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**NOVAS PERSPECTIVAS DE USO PRÉ-CLÍNICO DO CMS121 ENVOLVENDO O  
EFEITO TIPO ANTIDEPRESSIVO, MODELOS DE INFLAMAÇÃO E DE DOENÇAS  
METABÓLICAS**

Florianópolis

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EFEITO TIPO ANTIDEPRESSIVO, MODELOS DE INFLAMAÇÃO E DE DOENÇAS  
METABÓLICAS**

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Orientador(a): Prof. Alcir Luiz Dafré, Dr.(a)

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Jéssica Jorge Probst

**Novas perspectivas de uso pré-clínico do CMS121 envolvendo o efeito tipo antidepressivo, modelos de inflamação e de doenças metabólicas**

O presente trabalho em nível de Doutorado foi avaliado e aprovado, em 30 de julho de 2025, pela banca examinadora composta pelos seguintes membros:

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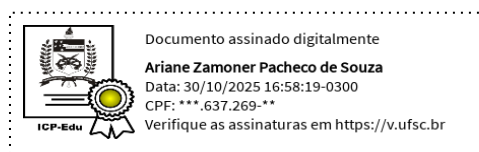
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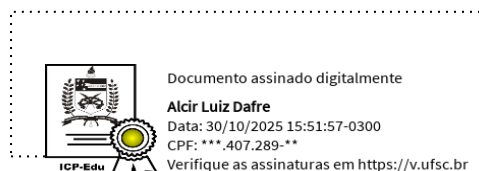
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Certificamos que esta é a versão original e final do trabalho de conclusão que foi julgado adequado para obtenção do título de Doutora em Bioquímica.



Coordenação do Programa de Pós-Graduação



Prof. Alcir Luiz Dafre, Dr.

Orientador

Florianópolis, 2025

Dedico esta tese à minha mãe, Elenita,  
que sempre sonhou em ter uma filha doutora.

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“Confie no Senhor de todo o coração e não se apoie na sua própria  
inteligência.” (Provérbios 3:5)

## RESUMO

O CMS121 é um derivado sintético do flavonoide fisetina, desenvolvido para otimizar propriedades terapêuticas e farmacocinéticas. Seu mecanismo de ação envolve a ativação da via da AMPK, levando à fosforilação inibitória da ACC1 e, consequentemente, à redução da conversão de acetil-CoA em malonil-CoA, limitando a lipogênese. Esse composto apresenta efeitos anti-inflamatórios e metabólicos, com potencial neuroprotetor, já demonstrado em modelos experimentais de Alzheimer, envelhecimento e obesidade diabética. Nessas condições, o CMS121 reduziu o declínio cognitivo e a inflamação hepática, além de melhorar o metabolismo da glicose, os perfis lipídicos e a função renal. Ensaios clínicos de fase I demonstraram boa tolerabilidade e ausência de toxicidade, reforçando seu potencial terapêutico. Este estudo teve como objetivo investigar os efeitos farmacológicos do composto CMS121 em camundongos em modelos de inflamação, de disfunção metabólicas e em testes preditivos de ação antidepressiva. Foram conduzidos três conjuntos experimentais independentes. No Capítulo 1, camundongos C57BL/6 receberam dieta controle ou suplementada com CMS121 por seis meses, sendo avaliados quanto a parâmetros metabólicos e inflamatórios. O tratamento reduziu em 40% o ganho de peso, melhorou índices glicídicos e lipídicos e diminuiu marcadores inflamatórios hepáticos (caspase-1, caspase-3 e NOX4). No tecido adiposo, observou-se aumento de Nrf1, TFAM, GLUT4 e proteínas mitocondriais, além de elevação de acilcarnitinas de cadeia curta e metabólitos de butirato, sugerindo um perfil bioenergético semelhante ao de uma dieta cetogênica. No Capítulo 2, camundongos Swiss foram submetidos a dieta hiperlipídica e hiperglicídica (HFFD) com ou sem CMS121. Embora a HFFD tenha aumentado peso corporal, ingestão calórica e níveis de colesterol, o CMS121 não preveniu essas alterações quando associado à dieta. Entretanto, em animais saudáveis, o CMS121 isolado reduziu triglicerídeos e colesterol, além de melhorar o aspecto histológico hepático, sugerindo eficácia em condições metabólicas basais. No Capítulo 3, avaliou-se o efeito do CMS121 em comportamento e inflamação sistêmica. Em animais saudáveis, o tratamento aumentou a atividade locomotora, sem alterar comportamentos depressivos ou ansiosos. No modelo de sepse bacteriana, o CMS121 não mitigou os efeitos inflamatórios agudos, porém, na inflamação induzida por LPS, o composto atenuou a perda de peso e reduziu a hiperatividade pós-desafio inflamatório. Em conjunto, os resultados indicam que o CMS121 apresenta ação farmacológica dependente do contexto fisiopatológico, com maior eficácia em disfunções metabólicas crônicas e menor impacto em inflamações agudas graves. Assim, o composto desponta como um candidato promissor para o manejo de doenças metabólicas e neurodegenerativas associadas à inflamação crônica e à disfunção mitocondrial.

**Palavras-chave:** CMS121; obesidade; inflamação; metabolismo energético.

## ABSTRACT

CMS121 is a synthetic derivative of the flavonoid fisetin, developed to optimize its therapeutic potential through improved efficacy and pharmacokinetics. Its mechanism of action involves the activation of the AMPK pathway, leading to the inhibitory phosphorylation of ACC1 and consequently reducing the conversion of acetyl-CoA to malonyl-CoA, thereby limiting lipogenesis. This compound exhibits anti-inflammatory and metabolic effects, along with neuroprotective potential, as demonstrated in experimental models of Alzheimer's disease, aging, and diabetic obesity. Under these conditions, CMS121 was shown to reduce cognitive decline and hepatic inflammation, while improving glucose metabolism, lipid profiles, and renal function. Phase I clinical trials have demonstrated good tolerability and no evidence of toxicity, reinforcing its therapeutic promise. This study aimed to investigate the pharmacological effects of CMS121 in murine models of inflammation, metabolic dysfunction, and in predictive tests of antidepressant-like activity. Three independent experimental sets were conducted. In Chapter 1, C57BL/6 mice received a control or CMS121-supplemented diet for six months and were evaluated for metabolic and inflammatory parameters. Treatment reduced body weight gain by 40%, improved glucose and lipid indices, and decreased hepatic inflammatory markers (caspase-1, caspase-3, and NOX4). In adipose tissue, increases were observed in Nrf1, TFAM, GLUT4, and mitochondrial complex proteins, along with elevated plasma levels of short-chain acylcarnitines and butyrate metabolites, suggesting a bioenergetic profile similar to that induced by ketogenic diets. In Chapter 2, Swiss mice were subjected to a high-fat, high-fructose diet (HFFD) with or without CMS121. Although HFFD increased body weight, caloric intake, and cholesterol levels, CMS121 did not prevent these alterations when combined with the diet. However, in healthy animals, CMS121 alone reduced triglycerides and cholesterol levels and improved hepatic histology, indicating its efficacy under basal metabolic conditions but limited response against severe dietary challenges. In Chapter 3, the effects of CMS121 on behavior and systemic inflammation were evaluated. In healthy mice, CMS121 supplementation increased locomotor activity without altering depressive- or anxiety-like behaviors. In the bacterial sepsis model, CMS121 did not mitigate the acute inflammatory response. Conversely, in the LPS-induced inflammation model, CMS121 attenuated body weight loss and reduced post-inflammatory hyperactivity. Collectively, the findings demonstrate that CMS121 exhibits context-dependent pharmacological actions, showing greater efficacy in chronic metabolic dysfunction and limited effects in acute inflammatory conditions. These results highlight CMS121 as a promising therapeutic candidate for the management of metabolic and neurodegenerative diseases associated with mitochondrial dysfunction and low-grade chronic inflammation.

**Keywords:** CMS121; obesity; inflammation; energy metabolism.

## RESUMO EXPANDIDO

### Introdução

O CMS121 é um derivado sintético do flavonoide fisetina, desenvolvido com o objetivo de otimizar propriedades terapêuticas, aprimorando sua eficácia e perfil farmacocinético. Seu mecanismo de ação envolve a inibição da lipogênese, a redução de processos inflamatórios e o aumento dos níveis de acetil-CoA. O composto atua predominantemente por meio da via da AMPK (proteína quinase ativada por AMP), que promove a fosforilação inibitória da enzima ACC1, reduzindo sua atividade e, consequentemente, limitando a conversão de acetil-CoA em malonil-CoA. Estudos anteriores demonstraram que o CMS121 é eficaz em modelos experimentais de Alzheimer, envelhecimento e obesidade diabética, apresentando capacidade de atenuar o declínio cognitivo, reduzir marcadores metabólicos e transcricionais de envelhecimento cerebral, diminuir a inflamação hepática, aumentar o metabolismo da glicose, melhorar o perfil lipídico e oferecer proteção renal. O composto já passou por um ensaio clínico de fase I envolvendo indivíduos jovens e idosos saudáveis, no qual foi avaliada sua interação com a alimentação, sem que fossem observados indícios de toxicidade clínica. Devido à sua elevada biodisponibilidade e às ações neuroprotetoras e metabólicas, o CMS121 surge como uma molécula promissora para o tratamento de diversas condições fisiopatológicas. Neste estudo, três condições foram escolhidas como foco de investigação — obesidade, depressão e sepse —, as quais, além de apresentarem alta prevalência e representarem um desafio relevante à saúde pública, compartilham mecanismos fisiopatológicos comuns, como disfunção mitocondrial, inflamação crônica e alterações metabólicas sustentadas.

### Objetivos

Este trabalho se propõe investigar os efeitos farmacológicos do composto CMS121 em camundongos em diferentes modelos: de inflamação, de disfunção metabólica e em testes preditivos de ação antidepressiva.

### Metodologia

O estudo foi estruturado em três capítulos experimentais independentes. No Capítulo 1, camundongos da linhagem C57BL/6 receberam dietas controle ou suplementadas com CMS121 durante seis meses, sendo eutanasiados aos sete meses de idade. Amostras de sangue, tecido adiposo e fígado foram coletadas para análises de marcadores de glicose, lipídios e proteínas relacionadas ao metabolismo energético. No Capítulo 2, camundongos Swiss foram divididos em cinco grupos experimentais: (1) Controle (CTL), alimentados com dieta padrão; (2) CMS121, alimentados com dieta padrão suplementada com CMS121 (400 ppm/kg); (3) HFFD, alimentados com dieta hiperlipídica e solução de frutose (10%) na água potável; (4) HFFD+CMS full, alimentados com a dieta HFFD suplementada com CMS121 durante todo o tratamento; e (5) HFFD+CMS half, alimentados com a dieta HFFD nas oito primeiras semanas, seguida por HFFD+CMS nas oito semanas finais. Peso corporal e consumo alimentar foram monitorados semanalmente. Níveis de glicose e testes comportamentais (campo aberto) foram avaliados ao longo do experimento. Após 16 semanas, os animais foram eutanasiados, e tecidos hepáticos e amostras sanguíneas foram analisados para parâmetros histológicos, glicídicos, lipídicos e de dano tecidual.

No Capítulo 3, camundongos Swiss foram submetidos inicialmente à avaliação comportamental e divididos em quatro grupos que receberam diferentes concentrações de CMS121 por sete dias, além do grupo controle. Testes comportamentais foram realizados em 2, 7 e 9 dias após o início da administração. Para o modelo de sepse bacteriana, os animais foram divididos em três grupos: sépticos sem tratamento e sépticos tratados com duas concentrações distintas de CMS121, administradas quatro horas após a inoculação bacteriana. Peso corporal, consumo, escore clínico e mortalidade foram acompanhados por 15 dias. Para o modelo de inflamação induzida por LPS, os animais foram alocados em quatro grupos: controle, CMS121, LPS e CMS121+LPS. O tratamento com CMS121 foi realizado por três dias antes da inoculação de LPS, e parâmetros como peso e consumo foram monitorados por sete dias. O teste de campo aberto foi realizado 24 horas após a inoculação, e o teste de suspensão pela cauda, sete dias depois.

### **Resultados e Discussão**

No Capítulo 1, a dieta suplementada com CMS121 promoveu redução de aproximadamente 40% no ganho de peso corporal e melhora significativa nos índices de glicose e lipídios. No fígado, observaram-se níveis reduzidos de caspase-1, caspase-3 e NOX4, indicando um estado inflamatório atenuado. No tecido adiposo, o CMS121 aumentou a expressão dos fatores de transcrição Nrf1 e TFAM, além de proteínas mitocondriais associadas ao transporte de elétrons e à glicose (GLUT4), acompanhadas de maior taxa metabólica de repouso. A análise metabolômica revelou elevação de acilcarnitinas de cadeia curta e metabólitos de butirato, compatíveis com um perfil bioenergético semelhante ao induzido por dietas cetogênicas. No Capítulo 2, a dieta HFFD elevou o peso corporal, a ingestão calórica e os níveis plasmáticos de LDL e colesterol. O tratamento com CMS121, parcial ou integralmente associado à dieta, não preveniu as alterações decorrentes da HFFD. Contudo, em animais saudáveis (grupos CMS121 e CTL), observou-se redução nos níveis de triglicerídeos e colesterol, além de melhora histológica hepática, sugerindo que o CMS121 apresenta eficácia em condições metabólicas fisiológicas, mas resposta limitada frente a insultos dietéticos intensos. No Capítulo 3, a suplementação de CMS121 em animais saudáveis aumentou a atividade locomotora, sem alterar comportamentos depressivos ou ansiosos. No modelo de sepse bacteriana, o intenso processo inflamatório induzido pela infecção sobrepôs-se aos possíveis efeitos anti-inflamatórios do CMS121. Já na inflamação induzida por LPS, o tratamento atenuou a perda de peso e mitigou a hiperatividade observada após o desafio inflamatório.

### **Conclusão**

Os resultados obtidos indicam que o CMS121 apresenta um perfil farmacológico dependente do contexto fisiopatológico, com maior eficácia em condições de disfunção metabólica crônica e efeitos mais discretos em processos inflamatórios agudos e graves. Esses achados reforçam o potencial do CMS121 como agente terapêutico promissor para o manejo de doenças metabólicas e neurodegenerativas associadas à disfunção mitocondrial e à inflamação crônica de baixo grau.

