(2), 115–124. <https://doi.org/10.1177/0748730416633552>

## ANEXO A - Carta de aprovação do projeto pela CEUA/HCPA referente ao Artigo

1



**GRUPO DE PESQUISA E PÓS GRADUAÇÃO**  
**COMISSÃO DE ÉTICA NO USO DE ANIMAIS**



Certificamos que o projeto abaixo, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) e pelas áreas de apoio indicadas pelo pesquisador.

**Projeto:** 160378

**Data de Aprovação do Projeto:** 15/03/2018

**Título:** AVALIAÇÃO DO EFEITO DA ILUMINAÇÃO COM RITMICIDADE CIRCADIANA NO DESENVOLVIMENTO PUBERAL DE RATAS WISTAR

**Data de Término:** 01/08/2018

**Pesquisador Responsável:** MARIA PAZ LOAYZA HIDALGO

**Equipe de pesquisa:**

ANDRE COMIRAN TONON DANILLO DE PAULA SANTOS DÉBORA BARROGGI CONSTANTINO FLAVIA ARAUJO DE AMORIM  
JULIANA CASTILHOS BEAUVALET JULIANA JURY FREITAS MELISSA ALVES BRAGA DE OLIVEIRA

Submissão	Documento	Especie/Linhagem	Sexo/Idade	Qtd.	Data Reunião	Situação
15/08/2016	APROVAÇÃO	RATO - WISTAR	F/21dias	104	23/08/2016	APROVADO
16/01/2018	EMENDA	RATO - WISTAR	M/60dias	7	27/02/2018	APROVADO
16/01/2018	EMENDA	RATO - WISTAR	F/60dias	32	27/02/2018	APROVADO
07/03/2018	EMENDA	RATO - WISTAR	M/60dias	52	13/03/2018	APROVADO
<b>Total de Animais:</b>				195		

*Ass. Heleena*  
SÂNIA HELENA DA ROSA PAZ

Coordenadora

Comissão de Ética no Uso de Animais

- Os membros da CEUA/HCPA não participaram do processo de avaliação onde constam como pesquisadores.

- Toda e qualquer alteração do Projeto deverá ser comunicada à CEUA/HCPA.



- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao CEUA/HCPA.

quinta-feira, 29 de março de 2018

Página 1 de 1



## ANEXO B – Comprovante de recebimento do Artigo 1 pela revista *Physiology and Behavior*



Confirming submission to Physiology & Behavior  Caixa de entrada 



Physiology & Behavior <em@editorialmanager.com>

16:52 (há 2 horas)



 para mim 

 inglês  > português  Traduzir mensagem

Desativar para: inglês 

\*This message was sent automatically. Please do not reply.\*

Ref.

Title: Effects Of Lighting Patterns In Pubertal Development And Metabolism Of Female Wistar Rats

Journal: Physiology & Behavior

Dear Ms. Constantino,

Thank you for submitting your manuscript for consideration for publication in Physiology & Behavior. Your submission was received in good order.

To track the status of your manuscript, please log into Editorial Manager: <https://www.editorialmanager.com/phb/>.

*Authors of papers submitted to Physiology and Behavior are entitled, and actively encouraged, to submit a preprint of their manuscript to their repository of choice. For full details please see [here](#).*

Thank you for submitting your work to this journal.

Kind regards,

Physiology & Behavior