**Palavras-chave:** CMS121; obesidade; inflamação; metabolismo energético.

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## LISTA DE ABREVIATURAS E SIGLAS

15-LOX 2: 15-lipoxigenase-2  
4E-BP1: proteína de ligação ao fator de iniciação eucariótica 4E 1  
5-LOX: 5-lipoxigenase  
ACC1: acetil-CoA carboxilase 1  
AKT: proteína quinase B  
AMPK: proteína quinase ativada por AMP  
AVC: Acidente vascular cerebral  
BDNF: fator neurotrófico derivado do cérebro  
CLP: Ligação e Punção Cecal, do inglês Cecal Ligation and Puncture  
COX2: ciclooxigenase 2  
DA: doença de Alzheimer  
DH: doença de Huntington  
DSM-5: do inglês *Diagnostic and Statistical Manual of Mental Disorders*  
ERK: cinase regulada por sinal extracelular  
ERK2: Cinase 2 regulada por sinal extracelular  
ERO: espécies reativas de oxigênio  
FASN: ácido graxo sintase  
FH: fumarato hidratase  
GFAP: Proteína Ácida Fibrilar Glial  
GSH: glutathiona  
HbA1c: hemoglobina glicada  
HF: dieta rica em gordura e frutose, do inglês high fat and high fructose  
HPA: hipotálamo-hipófise-adrenal  
IL-10: interleucina 10  
IL-1 $\beta$ : Interleucina-1 $\beta$   
IL-6: interleucina 6  
IL-8: interleucina 8  
IMC: Índice de Massa Corporal  
iNOS: óxido nítrico sintase induzível  
LDH-A: lactato desidrogenase A  
LDL: lipoproteína de baixa densidade  
LPS: lipopolissacarídeos

MAPKs: Proteínas cinases ativadas por mitógenos

MCP-1: proteína quimioatraente de monócitos-1

MPTP/MPP+: 1-metil-4-fenil-1,2,3,6-tetrahidropiridina / 1-metil-4-fenil-piridínio

mRNA: RNA mensageiro

mTOR: alvo da rapamicina em mamíferos

NAD(P)H: nicotinamida adenina dinucleotídeo fosfato reduzido

NADH: nicotinamida adenina dinucleotídeo reduzido

NF-κB: fator nuclear kappa B

NO: óxido nítrico

NOX4: NADPH oxidase 4

Nrf1: Fator de Respiração Nuclear 1

Nrf2: Fator Nuclear Eritroide 2-relacionado ao Fator 2

p38 MAPK: proteína quinase ativada por mitógeno p38

PAMPs: Padrões Moleculares Associados a Patógenos

PI3K: fosfoinositídeo 3-quinase

TDM: transtorno depressivo maior

TFAM: Fator de Transcrição A Mitocondrial

TNF-α: Fator de Necrose Tumoral alfa

TXNIP: Proteína que interage com tiorredoxina

UTI: unidades de terapia intensiva

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## 1 INTRODUÇÃO

### 1.1 FLAVANOIDES

Flavonoides são um grande grupo de metabólitos secundários de plantas, presentes em frutas, ervas, caules, cereais, nozes, vegetais, flores e sementes (Ullah *et al.*, 2020). São compostos por dois anéis fenólicos ligados a um anel heterocíclico (anel C), de estrutura básica C6–C3–C6 (Wang, Tian-yang; Li; Bi, 2018). A diversidade estrutural dos flavonoides resulta em múltiplas subclasses, incluindo flavonas, flavanonas, isoflavonas, flavonóis, chalconas, flavanóis e antocianinas, determinadas por variações no grau de insaturação, oxidação e padrão de substituição do anel central (Chen, Shen *et al.*, 2023; Ullah *et al.*, 2020). Os flavonoides exercem uma ampla gama de efeitos biológicos, que sustentam sua aplicação em formulações nutracêuticas, farmacêuticas, cosméticas e alimentares (Chen, Shen *et al.*, 2023). Esses efeitos incluem propriedades antioxidantes, antibacterianas, antifúngicas, antivirais, antidiabéticas, neuroprotetoras, cardioprotetoras e anti-inflamatórias (Ullah *et al.*, 2020).

A atividade antioxidante constitui um dos principais mecanismos de ação dos flavonoides (Zuo *et al.*, 2018). Devido a sua baixa biodisponibilidade, os flavonóis não tem ação direta como antioxidantes *in vivo* (Forman *et al.*, 2015). Muitos dos efeitos dos flavonoides derivam da ativação de vias de proteção celular, como a ativação do fator de transcrição Nrf2 (Michalak; Michalak, 2025; Wang, Sumei *et al.*, 2025). Muitas enzimas oferecem proteção celular, como as de fase II (ex.: NAD(P)H-quinona oxidoreductase e glutathione S-transferase), por meio da indução do elemento responsivo a eletrófilo (Lee-Hilz *et al.*, 2006). Adicionalmente, esses compostos inibem enzimas produtoras de radicais, como xantina oxidase, proteína cinase C, ciclooxigenase, NADH oxidase e lipoxigenase, reduzindo a formação de ânions superóxido e outros oxidantes (Md Idris *et al.*, 2022; Zeng *et al.*, 2019). Outro mecanismo importante é a atividade quelante de metais, como  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$  e  $\text{Zn}^{2+}$ , que impede reações redox indutoras de estresse oxidativo. Além disso, flavonoides regeneram radicais  $\alpha$ -tocoferol, prolongando a proteção antioxidante contra a oxidação da lipoproteína de baixa densidade (LDL) (Grin *et al.*, 2018; Torello; Alvarez; Olalla Saad, 2021). Estes também atenuam o estresse oxidativo mediado por óxido

nítrico (NO) ao inibirem a expressão da enzima óxido nítrico sintase induzível (iNOS) e reduzirem a formação de peroxinitrito, um potente agente citotóxico (Gutiérrez-Venegas *et al.*, 2014).

A atividade anti-inflamatória dos flavonoides é vasta e envolve diversos mecanismos. Estes incluem (1) atividade antioxidante (supressão de espécies reativas de oxigênio - ERO); (2) inibição de enzimas reguladoras, como as proteínas cinases; (3) interferência no metabolismo do ácido araquidônico (supressão da liberação de células pró-inflamatórias); (4) modulação da expressão gênica e (5) modulação de células imunes (diminuição da liberação de citocinas pró-inflamatórias) (Jomova *et al.*, 2025). Assim, os flavonoides reduzem a expressão de citocinas pró-inflamatórias como TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 e MCP-1 em células imunes, e aumentam a produção de IL-10, com efeito imunomodulador (Jomova *et al.*, 2025).

Os flavonoides também demonstram ação neuroprotetora significativa em doenças neurodegenerativas, como Alzheimer, Parkinson e Huntington, atuando na mitigação do estresse oxidativo e inflamação crônica que caracterizam essas condições (Jomova *et al.*, 2025). Sua capacidade de quelar metais redox-ativos, inibir a geração de ERO e modular vias inflamatórias (como fator nuclear kappa B e citocinas pró-inflamatórias IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) favorece a diminuição do dano neuronal, preservação da função mitocondrial e redução da ativação microglial (Chakraborty *et al.*, 2014). Assim, esses compostos se mostram promissores para retardar a progressão de neurodegeneração associada ao acúmulo de agregados proteicos e morte celular.

Na saúde mental, flavonoides promovem efeitos antidepressivos, ansiolíticos e moduladores de humor, atuando por meio de mecanismos semelhantes àqueles observados em distúrbios neurodegenerativos (Jia *et al.*, 2023). Eles reduzem o estresse oxidativo no sistema nervoso central, suprimem a neuroinflamação (especialmente via microglia) e regulam a expressão de fatores neurotróficos e neurotransmissores (Ben-Azu *et al.*, 2019). A modulação do equilíbrio entre citocinas pró- e anti-inflamatórias contribui para melhorar neuroplasticidade e resiliência frente a estresse, o que é crucial em condições como depressão, esquizofrenia e transtorno bipolar (Sawamoto *et al.*, 2016).

### 1.1.1 Fisetina

A fisetina é um flavonol natural, classificado como 3,3',4',7-tetrahidroxi-flavona, presente em frutas e vegetais como morangos, maçãs, cebolas, pepinos e caquis, em concentrações que variam de 2 a 160 µg/g (Khan *et al.*, 2013). Essa estrutura confere à fisetina propriedades antioxidantes significativas *in vitro* devido à presença de múltiplos grupos hidroxila, os quais contribuem para a doação de elétrons e a neutralização de espécies reativas de oxigênio (Hassan *et al.*, 2022). Entretanto, os efeitos *in vivo* da fisetina provavelmente advém da regulação de vias de sinalização (Fatima *et al.*, 2025). A ação antioxidante da fisetina é amplamente documentada e atribuída à modulação de vias intracelulares de defesa antioxidante, como a ativação do fator de transcrição Nrf2 (Fator Nuclear Eritroide 2-relacionado ao Fator 2) e a inibição da cascata inflamatória pela via do fator nuclear kappa B (NF-κB) (Khan *et al.*, 2013). Essas propriedades são centrais para sua atividade protetora em doenças crônicas relacionadas ao estresse oxidativo.

No sistema nervoso, a fisetina atua como neuroprotetora por meio de efeitos antioxidantes e anti-inflamatórios, além de modular vias intracelulares envolvidas na apoptose e sobrevivência neuronal. Em modelos de células PC12 expostas à corticosterona, a fisetina reduziu a produção de espécies reativas de oxigênio e impediu a ativação de vias de morte celular como ERK, p38 MAPK e PI3K/Akt/FOXO3a (Chang *et al.*, 2023). Esses achados sugerem aplicações potenciais em doenças neurodegenerativas como Alzheimer, Parkinson e esclerose múltipla (Hassan *et al.*, 2022).

No contexto da doença de Alzheimer, a fisetina atua em diversos alvos moleculares implicados na fisiopatologia da doença. Estudos demonstram que ela atenua a toxicidade induzida pelo peptídeo β-amiloide, preserva a função mitocondrial e melhora a plasticidade sináptica em modelos murinos (Maher, 2015, 2024). Além disso, a fisetina regula positivamente a expressão de fatores neurotróficos como o BDNF (fator neurotrófico derivado do cérebro), essenciais para a manutenção da integridade sináptica, ao mesmo tempo em que inibe vias inflamatórias como NF-κB e reduz a produção de citocinas pró-inflamatórias. Em modelos animais de Alzheimer, a administração crônica de fisetina foi capaz de prevenir a perda cognitiva e reduzir

significativamente a carga de placas amiloides, demonstrando eficácia mesmo quando o tratamento foi iniciado em estágios avançados da patologia (Maher, 2020).

Na doença de Parkinson, a fisetina também se mostrou eficaz em modelos pré-clínicos. Sua ação protetora sobre neurônios dopaminérgicos se dá por meio da redução da agregação de  $\alpha$ -sinucleína, diminuição da produção de ERO e modulação da inflamação neuroglial (Maher, 2017). Um pré-tratamento com fisetina antes da exposição a MPTP/MPP+ diminuiu significativamente a citotoxicidade induzida por MPTP/MPP+ e a morte celular, provavelmente pela diminuição da expressão de  $\alpha$ -sinucleína, conhecida como principal contribuinte para a morte neuronal (Patel *et al.*, 2012). Esses efeitos indicam que a fisetina pode retardar ou mitigar a progressão da degeneração neuronal característica do Parkinson, atuando através de múltiplos mecanismos na homeostase do sistema nervoso central.

Em relação à demência de forma geral, a fisetina exerce ações que reduzem fatores de risco fisiológicos associados ao declínio cognitivo, como inflamação sistêmica, disfunção endotelial e senescência celular (Maher, 2024). Ao atuar simultaneamente sobre vias metabólicas, inflamatórias e oxidativas, a fisetina contribui para a manutenção da função cognitiva em modelos de envelhecimento cerebral. Um dos efeitos mais notáveis é sua capacidade de atuar como um agente senolítico, eliminando seletivamente células senescentes, que se acumulam com a idade e contribuem para o estado pró-inflamatório crônico no cérebro, característico do envelhecimento (Maher, 2020). Em modelos murinos, a administração de fisetina em camundongos idosos resultou na redução da carga de células senescentes, melhora de parâmetros fisiológicos e extensão da expectativa de vida (Yousefzadeh *et al.*, 2018).

A fisetina também tem sido investigada por seus efeitos neuroprotetores e benefícios potenciais na saúde mental, especialmente em distúrbios como a depressão. Em modelos preditivos, a fisetina mostrou um efeito dependente da dose e produziu um aumento nos níveis de serotonina e noradrenalina no córtex frontal e no hipocampo (Zhen *et al.*, 2012). Os efeitos antidepressivos da fisetina envolvem a modulação do sistema serotoninérgico pela redução do metabolismo da serotonina, possivelmente por meio da inibição da monoamina oxidase A, uma enzima que metaboliza a serotonina (Zhen *et al.*, 2012). Em modelo de comportamento depressivo

induzido por lipopolissacarídeos (LPS) em camundongos, o pré-tratamento com fisetina foi efetivo devido a suas propriedades anti-inflamatórias, uma vez que reverteu a superexpressão de citocinas pró-inflamatórias (IL-1 $\beta$ , IL-6 e TNF- $\alpha$ ) induzida por LPS no hipocampo e no córtex pré-frontal, e em doses mais altas antagonizou efetivamente a expressão de mRNA da iNOS e os níveis de nitrito por meio da modulação do NF- $\kappa$ B nos mesmos tecidos (Yu *et al.*, 2016).

A fisetina tem sido investigada como um agente terapêutico promissor em modelos experimentais de inflamação sistêmica induzida por LPS, um componente da parede celular de bactérias gram-negativas amplamente utilizado para mimetizar sepse em modelos *in vivo*. Embora os estudos citados não enfoquem diretamente a sepse clínica, eles destacam mecanismos relevantes para esse contexto, como a potente atividade anti-inflamatória da fisetina por meio da modulação negativa de vias pró-inflamatórias como NF- $\kappa$ B, MAPKs e citocinas como TNF- $\alpha$  e IL-6 (Hassan *et al.*, 2022; Khan *et al.*, 2013). Em modelos celulares, a fisetina demonstrou capacidade de suprimir a liberação de mediadores inflamatórios induzidos por LPS, reduzindo o estresse oxidativo e a apoptose celular (Hassan *et al.*, 2022). Além disso, sua ação reguladora sobre vias como PI3K/Akt e Nrf2 contribui para restaurar o equilíbrio redox e a integridade celular, aspectos críticos na resposta à sepse. Esses dados sugerem que a fisetina pode atuar como modulador da resposta inflamatória exacerbada observada em condições sépticas, embora sejam necessários mais estudos *in vivo* específicos e ensaios clínicos para validar sua eficácia nesse cenário.

Ensaio clínico com fisetina ainda são incipientes, mas promissores. Um estudo multicêntrico de fase 2 está em andamento para avaliar a eficácia da fisetina no tratamento da sepse em idosos, com administração oral e avaliação de marcadores inflamatórios e desfechos clínicos (NCT04771611, 2024)(COVFIS-HOME: A Phase 2 Placebo-Controlled Pilot Study in COVID-19 of Fisetin to Alleviate Dysfunction and Decrease Complications in At-Risk Outpatients, [s. d.]). Essas investigações poderão confirmar se os efeitos observados em modelos pré-clínicos são replicáveis em humanos, e definir parâmetros seguros e eficazes de uso.

Em síntese, a fisetina representa um flavonol com ampla gama de propriedades farmacológicas, incluindo ação antioxidante, anti-inflamatória, antitumoral, senolítica, neuroprotetora e ortopédica. Embora os dados pré-clínicos

sustentem seu potencial terapêutico, ainda são necessárias evidências clínicas robustas para sua incorporação como agente farmacológico. Na busca pela melhoria das propriedades farmacológicas e eficácia, novos fármacos vem sendo pesquisado extensivamente (Currais; Raschke; Maher, 2024).

### 1.1.2 CMS121

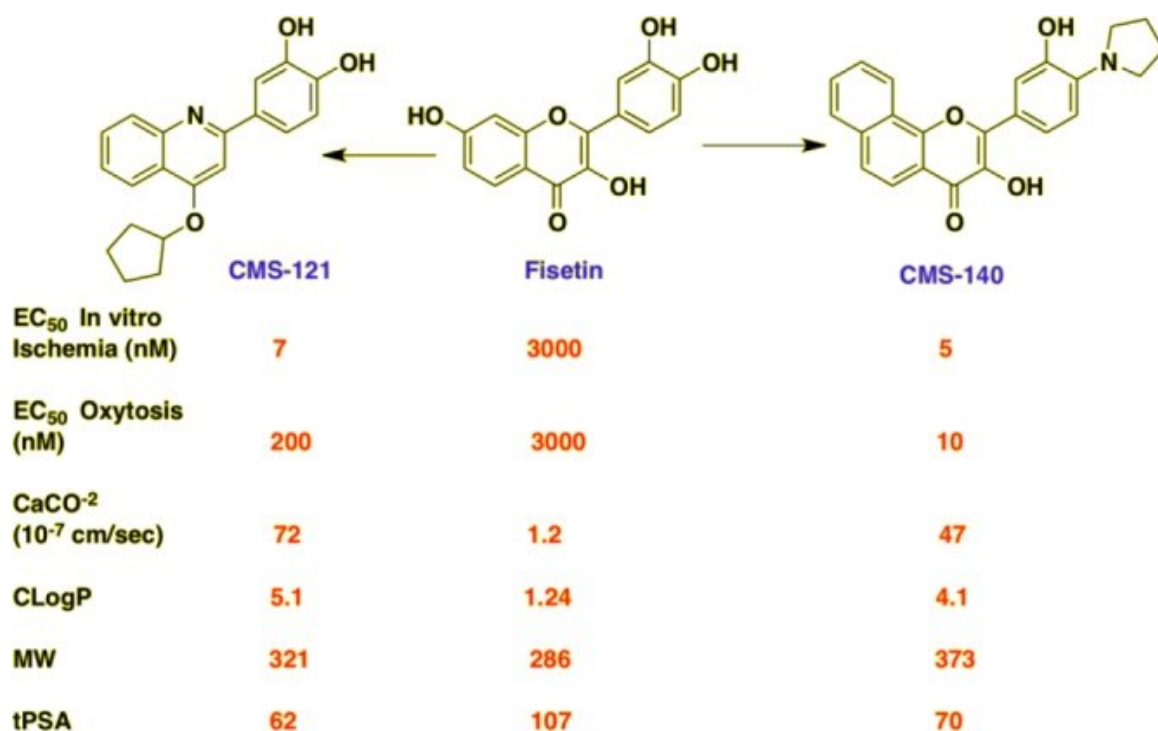
Apesar de seu grande potencial terapêutico, a aplicação clínica dos flavonoides é limitada por suas características farmacocinéticas, como baixa solubilidade aquosa, fraca absorção intestinal e metabolismo rápido (Jannat *et al.*, 2021; Teng *et al.*, 2023). A exemplo da fisetina, que possui baixa solubilidade aquosa (menos de 1 mg/mL), o que resulta em baixa biodisponibilidade, e acaba limitando seu uso (Jannat *et al.*, 2021). Nesse cenário, a criação de novas moléculas baseadas em flavonóides tem emergido como estratégia promissora para superar essas limitações.

O CMS121 é um derivado sintético da flavonóide fisetina, desenvolvido para otimizar potenciais terapêuticos por meio de melhor eficácia e farmacocinética (Currais *et al.*, 2019). A seleção do CMS121 ocorreu por meio de avaliação de 160 derivados, priorizando aqueles com ação anti-inflamatória, alta biodisponibilidade oral e habilidade de atravessar a barreira hematoencefálica (neuropenetrância) (Maher *et al.*, 2025).

A estrutura de flavona foi alterada para uma quinolina, enquanto, ao mesmo tempo, mantiveram-se os principais elementos estruturais da fisetina (Prior *et al.*, 2014). Três propriedades da fisetina foram mantidas nos derivados, incluindo modulação da síntese de GSH, a inibição da ativação microglial induzida por LPS e a diferenciação de células PC12, uma medida da atividade neurotrófica.

O derivado de quinolona CMS121 é 400 vezes mais potente do que a fisetina no ensaio de isquemia (Figura 1). Assim, é possível manter as qualidades multialvo de um polifenol, ao mesmo tempo em que se melhoram as propriedades físico-químicas e farmacológicas do composto.

Figura 1 – Melhoria da fisetina



Melhoria da fisetina em termos de potência (isquemia e oxitose in vitro), propriedades químicas medicinais (clogP, MW, tPSA) e transporte através de células epiteliais intestinais CaCO-1. Fonte: Adaptado de Prior e colaboradores [2014].

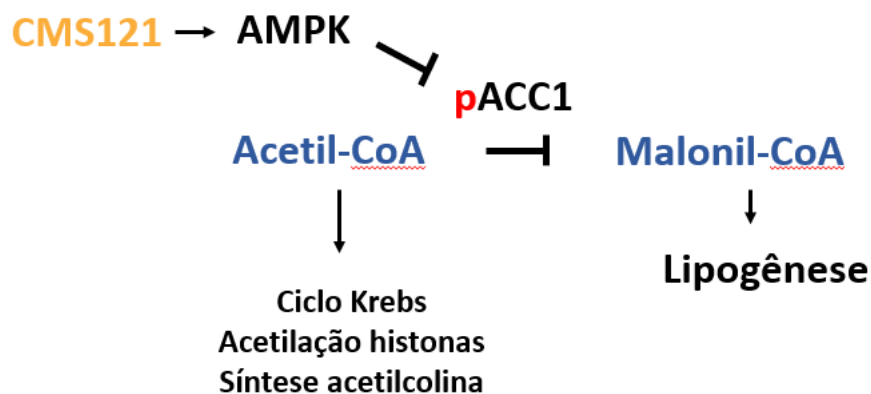
Estudos farmacocinéticos dos seis derivados mais promissores da fisetina mostraram que o CMS121 possui potência melhorada (EC<sub>50</sub>=200nM) contra a oxitose/ferroptose (Chiruta *et al.*, 2012). Ademais, após uma dose oral única de 20 mg/kg, o CMS121 alcançou concentrações cerebrais que excederam amplamente sua EC<sub>50</sub> média *in vitro*, com boa biodisponibilidade oral e capacidade de atravessar a barreira hematoencefálica (Maher; Currais; Schubert, 2020).

O CMS121 já finalizou um ensaio clínico de fase 1 (NCT05318040) em voluntários jovens e saudáveis, demonstrando excelente tolerabilidade, com doses únicas de até 1800 mg e múltiplas de até 900 mg/dia por sete dias, sem eventos adversos graves relatados (Maher *et al.*, 2025). Em dose única o CMS e seus metabólitos apresentaram pico de concentração plasmática em aproximadamente 1,5h após a dose e excreção urinária mínima com a fração cumulativa média excretada 72 horas após a dose. Em doses múltiplas o pico plasmático aconteceu em 2-3h após a última dose e manteve o tempo de excreção urinária. Já em idosos

permaneceu quantificável no plasma por mais tempo e com meia-vida de eliminação terminal maior. O estado alimentado atrasou a absorção para 4h, enquanto em jejum ocorreu de 2-3h, entretanto o CMS apresentou maior biodisponibilidade em estado alimentado, em torno de 50%. Tais resultados demonstram que o composto não tem indícios de toxicidade clínica.

O CMS121 atua inibindo a lipogênese, reduzindo inflamação e aumentando níveis de acetil-CoA, conforme o esquema abaixo (Figura 2). A acetil-CoA carboxilase 1 (ACC1) é a enzima responsável por converter a acetil-coa em malonil-coa, etapa inicial da síntese de ácidos graxos. O CMS121 ativa a via da AMPK (proteína quinase ativada por AMP), que fosforila a ACC1, reduzindo sua atividade. A inibição de ACC1 limita a conversão de acetil-CoA em malonil-CoA e evita o consumo de acetil-CoA para lipogênese, preservando ou elevando os níveis intracelulares. Assim, a acetil-coa permanece disponível para o ciclo de Krebs e consequente produção de energia; acetilação de histonas e consequente regulação epigenética da memória; e síntese de acetilcolina.

Figura 2 - Atuação do CMS121



Mecanismo de ação do CMS121: o CMS121 ativa a via da AMPK, fosforilando a ACC1, limitando a conversão de acetil-coA em malonil-coA. Fonte: Adaptado de Currais *et al.*, [2019] e Ates *et al.*, [2020].

Estudos *in vitro* com células HT22 mostraram que o CMS121 protege durante a oxitose/ferroptose, reduzindo a morte celular induzida por glutamato, reduzindo peroxidação lipídica, com o aumento da acetil-CoA e promovendo fosforilação da acetil-CoA carboxilase 1 (ACC1) (Ates *et al.*, 2020). Esses mecanismos estão relacionados à inibição da lipogênese e ao aumento da acetilação de histonas, um

mecanismo epigenético crucial para regulação da expressão gênica e formação de memória (Ates *et al.*, 2020).

Modelos pré-clínicos têm reforçado o potencial do CMS121 em retardar os efeitos do envelhecimento. Em camundongos SAMP8, que exibem envelhecimento acelerado, a administração oral de CMS121 (400 ppm) resultou em preservação da função cognitiva, estabilização dos metabólitos cerebrais e aumento dos níveis de acetil-CoA, essencial para a síntese de neurotransmissores e acetilação de histonas (Currais *et al.*, 2019). A elevação de acetil-CoA está associada à inibição da ACC1 via ativação da AMPK, favorecendo a preservação mitocondrial e redução da síntese de lipídios, efeitos que explicam parcialmente os benefícios cognitivos observados.

Além disso, em estudo com camundongos transgênicos APP<sup>swe</sup>/PS1 $\Delta$ E9, modelo de doença de Alzheimer (DA), o CMS121 melhorou a função cognitiva, permitindo que eles se comportassem de maneira semelhante aos camundongos selvagens em testes de memória espacial, memória contextual e desinibição (Ates *et al.*, 2020). Ainda reduziu a peroxidação lipídica, neuroinflamação e acúmulo de  $\beta$ -amiloide, além de normalizar marcadores inflamatórios como GFAP e 15-LOX2, resultando em melhora significativa da memória espacial e contextual (Ates *et al.*, 2020).

No estudo conduzido por Kepchia *et al.* (Kepchia *et al.*, 2021), o CMS121 também demonstrou efeitos benéficos sobre a função renal em camundongos SAMP8, mas também para saúde do cérebro, capaz de promover um fenótipo mais jovem nestes tecidos. O tratamento restaurou a massa renal, reduziu a creatinina plasmática e reverteu alterações em proteínas inflamatórias (TNF $\alpha$ , iNOS, 5-LOX, caspase-1, COX2) e da via MAPK (ERK2) nos rins. No cérebro, normalizou proteínas associadas às vias mTOR e AKT, com destaque para redução da fosforilação de 4E-BP1 e AKT, e aumento da HSP90, associada à manutenção da proteostase.

A eficácia do CMS121 também foi testada em camundongos com deficiência auditiva relacionada à idade (DARI), onde se observou melhora nos limiares auditivos e no número de sinapses funcionais, indicando neuroproteção na via auditiva (Pham *et al.*, 2023). Em modelo de doença de Huntington (DH) com camundongos R6/2, o CMS121 reduziu a progressão da doença, melhorando a força de preensão, embora sem prolongar significativamente a sobrevivência (Ates; Taguchi; Maher, 2024).

Adicionalmente, o CMS121 demonstrou impacto positivo em um modelo de síndrome metabólica com camundongos db/db, animais que não apresentam receptor de leptina e apresentam hiperfagia, resultando em distúrbios do metabolismo. O tratamento por 6 meses reduziu a hemoglobina glicada, melhorou a tolerância à glicose, diminuiu marcadores inflamatórios hepáticos e promoveu nefroproteção, com redução de albumina e colágeno I urinário, indicando menor fibrose e inflamação renal (Zahid *et al.*, 2023). Entretanto, não foram observadas mudanças significativas no consumo de oxigênio nem na atividade locomotora, apesar da redução de 5% no peso corporal.

De forma geral, o CMS121 representa um avanço promissor na terapêutica de condições relacionadas ao envelhecimento, associando segurança, biodisponibilidade e ação neuroprotetora e metabólica, abrindo perspectivas para a ampliação de estudos pré-clínicos e estudos clínicos em larga escala.

## 1.2 OBESIDADE E ALTERAÇÕES METABÓLICAS

A obesidade é popularmente conhecida como uma condição crônica caracterizada pelo acúmulo excessivo de gordura corporal. Atualmente a obesidade é classificada como uma doença crônica progressiva, com influências complexas entre fatores genéticos, comportamentais e ambientais (Sbraccia; Dicker, 2023).

Os níveis de sobrepeso da população vêm aumentando em todo mundo, e as estimativas para 2030 são de 1 bilhão de pessoas obesas, podendo já ser considerada uma pandemia global (World Obesity Federation, 2020). Proporcional ao aumento da quantidade de pessoas obesas, os gastos em serviços de saúde para essa parte da população também aumentam, com estimativa de gasto de 990 bilhões de dólares anual, o que equivale a mais que 13% dos gastos de saúde mundiais (World Obesity Federation, 2020). A condição está associada a custos diretos com tratamentos médicos e indiretos devido à perda de produtividade e ao aumento da mortalidade prematura. Estima-se que a obesidade contribua significativamente para os gastos com doenças não transmissíveis, como diabetes tipo 2, doenças cardíacas e alguns tipos de câncer (Pan; Wang; Pan, 2021). Na América Latina, o impacto econômico da obesidade nos sistemas de saúde é alarmante, considerando o aumento das taxas de obesidade em adultos e crianças (O'Lawrence, Henry *et al.*, 2020).

A classificação da obesidade tradicionalmente se baseia no Índice de Massa Corporal (IMC), que categoriza os indivíduos segundo a razão entre peso e altura ao quadrado ( $\text{kg/m}^2$ ), variando de baixo peso ( $\text{IMC} < 18,5$ ) a obesidade mórbida ( $\text{IMC} > 40$ ) (Lorenzo, 2016). Embora amplamente utilizada, essa abordagem não diferencia entre massa magra e gordura corporal, o que limita sua acurácia na avaliação do risco metabólico individual. Por isso, métodos adicionais como a circunferência abdominal e a análise de composição corporal via bioimpedância, pregas cutâneas, DEXA (densitometria óssea por absorção de raios-x de dupla energia), tomografia ou ressonância magnética são empregados para refinar o diagnóstico (Garvey *et al.*, 2016; Jensen *et al.*, 2014).

A abordagem fenotípica da obesidade, que considera a distribuição da gordura visceral e subcutânea, permite uma avaliação mais precisa e a individualização do tratamento. Quatro fenótipos principais são descritos com base na composição e distribuição da gordura corporal: obeso com peso normal, metabolicamente obeso com peso normal, obeso metabolicamente saudável e obeso metabolicamente não saudável, sendo este último associado a maior risco de doenças crônicas (Lorenzo, 2016).

A distribuição de gordura afeta o risco metabólico em indivíduos com obesidade, pois a gordura visceral, acumulada ao redor dos órgãos abdominais, está associada a um maior risco de alterações metabólicas, como resistência à insulina e síndrome metabólica, enquanto a gordura subcutânea, que reside abaixo da pele, tende a ter um efeito positivo na sensibilidade à insulina (Lorenzo, 2016). Assim, a localização do excesso de gordura corporal é um fator crítico na determinação do risco de doenças metabólicas.

O acúmulo excessivo de tecido adiposo, particularmente na região visceral, promove a secreção de adipocinas pró-inflamatórias, como TNF- $\alpha$ , IL-6 e resistina, ao mesmo tempo em que reduz a produção de adiponectina, um hormônio com ação anti-inflamatória e sensibilizadora da insulina (Lumeng; Saltiel, 2011; Ouchi *et al.*, 2011). Esse estado inflamatório favorece o recrutamento de macrófagos para o tecido adiposo, intensificando a inflamação local e sistêmica. Além disso, a obesidade compromete o metabolismo lipídico, levando à elevação de ácidos graxos livres circulantes, que interferem na sinalização da insulina em tecidos periféricos, como

fígado e músculo esquelético, contribuindo para a resistência insulínica (Samuel; Shulman, 2016). Em conjunto, essas alterações bioquímicas sustentam um ciclo patológico que perpetua a obesidade e suas complicações metabólicas.

Modelos experimentais de obesidade são fundamentais para o estudo dos mecanismos fisiopatológicos e terapêuticos associados à doença. Esses modelos podem ser classificados em genéticos, dietéticos ou farmacológicos. Os modelos genéticos, como os camundongos ob/ob (deficientes em leptina) e db/db (com mutação no receptor de leptina), desenvolvem obesidade espontaneamente e apresentam hiperglicemia, hiperinsulinemia e resistência à insulina, mimetizando características da obesidade humana associada à síndrome metabólica (Chen, Hong *et al.*, 1996; Zhang, Yiyang *et al.*, 1994). Por outro lado, os modelos induzidos por dieta, particularmente por dietas hiperlipídicas ou ricas em gordura e frutose, reproduzem de forma mais próxima a obesidade adquirida por fatores ambientais, promovendo ganho de peso progressivo, disfunção metabólica e inflamação de tecido adiposo (Buettner; Schölmerich; Bollheimer, 2007). Esses modelos dietéticos são amplamente utilizados por refletirem melhor a complexidade da obesidade humana, incluindo alterações no eixo neuroendócrino e na microbiota intestinal. Modelos farmacológicos, como a administração de glutamato monossódico, também são empregados, embora menos comuns (Sasaki *et al.*, 2009). A escolha adequada do modelo é essencial, considerando o objetivo do estudo e os mecanismos específicos a serem investigados, visto que cada modelo possui vantagens e limitações translacionais distintas.

### 1.3 DEPRESSÃO

O transtorno depressivo maior (TDM) é uma condição psiquiátrica debilitante caracterizada por episódios depressivos distintos, com duração mínima de duas semanas e presença de humor deprimido, perda de interesse, alterações cognitivas e sintomas vegetativos, como distúrbios do sono e apetite (Otte *et al.*, 2016). O diagnóstico é definido pela presença de pelo menos cinco sintomas durante um período de duas semanas, conforme os critérios do *Diagnostic and Statistical Manual of Mental Disorders* – quinta edição (DSM-5) (American Psychiatric Association,

2013). A condição pode ser subclassificada por especificadores clínicos, como sintomas ansiosos, psicóticos, melancólicos, sazonais ou atípicos.

A prevalência global do TDM é de aproximadamente 6% ao ano, com cerca de um em cada seis adultos sendo afetado ao longo da vida (Kessler; Bromet, 2013). O distúrbio é duas vezes mais prevalente em mulheres e frequentemente se inicia entre a adolescência tardia e os 40 anos (Otte *et al.*, 2016). A depressão está entre as principais causas de incapacidade no mundo e está associada a múltiplas comorbidades clínicas, como doenças cardiovasculares, diabetes tipo 2, obesidade, acidente vascular cerebral (AVC) e demência, além de representar um fator de risco importante para suicídio, sendo responsável por até 50% dos suicídios anuais no mundo (Cavanagh *et al.*, 2003; World Health Organization, 2025).

A etiologia do TDM é multifatorial. A hereditariedade responde por cerca de 35% do risco, com estudos de famílias e gêmeos apontando para um componente genético importante, embora altamente poligênico e de difícil replicação em estudos genômicos (Hyde *et al.*, 2016; Sullivan; Neale; Kendler, 2000). Fatores ambientais, especialmente eventos estressores precoces, como abusos físicos, emocionais ou sexuais na infância, estão fortemente associados à vulnerabilidade ao transtorno (Heim *et al.*, 2008). Esses eventos atuam sobre sistemas neurobiológicos como o eixo hipotálamo-hipófise-adrenal (HPA), induzindo hiperatividade do eixo, resistência aos glicocorticoides e inflamação sistêmica (Miller; Maletic; Raison, 2009; Pariante; Lightman, 2008).

Do ponto de vista fisiopatológico, a depressão está associada a alterações neuroanatômicas e funcionais. Estudos de neuroimagem revelam redução volumétrica do hipocampo, alterações em áreas frontais, tálamo e gânglios da base, bem como disfunções em redes neurais como a rede de saliência, rede de modo padrão e circuito de controle cognitivo (Schmaal *et al.*, 2016). As alterações estruturais e funcionais parecem estar relacionadas à severidade, duração e recorrência dos episódios depressivos (Sheline *et al.*, 1999). A nível molecular, observam-se disfunções nos sistemas monoaminérgicos, com destaque para serotonina, noradrenalina e dopamina, embora a hipótese monoaminérgica não explique isoladamente a complexidade da doença (Otte *et al.*, 2016). Evidências também apontam para o envolvimento da neuroinflamação, com aumento de citocinas pró-

inflamatórias como TNF- $\alpha$  e IL-6 (Dowlati *et al.*, 2010), além de redução da neurogênese e de fatores neurotróficos como o BDNF (Krishnan; Nestler, 2008).

Os modelos experimentais em animais são fundamentais para o entendimento dos mecanismos biológicos da depressão. Apesar das limitações em transpor sintomas subjetivos para animais, diversos modelos, como o estresse crônico imprevisível, a exposição precoce a estressores ou a administração de lipopolissacarídeo (LPS), têm contribuído para a identificação de vias implicadas, incluindo o eixo HPA, inflamação, neuroplasticidade, epigenética e microbiota intestinal (Otte *et al.*, 2016). Esses modelos permitiram testar alvos terapêuticos, como receptores de glicocorticoides, citocinas inflamatórias, e agentes que estimulam a neurogênese.

O tratamento do TDM inclui abordagens psicoterapêuticas e farmacológicas. A psicoterapia cognitivo-comportamental, a terapia interpessoal, a terapia psicodinâmica e a terapia de resolução de problemas demonstraram eficácia comparável à farmacoterapia em casos leves a moderados (Cuijpers *et al.*, 2008; Otte *et al.*, 2016). Para casos moderados a graves, recomenda-se o uso de antidepressivos, sendo os inibidores seletivos da recaptação de serotonina (ISRS) os mais prescritos devido à sua eficácia e melhor perfil de tolerabilidade em relação aos tricíclicos. Contudo, aproximadamente 30% dos pacientes não alcançam remissão mesmo após múltiplas tentativas de tratamento (Rush *et al.*, 2006).

Em quadros resistentes, estratégias como aumento da dose, troca ou combinação de antidepressivos, uso de antipsicóticos atípicos ou estabilizadores de humor, bem como a eletroconvulsoterapia, são recomendadas. Recentemente, terapias emergentes como cetamina, agentes anti-inflamatórios, psicodélicos, estimulação magnética transcraniana e compostos neurogênicos estão sendo investigadas com resultados promissores (Otte *et al.*, 2016).

Assim, a depressão é uma condição multifatorial com repercussões amplas, que exige abordagens terapêuticas integradas e personalizadas, baseadas em biomarcadores clínicos, genéticos e neurobiológicos.

## 1.4 SEPSE

Em termos conceituais, a sepse é uma condição grave que ameaça a vida, advinda de um foco infeccioso e de sua consequente resposta à injúria. Sua complexidade não abarca somente os órgãos e tecidos de origem afetados, mas desdobra-se em uma reação orgânica intrincada, com alterações fisiopatológicas, celulares e bioquímicas de amplo escopo (Singer *et al.*, 2016).

Esta síndrome é um problema clínico de grande relevância nas unidades de terapia intensiva (UTI) devido a sua alta taxa de incidência e morbimortalidade (Borges *et al.*, 2015; Lobo *et al.*, 2019). No Brasil, de acordo com o banco de dados do Instituto Latino Americano de Sepsis, os 84 centros de saúde credenciados (dentre hospitais públicos e privados) registraram 15.571 casos de sepse e choque séptico, com letalidade de 20,4% na sepse e 56,7% no choque séptico (INSTITUTO LATINO AMERICANO DE SEPSE (ILAS), 2019).

Os indivíduos sépticos apresentam os piores desfechos clínicos, em particular, nos domínios físico e cognitivo, quando comparados a outros doentes hospitalizados (Comim *et al.*, 2009; Hofhuis *et al.*, 2008; Iwashyna *et al.*, 2010). Pacientes sobreviventes à sepse podem apresentar limitações cognitivas e funcionais à longo prazo, como distúrbios de aprendizado e memória, transtornos de atenção e humor, disfunções como fadiga, dispneia, fraqueza muscular, diminuição da capacidade física e da qualidade de vida, com importantes repercussões interativas no convívio familiar e social (Borges *et al.*, 2015; Perl *et al.*, 1995; Singer *et al.*, 2016).

Fisiopatologicamente, os principais componentes bacterianos responsáveis por desencadear o processo inflamatório na sepse são o lipopolissacarídeos (LPS), peptidoglicanos, ácido lipoproteico e flagelina, coletivamente denominados Padrões Moleculares Associados a Patógenos (PAMPs, do inglês Pathogen-Associated Molecular Patterns) (Hotchkiss; Monneret; Payen, 2013). Como consequência do reconhecimento dos PAMPs, há a ativação e recrutamento dos leucócitos aos tecidos afetados diretamente pela infecção, bem como para outros órgãos. A ativação dos receptores celulares, por sua vez, induz a produção de citocinas pró-inflamatórias, como o TNF- $\alpha$ , IL-1 $\beta$  e IL-6, e desta forma, inicia-se a ativação da imunidade inata do hospedeiro (Chatre *et al.*, 2017; Rolli *et al.*, 2010). Resumidamente, as citocinas iniciam a ativação do sistema imune inato, com aumento da produção de ERO, que,

por sua vez, também influenciam o equilíbrio dos processos pró e anti-inflamatório (Chatre *et al.*, 2017). Deste modo, a regulação pró e anti-inflamatória é complexa, e a resultante deste equilíbrio delicado delineará a resolução ou não da inflamação (Hotchkiss; Monneret; Payen, 2013).

Os modelos experimentais de sepse são fundamentais para investigar tanto os mecanismos fisiológicos de defesa do hospedeiro nas fases iniciais da infecção, quanto os processos de desregulação imunológica durante a progressão da sepse, além de possibilitar a avaliação da eficácia e segurança de agentes terapêuticos (Van Der Poll, 2012). Entre os modelos mais utilizados está a endotoxemia induzida por LPS, administrado por via intraperitoneal ou intravenosa, que provoca uma ativação sistêmica rápida e intensa do sistema imune inato, com elevação abrupta de citocinas como TNF- $\alpha$  e IL-6, além de alterações hemodinâmicas típicas da sepse. Apesar de ser um modelo reprodutível e de fácil controle, não reproduz com fidelidade a complexidade e a cinética da resposta inflamatória observada na sepse humana (Fink, 2014; Van Der Poll, 2012). Por sua vez, o modelo de Ligação e Punção Cecal (CLP) é considerado mais representativo da sepse clínica, pois induz peritonite polimicrobiana com progressiva disseminação bacteriana, mimetizando o quadro clínico de sepse abdominal; no entanto, apresenta desafios relacionados à padronização e reprodutibilidade (Dejager *et al.*, 2011). Em casos de sepse pulmonar, a inoculação intratraqueal de bactérias é capaz de reproduzir as manifestações pulmonares e sistêmicas observadas em pacientes, reduzindo limitações translacionais frequentemente presentes em outros modelos (Fink, 2014; Supinski; Callahan, 2007).

## **2 JUSTIFICATIVA**

A crescente prevalência de doenças crônicas associadas ao envelhecimento, como obesidade, diabetes tipo 2, doenças neurodegenerativas e síndromes inflamatórias, representa um desafio global significativo à saúde pública. Tais condições compartilham mecanismos fisiopatológicos comuns, incluindo disfunção mitocondrial, inflamação crônica e alterações metabólicas sustentadas. O uso de compostos bioativos naturais como os flavonoides têm ganhado destaque por seu potencial de atingir múltiplos alvos, atuando simultaneamente em vias antioxidantes,

anti-inflamatórias e metabólicas. Dentre eles, a fisetina tem se mostrado promissora, mas sua baixa biodisponibilidade limita sua aplicação clínica.

Nesse contexto, o CMS121, um derivado sintético da fisetina, foi desenvolvido para superar essas limitações farmacocinéticas, apresentando melhor estabilidade, penetração no sistema nervoso central e eficácia terapêutica. Estudos pré-clínicos demonstram que o CMS121 exerce efeitos benéficos sobre o metabolismo energético, reduz inflamação hepática e melhora marcadores mitocondriais, especialmente em modelos de envelhecimento

Dada a complexidade das doenças abordadas e a ausência de terapias eficazes com baixa toxicidade para abordá-las simultaneamente, a avaliação sistemática dos efeitos do CMS121 em diferentes modelos experimentais oferece uma oportunidade valiosa para compreender suas múltiplas ações e potencial terapêutico. Esse conhecimento pode contribuir para o desenvolvimento de novas estratégias farmacológicas direcionadas a doenças crônicas associadas ao envelhecimento e à disfunção metabólica.

### **3 OBJETIVOS**

#### **3.1 OBJETIVO GERAL**

Investigar os efeitos farmacológicos do composto CMS121 em camundongos em modelos de inflamação, de disfunção metabólicas e em testes preditivos de ação antidepressiva.

#### **3.2 OBJETIVOS ESPECÍFICOS**

Os objetivos específicos serão apresentados em cada um dos capítulos no desenvolvimento deste trabalho.

## 4 DESENVOLVIMENTO

Este trabalho será dividido em três capítulos, com metodologia, resultados e discussões escritos em língua inglesa, e ao final uma discussão integrada desses capítulos em língua portuguesa. O primeiro capítulo aborda resultados já publicados na revista *Aging*, no ano de 2024, titulado “CMS121: a novel approach to mitigate aging-related obesity and metabolic dysfunction”. O segundo capítulo abordará os efeitos do CMS121 em modelo de obesidade e disfunção metabólica induzida por dieta hiperlipídica contendo frutose. O terceiro capítulo abordará os efeitos da administração do CMS121 em testes preditivos de ação antidepressiva e em modelos de inflamação.

### 4.1 CAPÍTULO 1

#### 4.1.1 Objetivos específicos

- Avaliar a ação de CMS121 sobre a composição corporal (massa magra e gorda), consumo energético e atividade metabólica em camundongos C57BL/6 submetidos a dieta crônica.
- Caracterizar os efeitos do CMS121 sobre marcadores moleculares relacionados à inflamação hepática, metabolismo de glicose e lipídios, e biogênese mitocondrial em tecidos periféricos (fígado e tecido adiposo).
- Realizar análise metabolômica plasmática para identificar alterações em metabólitos relacionados ao metabolismo de lipídios, corpos cetônicos, aminoácidos e nucleotídeos induzidas pelo tratamento com CMS121.

#### 4.1.2 Material and Methods

##### 4.1.2.1 *Animals and treatment*

At 5 weeks of age, male C57BL/6 male mice were started on a standard rodent diet (LabDiet 5015, TestDiet, Richmond, IN, USA) with or without CMS121 for 6 months. Mice (12 per group) were assigned randomly to the CMS121 diet or the control diet and housed with 3 mice per cage. Body weight and food consumption were evaluated weekly. Based on the cage average food consumption, the CMS121

ingestion was 9.4 mg/kg/day during the first 17 weeks of treatment (200 ppm) and 18.8 mg/kg/day CMS121 for weeks 18–24 (400 ppm). CMS121 at the indicated doses was previously shown to be effective in mouse models of ageing and AD (Ates *et al.*, 2020a; Currais *et al.*, 2019) and diabetes (Zahid *et al.*, 2023). Behavioral testing and data collection were performed with the researcher blinded to the treatment. Body mass and metabolic status were evaluated at the 13th and 15th weeks of treatment, respectively. After 6 months of treatment, animals were sacrificed, and blood, liver, and subcutaneous adipose tissue were collected for further analyses.

#### 4.1.2.2 *Metabolic evaluation*

Echo-MRI analysis was performed at the 13th treatment week to evaluate body mass using an EchoMRI 100 apparatus (Echo Medical Systems, Houston, TX, USA). At the 15th week of treatment animals were placed for 5 days in a metabolic cage system apparatus (LabMaster, TSE-Systems Inc., Chesterfield, MO, USA) equipped to detect indirect calorimetry, measure food and water intake, and monitor activity. Metabolic parameters, such as oxygen consumption (VO<sub>2</sub>), carbon dioxide production (VCO<sub>2</sub>), respiratory exchange rate (RER), and energy expenditure were obtained and data analyzed with CalR (Mina *et al.*, 2018).

#### 4.1.2.3 *Glucose and lipid status*

The enzymatic Infinity Glucose Hexokinase Liquid Stable Reagent (Thermo Scientific, Middletown, VA, USA) or Accu-Check Aviva test strips (Roche, Indianapolis, IN, USA) were used to assay blood and tail blood glucose, respectively. Hemoglobin A1c (HbA1c) was measured by an enzymatic assay, and insulin by ELISA, both from CrystalChem (Elk Grove Village, IL, USA). Triglycerides (TG), and plasma cholesterol were assayed by commercial kits (Pointe Scientific, Canton, MI, USA). Liver cholesterol was assayed with a fluorometric assay (Cell Biolabs, San Diego, CA, USA). Free fatty acids (FFA) were evaluated by a fluorescent method (BioAssays Systems, Hayward, CA, USA).

Mice were deprived of food overnight to perform the glucose tolerance test (GTT) on the next day. A glucose solution was given by gavage (0.5 g/kg), as previously described (Benedé-Ubieto *et al.*, 2020). Blood glucose was sampled at 0,

30, 60, 90 and 120 min by tail vein puncture and evaluated by Accu-Check Aviva test strips (Roche, Indianapolis, IN, USA).

At the end of the experiment, mice were anesthetized, and their blood collected by cardiac puncture. Red blood cells and plasma were separated by using Microvette CB 300 K2E blood separation tubes (Sarstead, Nübrecht, Germany). After perfusion with phosphate buffered saline, adipose tissue and liver were removed and stored at  $-80^{\circ}\text{C}$  until further use.

#### 4.1.2.4 *Western blotting and slot blot*

Tissue samples were mechanically homogenized with a TissueRuptor (Qiagen, Hilden, Germany) at a 1:5 w/vol in mitochondria buffer (Hepes 5 mM, EGTA 1 mM, mannitol 220 mM, sucrose 70 mM) with added Roche (Indianapolis, IN, USA) protease and phosphatase inhibitors cocktail. After centrifugation (800 g, 10 min,  $4^{\circ}\text{C}$ ), the supernatant was centrifuged at 10000 g for 10 min at  $4^{\circ}\text{C}$ , and the supernatant was considered as the cytosolic fraction, while the pellet was the mitochondrial fraction. Protein was assayed by the BCA method (Invitrogen, Waltham, MA, USA).

Samples were prepared in sample buffer (66 mM Tris/HCl pH 6.8, 10% glycerol, 2% SDS, 2% 2-mercaptoethanol, 0.1% bromophenol blue) and analyzed by SDS-PAGE using 4–12% gradient gels (Criterion XT Precast Bis-Tris Gels, Bio-Rad, Hercules, CA, USA). Proteins were transferred to polyvinylidene fluoride membranes and probed with the desired primary antibody. Stain-free gels were used to quantify proteins (Bio-Rad). Horseradish peroxidase-conjugated secondary antibodies were diluted 1/5000 in 2% skim milk in Tris buffered saline/0.1% Tween 20 (TTBS) prior to use. Images were obtained by the Chemidoc MP Imaging System (Bio-Rad) and quantified with the ImageJ software (<https://imagej.nih.gov/ij/>).

We have previously validated a few antibodies for slot blot analysis and therefore, for these antibodies, this technique was used instead of Western blots. A nitrocellulose membrane was pre-wet in TTBS (150 mM NaCl, 25 mM Tris/HCl pH 7.4, 0.5% Tween 20) and assembled along with the 2 filter papers (#1620161, Bio-Rad, Hercules, CA, USA) in the Bio Dot SF apparatus. To wash the membrane, 500  $\mu\text{L}$  of TTBS was passed through the membrane by suction and kept under negative pressure for 10 min to completely remove the TTBS. Sample (20  $\mu\text{L}$ ) was applied directly to the

membrane using a multichannel pipette and kept for 15 min in a fume hood to allow the samples to pass through the membrane. Membranes were washed with deionized water for 5 min until the bromophenol blue was completely removed, and soaked in a fixative solution (50% methanol, 6% acetic acid, and 44% water), and kept under agitation for 5 min on a rocking shaker. After a 5 min wash in TTBS, samples were incubated with the primary antibody. The remaining procedures were identical to those used in Western blotting. Ponceau S staining was used as the loading control.

The following antibodies were used: caspase 1 (#4199), caspase 3 (#9661), TOM20 (#42406), VDAC (#4866), ACC1 phosphorylated at serine 79 (#3661), GLUT4 (#2213), FASN (#3189), LDH-A phosphorylated at tyrosine 10 (#8176), PFKFB3 (#13123), TXNIP (#14715), MAL-K (#14942), FH (#4567) were from Cell Signaling (Danvers, MA, USA); Nrf1 (#175932), TFAM (#131607), mitochondrial electron transport chain (ETC) markers (#110413) including complex I (CI, NDUFB8), CII (SDHB), CIII (UQCRC2), CV (ATP5A) were obtained from Abcam (Waltham, MA, USA); NOX4 (#C313066) was obtained from Life Span Biosciences (Mandideep, India); phosphoenolpyruvate carboxylase 1 PEPCK1/PCK1 (#10004943) was obtained from Cayman (Shirley, MA, USA); MLYCD (#pro15265-1-ap) was obtained from Proteintech (Rosemont, IL, USA).

#### 4.1.2.5 *Metabolomic analyses of plasma samples*

Metabolomic analysis was performed at Metabolon with plasma samples from an independent experiment, as previously published (Ates *et al.*, 2020). Raw data are available in Supplementary Table 1. At 9 months of age, male controls (C57BL/6J) were fed a diet (Harlan Teklad; Envigo, Indianapolis, IN, USA) with or without 400 ppm CMS121, leading to an average consumption of 34 mg/kg/day CMS121, based on the measurement of the overall food consumption of the mice housed together in each cage. Treatment lasted for 3 months, and mice were assigned randomly to treatment or control groups and data analysis was performed by different blinded researchers. The sample preparation and initial analysis were done by Metabolon, as previously published (Ates *et al.*, 2020). Datasets with 3 or more missing values were excluded from the analysis, as well as outliers. The outliers were identified using the interquartile range (IQR) of data, which is the range between the first (Q1) and the third (Q3)

quartiles ( $IQR = Q3 - Q1$ ). The data points which fall below  $Q1 - 1.5 \times IQR$  or above  $Q3 + 1.5 \times IQR$  are defined as outliers. After filtering out 10 metabolites, from a total of 714 metabolites, 107 presented differences between control and CMS121-treated mice. Differential test was performed by the Wilcoxon rank-sum test and considered significant when  $p < 0.05$ .

#### 4.1.2.6 Statistical analyses

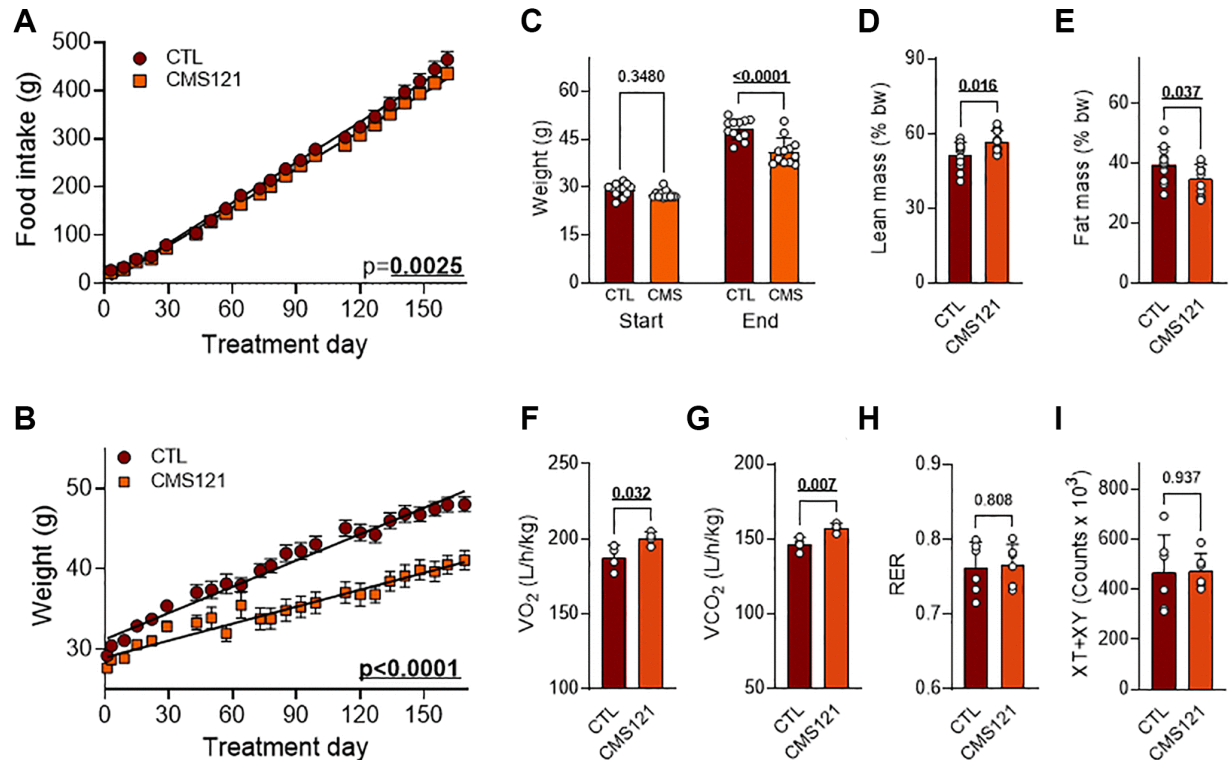
Weight and food intake data were adjusted to a linear regression and slopes of linear regressions were compared. Pairwise samples were compared by the student's t test and significant differences were set at  $p < 0.05$ . For the GTT, two-way ANOVA was used followed by the Bonferroni post hoc test for multiple comparison analysis. Unless stated, data are presented as average  $\pm$  SD, and sample number is indicated in the figure legends.

#### 4.1.3 Results

In the course of the study that examined the effects of the CMS121 diet on leptin insensitive db/db mice (Zahid *et al.*, 2023), we noticed that CMS121 presented a clear effect in WT mice regarding glucose and lipid status as well. Therefore, we performed additional analyses to characterize the metabolic changes induced by CMS121 in WT C57/Bl6 mice.

To uncover any possible differences in food intake, linear regressions were compared and found to be different (Figure 1A), indicating a slightly lower food intake in the CMS121-treated mice. Linear regression comparison also indicated that control mice presented a consistently higher weight gain (Figure 1B). Control mice started the experiment weighing  $29.2 \pm 2.0$  g (average  $\pm$  SEM) at the age of 5 weeks and reached  $48.0 \pm 3.2$  g by the 6th experimental month, with an average weight gain of  $18.8 \pm 2.6$  g (Figure 1C). The starting weight increased from  $27.6 \pm 1.3$  g to  $41.1 \pm 4.2$  g in mice receiving the CMS121 diet (Figure 1C). The lower weight gain of  $13.4 \pm 2.9$  g was statistically significant as compared to the control mice (CTL). In addition, the mice receiving the CMS121 diet presented significantly greater lean mass (Figure 1D), and less fat mass (Figure 1E). The metabolic activity of the mice was also increased by the CMS121 diet with higher oxygen consumption (Figure 1F), and carbon dioxide

production (Figure 1G), without an appreciable effect on the RER (Figure 1H) and without affecting the overall locomotor activity (Figure 1I).

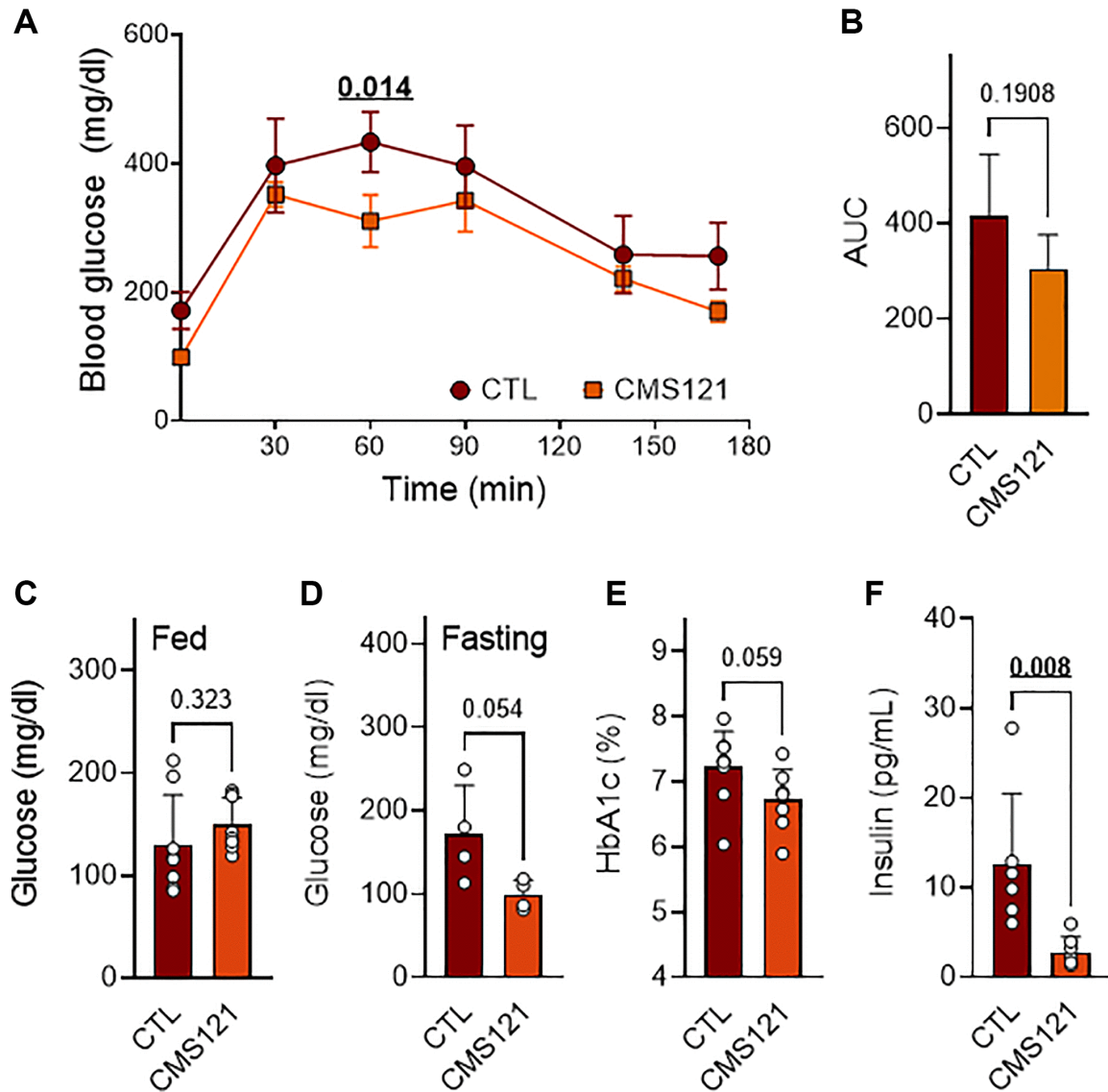


Capítulo 1 - Figure 1 – Nutritional and body parameters

Nutritional, body mass, locomotor activity, and metabolic activity of mice fed a control diet or a diet containing the geroneuroprotective drug candidate CMS121 for 6 months. Beginning at the 5th week after birth, mice were either kept on the control diet (CTL) or put on a diet containing CMS121 ad libitum. Cumulative food intake (A) and body weight (B). Food intake per animal was based on the average food consumption in cages holding 3 mice allocated among a total of 4 cages, and the body weight of 12 mice was followed. Differences between linear regression slopes of CTL and CMS121-treated mice were analyzed and the p-values are shown. Initial and final body weights are presented (C). During the 13th week of treatment body mass (n = 12) indexes were obtained: lean mass (D), and fat mass (E). Metabolic activity (F–H) was evaluated at the 15th week of treatment for oxygen consumption ( $VO_2$ ) (F); carbon dioxide production ( $VCO_2$ ) (G); respiratory exchange ratio (RER) (H), and ambulatory activity (I); Data are presented as mean ± SD (n = 4). Bold underlined p-values indicate statistical differences.

Glucose presented a tendency to lower levels in the glucose tolerance test (GTT, Figure 2A, 2B), and when evaluated in the blood after fasting (Figure 2D), which was not seen when glucose was assessed in fed animals (Figure 2C). In agreement with a lower glycemic status, glycated hemoglobin also presented a tendency to

decreased levels (Figure 2E), while insulin levels were markedly decreased (Figure 2F) in the mice fed with the CMS121 diet.

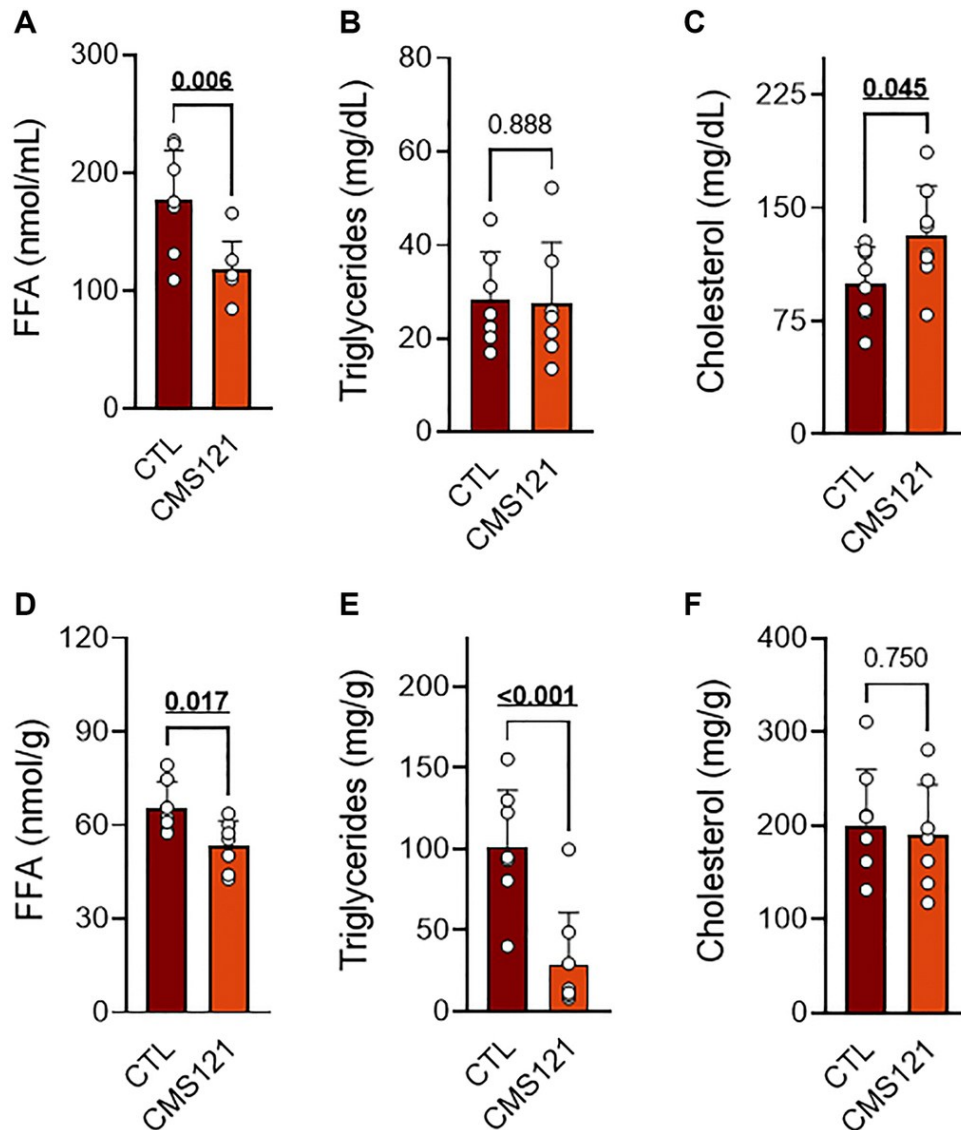


Capítulo 1 - Figure 2 - Glucose status

Glucose status in mice treated with CMS121 for 6 months. The glucose tolerance test (GTT,  $n = 4-5$ ) was performed at the 6th month of treatment (**A**). The area under the curve (AUC) is presented in (**B**). Glucose was evaluated by caudal vein puncture after the 5th month of treatment in fed mice (**C**) ( $n = 8$ ). At the end of the study, blood was collected for the measurement of fasting glucose (**D**) ( $n = 4$ ), glycated hemoglobin (**E**) (HbA1c;  $n = 8-9$ ), and insulin levels (**F**) ( $n = 6-7$ ). Data are presented as mean  $\pm$  SD, except for GTT (mean  $\pm$  SEM). Bold underlined  $p$ -values indicate statistical differences.

The CMS121 diet also altered the lipid status in blood and liver as compared to the control mice (Figure 3). A significant decrease in free fatty acids (FFA, Figure

3A) was seen in blood, while TG (Figure 3B) remained at control levels, and the cholesterol levels were increased (Figure 3C). A significant decrease in FFA (Figure 3D) and TG (Figure 3E) was also observed in the liver, while cholesterol (Figure 3F) remained unaltered.

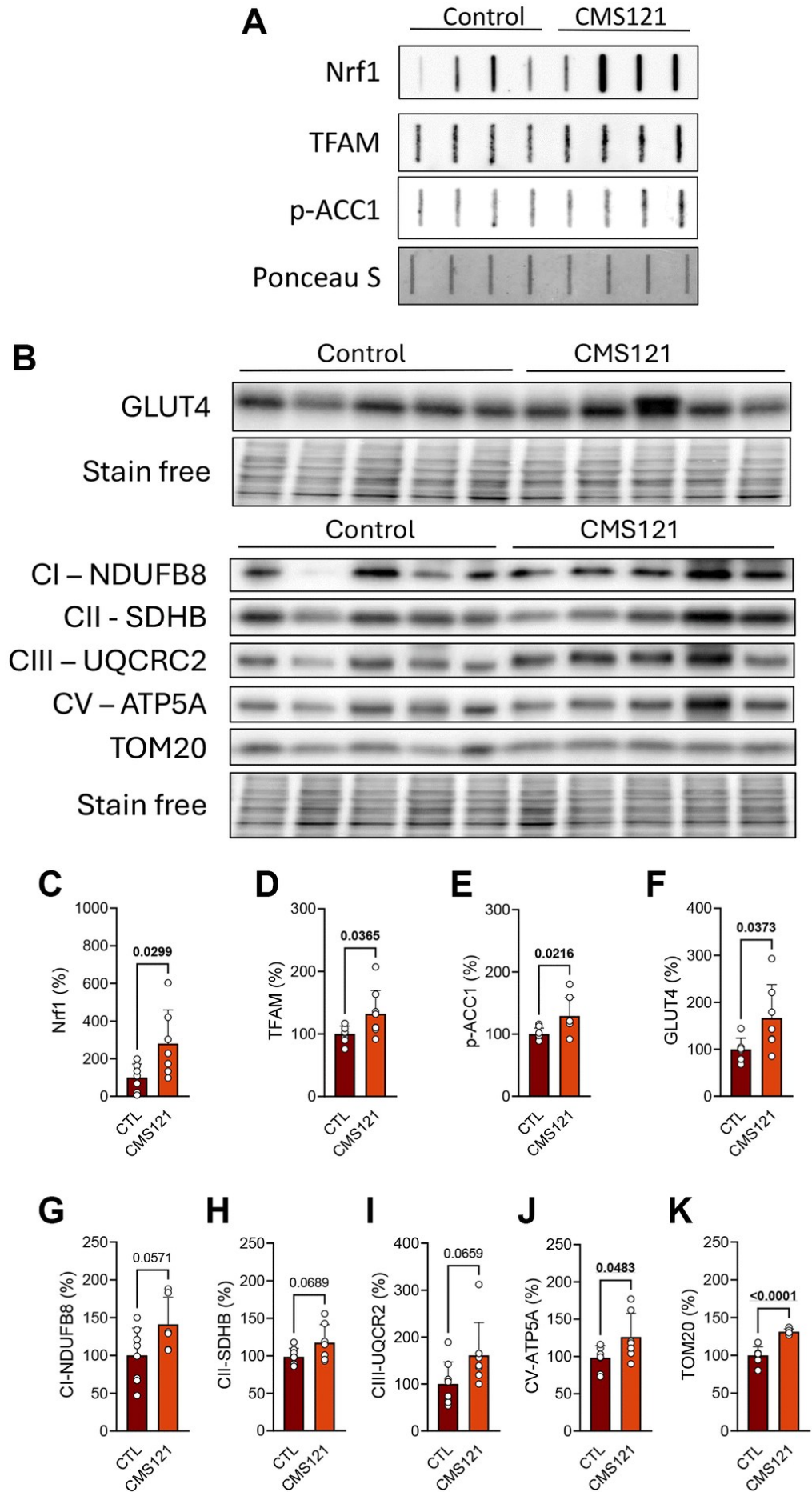


Capítulo 1 - Figure 3 - Blood and liver lipids

Blood and liver lipids of mice fed control diet or a diet containing CMS121 for 6 months. Plasma (A–C) and liver (D–F) were evaluated for free fatty acids (FFA (A, D)), triglycerides (B, E), and cholesterol (C, F) at the end of the experiment. Data are presented as mean ± SD ( $n = 7-8$ ). Bold underlined  $p$ -values indicate statistical differences.

The clear metabolic changes, such as increased lean mass and metabolic activity, allied to lower glucose and lipid status, prompted us to investigate possible mechanisms underlying such alterations. We first investigated the status of

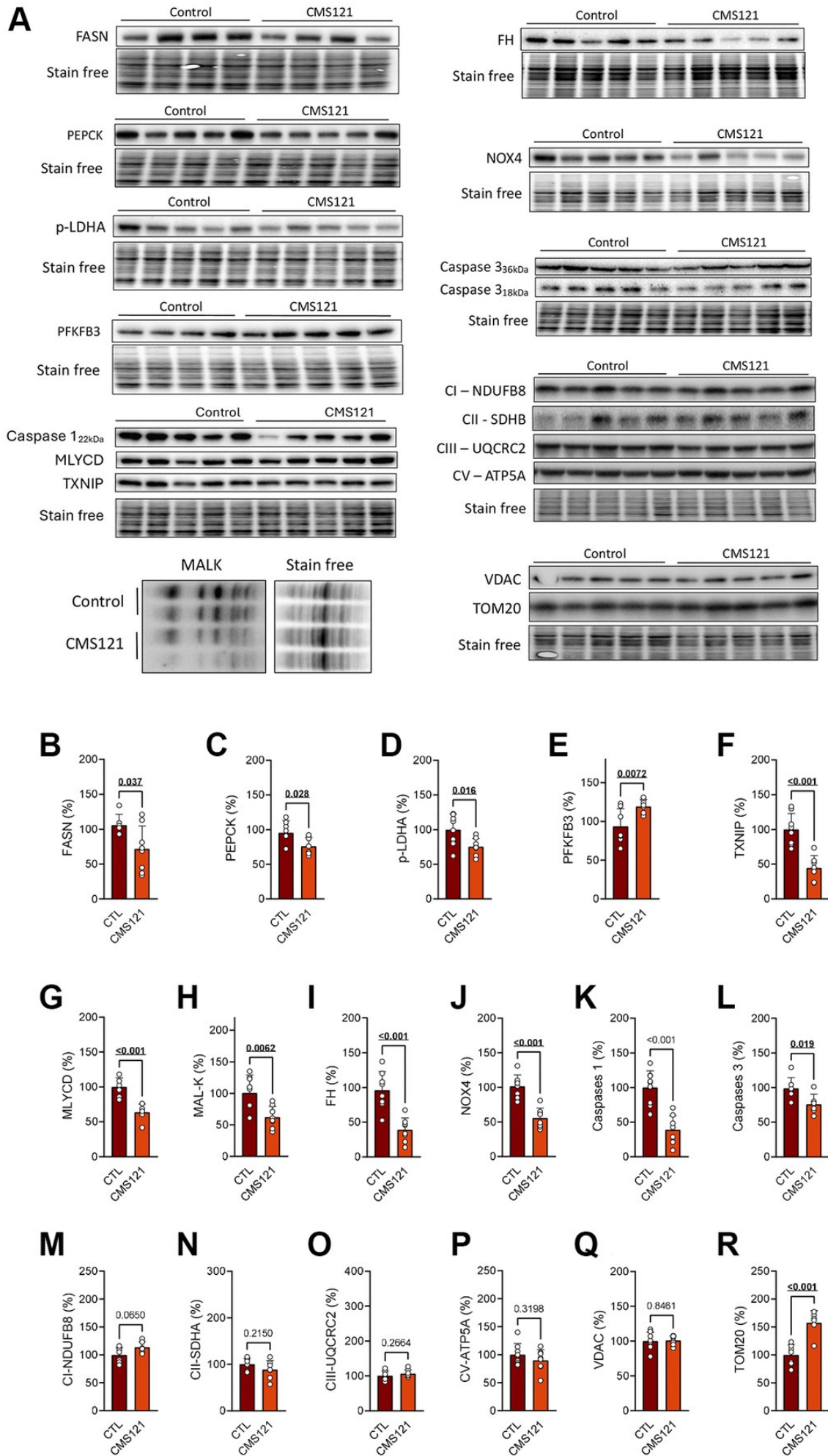
mitochondrial proteins, transcription factors associated with mitochondria biogenesis, and markers of lipid and glucose metabolism in the adipose tissue (Figure 4), where representative blot images are presented in panels A and B, and their respective quantifications in C-K. Interestingly, two transcription factors associated with mitochondrial biogenesis were increased by the CMS121 diet, namely nuclear respiratory factor 1 (Nrf1, Figure 4A, 4C) and transcription factor A, mitochondrial (TFAM, Figure 4A, 4D), a response that is in line with an increase in mitochondrial activity, based on the tendency towards increased levels of respiratory complexes I, II, III and V (Figure 4B, 4G–4J). The translocase of outer mitochondrial membrane 20 (TOM20) was also increased by the diet (Figure 4A, 4K). The known inhibitory effect of CMS121 on lipid synthesis [14] was also observed in the adipose tissue, as phosphorylation of ACC1 was increased (Figure 4A, 4E). The levels of the insulin responsive glucose transporter solute carrier family 2 (facilitated glucose transporter), member 4 (GLUT4, Figure 4B, 4F) were also increased by the CM121 diet.



#### Capitulo 1 - Figure 4 - Adipose tissue metabolic markers

Adipose tissue metabolic markers were evaluated in mice fed with CMS121 for 6 months. Representative slot blot (A) or Western blot (B) images of mitochondrial fractions from adipose tissue are presented. Quantitative results (C–K) of proteins related to mitochondrial metabolism, such as transcription factors Nrf1 (C) and TFAM (D), markers of lipid (p-ACC1, (E)) and glucose (GLUT4, (F)) metabolism, and markers of mitochondrial complex I ((G); NDUFB8), complex II ((H); SDHB), complex III ((I); UQCRC2), and complex V ((J); ATP5A) are presented, as well as the levels of the outer membrane translocase TOM20 (K). Data are presented as mean + SD ( $n = 6-8$ ). Bold underlined values of  $p$  indicate statistical differences.

We also examined protein markers of metabolic changes in the liver as shown in Figure 5A, where present representative blot images, and their respective quantification in graphs B-R. The same inhibitory effect on lipid synthesis that was observed in the adipose tissue was also observed in the liver, as indicated by lower levels of FASN (Figure 5B). In addition, the gluconeogenesis marker phosphoenopyruvate carboxylase 1 (PEPCK) was decreased (Figure 5C), indicating decreased gluconeogenesis, while the lower phosphorylation of lactate dehydrogenase A (LDH-A) indicates a decrease in liver lactate production (Figure 5D). An increase in 6-phosphofructo-2-kinase/fructose 2,6-biphosphatase 3 (PFKFB3, Figure 5E) indicates an increase in glycolytic flux, while the decreased thioredoxin interacting protein (TXNIP) levels (Figure 5F) would suggest lower removal of glucose transporters from the plasma membrane, thus favoring glucose uptake. Although the enzyme malonyl-CoA decarboxylase (MLYCD) was decreased by CMS121 (Figure 5G), the concomitant decrease in protein malonylation (Figure 5H) suggests a decrease in malonyl CoA synthesis. The tricarboxylic acid cycle (TCA) enzyme fumarate hydratase (FH) levels were also decreased by CMS121 (Figure 5I).



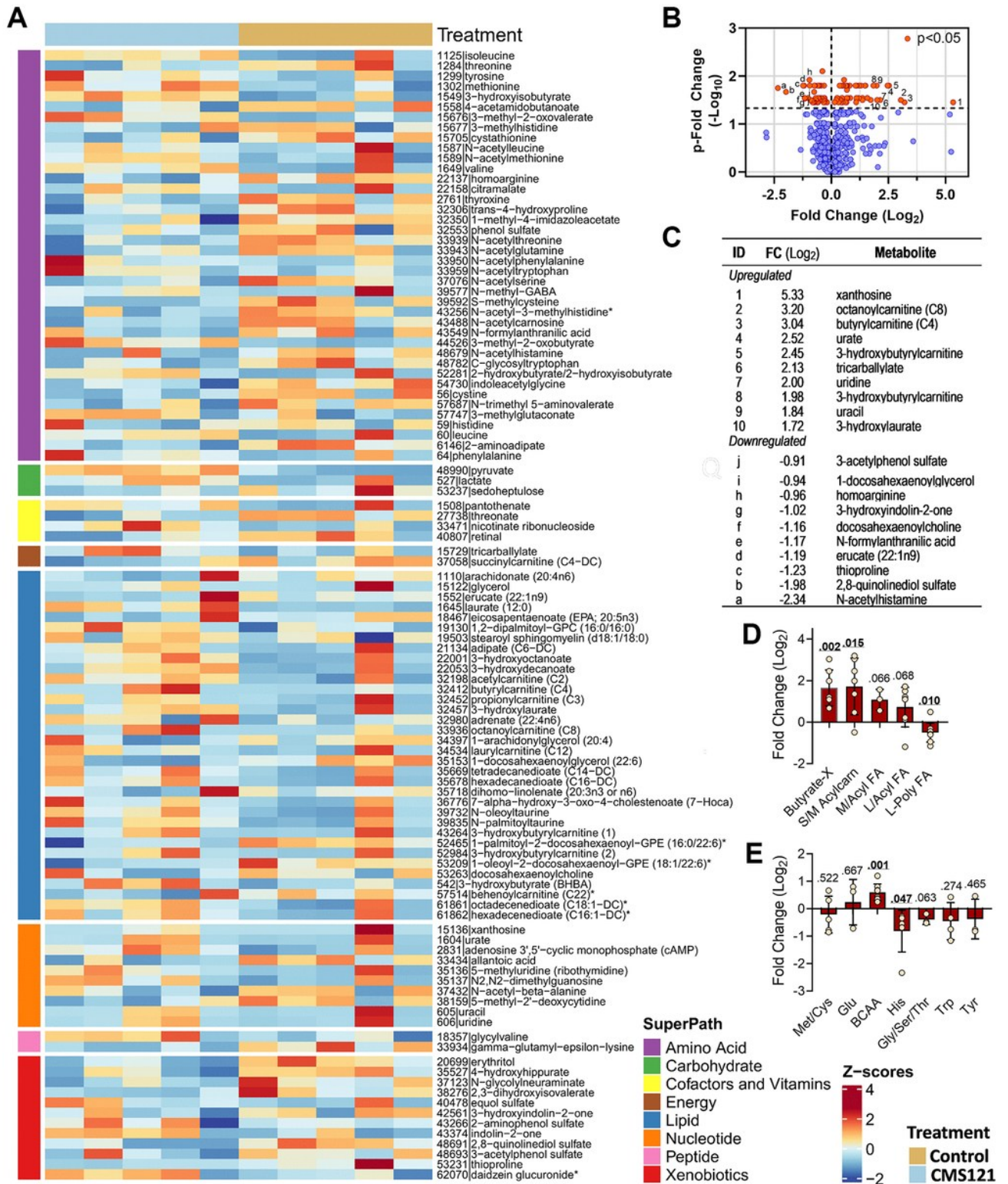
### Capitulo 1 - Figure 5 - Liver protein markers

Liver protein markers were evaluated in mice fed with CMS121 for 6 months. Representative blot images (A), and the corresponding quantification (B–R) of metabolism markers in the hepatic tissue: FASN (B), PEPCK (C), p-LDH-A (D), PFKFB3 (E), TXNIP (F), MLYCD (G), malonylation of proteins at lysine residues (MAL-K, (H)), FH (I), Nox4 (J), caspase 1 (K), caspase 3 (L), and markers of mitochondrial complex I ((M); NDUFB8), complex II ((N); SDHB), complex III ((O); UQCRC2), and complex V ((P); ATP5A), as well as the voltage dependent anion channel VDAC (Q), and the outer membrane translocase TOM20 (R). Liver cytosolic fractions were used for blotting, except for the mitochondrial markers (M–R), FH (I), and NOX4 (J) that used mitochondrial fractions. Data are presented as mean  $\pm$  SD ( $N = 6-8$ ). Bold underlined  $p$ -values indicate statistical differences.

It was previously noted that CMS121 produced a decreased inflammatory status in the liver of db/db mice [17], and this was also observed in the WT mice receiving CMS121 in the diet, as mitochondrial NADPH oxidase 4 (NOX4) (Figure 5J), caspase 1 (Figure 5K), and caspase 3 (Figure 5L) presented values below those of the control diet group.

None of the mitochondrial respiratory complexes (I, II, III and V) or the voltage-dependent channel were altered in the liver tissue (Figure 5M–5Q), contrasting with the clear increase in the outer mitochondrial translocase TOM20 (Figure 5R), that was seen with the CMS121 diet.

To gain further insight into the metabolic effects of CMS121, we performed a non-targeted metabolomic study of the mouse plasma. A total of 704 metabolites were identified and 107 (15.2%) of these were altered by the CMS121 diet (Figure 6). Forty-one differentially expressed amino acids represent 38.3% of the total changes. Amino acids, along with 33 lipids representing 30.8% of the total changes, 11 nucleotides representing 10.3% of the total changes and 12 xenobiotics representing 11.2% of the total changes were the most abundant groups presenting differentially expressed metabolites within the super pathways (Figure 6A).



Capítulo 1 - Figure 6 - Plasma metabolites

Differentially expressed metabolites are presented as (A) heat map; (B) volcano plot; (C) table presenting the top ten upregulated or downregulated metabolites, and metabolites related to lipids (D) or amino acids (E). Only sub-pathways with 3 or more differentially expressed metabolites are presented in (D and E), and represent the average  $\pm$  SD. Abbreviations are: BCAA – branched chain amino acids;

Butyrate-X – indicates 4 carbon metabolites related to  $\beta$ -hydroxybutyrate; S/M Acylcarn – small or medium acylcarnitine metabolites; L/Acyl FA – long saturated/monounsaturated free or acyl fatty acids; L-Poly FA – long polyunsaturated free or acyl-fatty acids. \*Indicates compounds that have not been confirmed based on a standard, but mass spectral data were appropriate to reveal their identity.

The volcano plot of the results (Figure 6B) shows the metabolites that were significantly influenced by the CMS121 diet (red circles). A similar number of metabolites were found to be increased or decreased by CMS121. When the top 10 increased metabolites (Figure 6C) were analyzed, it was found that acylcarnitine (4) and nucleotide (4) metabolites were the major groups. The top 10 decreased metabolites (Figure 6C) include 4 xenobiotics (3-acetylphenol sulfate, 3-hydroxyindolin-2 one, 2,8-quinolinediol sulfate, thioproline), and 3 lipids (docosahexaenoylcholine, 1-docosahexaenoylglycerol, and erucate).

Although one of the most abundant groups of metabolites influenced by CMS121 are the amino acids, there is no specific pattern of changes when they are analyzed as a group (Figure 6E), except for an increase in the branched chain amino acids (BCAA), and a decrease in the levels of histidine metabolites. The subpathways Met/Cys/SAM/Tau, Glu, Gly/Ser/Thr, Trp and Tyr presented a limited number of metabolites that were altered by the CMS121 diet, precluding further inferences. Interestingly the increase in BCAAs is consistent with an increased mobilization of protein for the production of TCA intermediates and ketone bodies (Ruiz-Sala; Peña-Quintana, 2021), but further experimentation on this topic is required to confirm this idea.

The CMS121 diet also resulted in increased plasma levels of the degradation products of purine (xanthosine) and pyrimidine (uridine and uracil) nucleotides, as well as increased levels of uric acid (Figure 6C). Increased ingestion or catabolism of nucleotides, and/or decreased salvage pathway activity can lead to increased levels of uric acid. Another possible mechanism is increased plasma levels of lactate, which leads to increased renal reabsorption of filtered urate (Mandal; Mount, 2015). Nevertheless, purine metabolism is tightly regulated, as is uric acid production and excretion. Elevated concentrations of uric acid are frequently found in subjects consuming a ketogenic diet (Li *et al.*, 2022; Schwartz; Boyes; Aynsley-Green, 1989), although the mechanisms underlying this effect were not described. Therefore, any

increase in purine catabolism or decreased excretion would promote the accumulation of purine metabolites and uric acid. Without further experimentation, it is not possible to have a clear interpretation of these findings.

When the lipids were grouped, a clear pattern emerged (Figure 6D). Metabolites related to  $\beta$ -hydroxybutyrate (BHB) and acylcarnitines were increased, which is consistent with increased ketone metabolism. Monounsaturated or saturated fatty acids presented a tendency to increase as a group ( $p = \sim 0.067$ ), but without statistical significance, while the group of PUFA presented a significant decrease.

#### 4.1.4 Discussion

The CMS121 prototype molecule fisetin protected from high fat diet-induced nephropathy demonstrating clear metabolic effects such as preventing non-alcoholic fatty liver disease, decreasing hepatic fat, increasing muscle mass, and inducing adipose tissue browning (Chenxu *et al.*, 2021; Choi; Choi; Kwon, 2020; Gaballah; El-Horany; Helal, 2019). CMS121 presented related effects, such as a clear anti-diabetic effect in the db/db obese mouse (Zahid *et al.*, 2023), indicating its potential as a possible treatment for metabolic diseases. Better glycemic and lipid status, decreased liver inflammation, and renal protection were the main effects of CMS121 found in the db/db mice (Zahid *et al.*, 2023). Here, the potential metabolic effects of CMS121 were explored in WT mice (C57/BL6) treated for 6 months with CMS121 in the diet.

During normal ageing, locomotor activity declines with progressive body weight gain (Benfato *et al.*, 2017; Chiang *et al.*, 2023; Gonçalves *et al.*, 2017), indicating that physical activity is an important health factor (Izquierdo; Fiatarone Singh, 2023). In this regard, laboratory mice have lifelong food availability and generally a sedentary lifestyle. The WT mice in the C57/BL6 background have an average body weight of  $33.3 \pm 2.8$  g at 180 days of age (Jackson Laboratory, 2023). The same average body weight of C57/BL6 mice at 6 months of age, as reported by the Jackson Laboratory, is frequently observed in the literature (Sanger Mouse Genetics Project *et al.*, 2013; Seimon *et al.*, 2016; Song *et al.*, 2010; Zhang, Qian *et al.*, 2021). However, in our study, the final weight of the WT mice ( $48.0 \pm 3.2$  g) indicates that the mice became overweight over the experimental period. The reasons for this weight gain in the WT C57/BL6 mice above what is reported by the Jackson

Laboratory, are not clear and warrant further experimentation. Nevertheless, it allowed us to investigate the effects of CMS121 on age-dependent weight gain. The final body weight of mice given the CMS121 diet ( $41.1 \pm 4.2$  g) approached the literature values but remained elevated above the expected values ( $\sim 30\text{--}35$  g), despite a 40% lower weight gain ( $13.4 \pm 2.9$  g) as compared to untreated controls ( $18.8 \pm 2.6$  g). We hypothesize that the lower weight gain likely contributed to the improved glucose and lipid status of the CMS121-treated mice. Besides being lighter, the mice given the CMS121 diet also had more muscle and less fat, as well as an increased metabolic rate under basal conditions.

A slight decrease in food consumption cannot explain the much lower weight gain in the mice receiving CMS121. Locomotor activity was not altered by the diet, as evaluated at the 15th treatment week in metabolic cages. However, the limited number of animals evaluated and the stressful condition of the metabolic cage precludes a definitive conclusion. In this regard, two weeks after the diet was introduced to the mice, locomotor activity was increased in the open field test (Supplementary Figure 1), keeping open the possibility that CMS121 can induce an increase in locomotor activity, which certainly would be relevant to the observed metabolic improvements. Unfortunately, we do not have a later evaluation of locomotor activity in the open field test. Despite only finding a statistical decrease in glucose levels at 60 min in the GTT, there is a clear tendency to lower glucose levels during fasting. This trend is reinforced by a tendency to lower levels of HbA1c and markedly decreased levels of insulin in the mice receiving CMS121. If the lipid profile is included, with lower FFA and TG in liver and lower FFA in blood, it comprises a picture indicating that CMS121 may bring about several metabolic improvements that were also seen in the db/db mice treated with the same CMS121 diet (Zahid *et al.*, 2023).

The increased GLUT4 levels in the adipose tissue may indicate an increase in insulin sensitivity which is corroborated by the low basal levels of insulin and may contribute towards improved glucose tolerance and lower plasma levels of glucose. Obesity leads to general mitochondrial dysfunction, including decreased mitochondrial mass and downregulated biological function and biogenesis in adipose tissue (Heinonen *et al.*, 2020). The observed increase in transcription factors associated with mitochondrial biogenesis (Nrf1 and TFAM) in adipose tissues, in response to the

CMS121 diet, is in line with the observed increase in mitochondrial markers, thus, possibly counteracting the limitation in adipocyte respiration in the heavier WT mice. Increased mitochondrial activity in adipose tissue may also contribute to the observed increase in oxygen consumption, as detected in the metabolic cage analysis, however locomotor activity as a factor cannot be excluded.

Decreased fatty acid synthesis was indicated by increased phosphorylation of ACC1 in adipose tissue, which leads to its inhibition (Chen, Leyuan *et al.*, 2019) and a decrease in lipogenesis. Plasma metabolomic data showed increased plasma levels of short chain acylcarnitines and several butyrate metabolites, which are typically found following consumption of a ketogenic diet (Hung *et al.*, 2020). Interestingly, BHB can be produced by non-canonical sources such as adipocytes (Wang, Wenshan *et al.*, 2019).

Fatty acid synthesis inhibition was previously associated with a decrease in free PUFA in primary neurons and in the brains of transgenic AD mice (Ates *et al.*, 2020), decreased FFA in the plasma of db/db obese mice (Zahid *et al.*, 2023), and in the cerebral cortex and plasma of the ageing accelerated SAMP8 mice (Currais *et al.*, 2019). Here, these findings are extended to the plasma of CMS121-treated WT mice, as lower FFA levels were seen in the plasma and liver, and lower PUFA in the plasma. PUFA peroxidation is considered an essential component of cell death dependent on oxytosis/ferroptosis (Ursini; Maiorino, 2020). It would be interesting to know how the peripheral PUFA interacts with the brain, and if the lower plasma levels of PUFA represent a possible mechanism of protection against oxytosis/ferroptosis in peripheral tissues, such as the liver and adipose tissue.

Increased lipid mobilization promotes fatty acid oxidation leading to higher acetyl-CoA levels in hepatocytes (Guertin; Wellen, 2023), which is the substrate for the formation of ketone bodies, such as acetoacetate and butyrate metabolites that were found increased in the plasma of mice on the CMS121 diet. A ketogenic diet is known to drastically decrease hepatic TG (Luukkonen *et al.*, 2020), which was also observed with the CMS121 diet. Reductions in liver fat and de novo lipogenesis were also observed in humans on a ketogenic diet (Mardinoglu *et al.*, 2018), in line with the decreased FASN levels found in the liver of CMS121-treated mice. Interestingly, a ketogenic diet can also increase the hydrolysis of hepatic lipids which was associated

with lower serum insulin levels, and increased glucose tolerance (Mooli; Ramakrishnan, 2022), in agreement with the lower plasma insulin levels and improvements in the glucose status found in mice on the CMS121 diet.

Ketogenic metabolism is well-known for promoting health, particularly in obese and elderly individuals (Mentzelou *et al.*, 2023; Xu *et al.*, 2023). Several studies also showed benefits for middle aged rodents suggesting that consumption of a ketogenic diet in middle age can reduce the ageing and neurodegenerative effects of an unhealthy diet. Moreover, there are a growing number of preclinical and clinical studies showing that a ketogenic diet is beneficial in AD by numerous mechanisms such as the anti-ageing effect of BHB, improved mitochondrial function, changes in the gut microbiota, reduced neuroinflammation, and decreased oxidative stress (Henderson *et al.*, 2009; Krikorian *et al.*, 2012; Wang, Lian; Chen; Xiao, 2021; Xu *et al.*, 2023). CMS121 seems to limit fatty acid synthesis by both an inhibitory effect on FASN and by activating AMPK, thus increasing ACC1 inhibition by phosphorylation (Ates *et al.*, 2020). Increased ACC1 phosphorylation was observed in adipose tissue and decreased levels of FASN were observed in the liver, both of which would lead to increased levels of acetyl-CoA (Guertin; Wellen, 2023) indicating CMS121 induces the same inhibitory effects on lipid synthesis in hepatic and adipose tissue as found in the brain (Ates *et al.*, 2020). Interestingly, CMS121 has been shown to be neuroprotective in ageing and Alzheimer's disease models, possibly by increasing in acetyl-CoA levels in the brain (Currais *et al.*, 2019).

The investigation into metabolic reprogramming by CMS121 in hepatic tissue also revealed that gluconeogenesis is probably diminished, as evidenced by lower levels of PEPCK, while the increased levels of PFKFB3 suggest increased glycolysis. Through the production of fructose 2,6-bisphosphate, PFKFB3 is a known activator of PFK1 triggering aerobic glucose oxidation (Duran *et al.*, 2009). Increased glycolysis could also be promoted by the decreased expression of TXNIP in the livers of the CMS121-treated mice. TXNIP is a known arrestin that promotes the recycling of glucose receptors, leading to decreased glucose uptake (Wu *et al.*, 2013). The lower phosphorylation of LDH-A decreases its activity (Hui *et al.*, 2017) and would also favor glucose-mediated respiration in the livers of CMS121-treated mice. Nevertheless, the CMS121 diet did not alter the expression of ETC markers which normally would be

expected in a scenario of decreased lactate production and increased glycolysis, showing that the effect of CMS121 on liver metabolism is complex and requires further investigation.

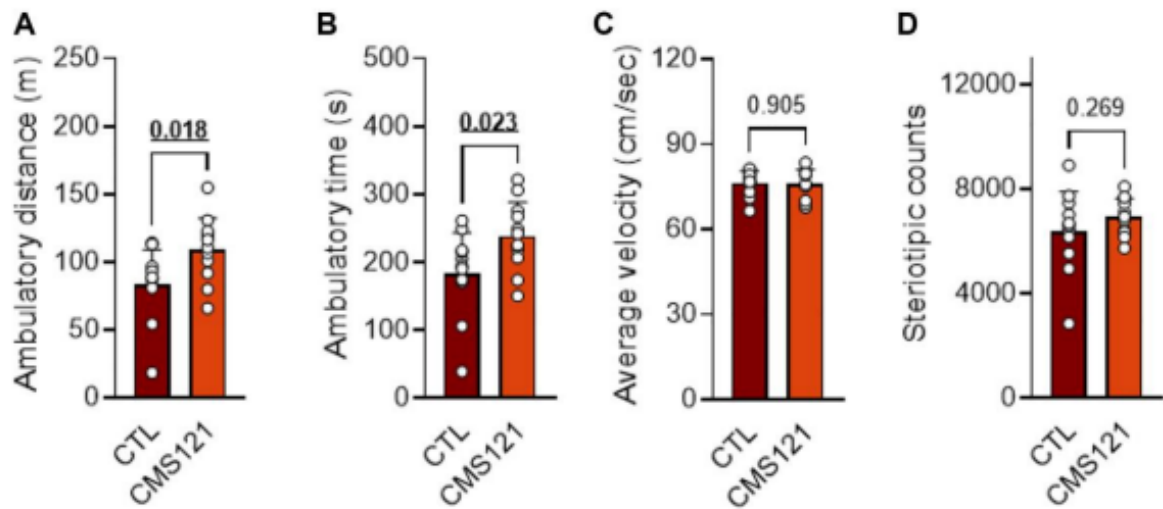
The observed increases in the plasma levels of pyruvate and lactate after consumption of the CMS121 diet are also compatible with the idea of increased lactate production and release to the circulation (Ferguson *et al.*, 2018). Given that hepatic LDH-A phosphorylation was decreased, suggesting an inhibition (Fan, Jun *et al.*, 2011), it is possible that the origin of the plasma lactate is extra-hepatic. In the traditional view, increased lactate levels are taken up by the liver and passed to the Cori cycle to increase glucose production and its release to the blood, which spares glucose to extrahepatic tissues, and thereby limits protein degradation (Soeters *et al.*, 2021). However, pyruvate and lactate can directly enter the TCA cycle and are readily oxidized by skeletal muscle during rest and contraction (Bertocci; Lujan, 1999), reinforcing the notion that circulating lactate can be a primary source of carbon for the TCA cycle and thus a valuable energy source (Brooks, 2018; Hui *et al.*, 2017). Furthermore, the overall picture indicates that mice receiving CMS121 are in a metabolic state resembling that produced by a ketogenic diet which is also consistent with preferential fatty acid oxidation usually found during anabolic metabolism [53]. The increased lean mass and decreased fat mass induced by the CMS121 diet is an interesting outcome that could be explored further in the context of the age-related decline of skeletal muscle in osteosarcopenic obesity patients (Baumgartner, 2000).

A deficiency in FH was reported to strongly protect against obesity, insulin resistance, and fatty liver (Yang, Hao *et al.*, 2016). Conversely, increased FH activity impairs the antioxidant response in obesity (Azzimato *et al.*, 2021). Itaconate is known to modulate the immune and antioxidant responses (Fan, Kerui *et al.*, 2022), and miR-144, by preventing itaconate synthesis, increases FH activity, potentially contributing to non-alcoholic fatty liver disease (Azzimato *et al.*, 2021; Weiss *et al.*, 2023; Yi *et al.*, 2020). Elevated levels of fumarate due to lower levels or activity of FH drives the reversal of mitochondrial complex II, promoting the conversion of fumarate to succinate (Fan, Kerui *et al.*, 2022; Weiss *et al.*, 2023), which was also observed during renal  $\beta$ -oxidation of long- and medium-chain fatty acids (Panov *et al.*, 2022). Succinate accumulation leads to increased itaconate levels, which can promote fatty acid

oxidation in hepatocytes (Weiss *et al.*, 2023). The CMS121 diet induced a decrease in the hepatic levels of mitochondrial FH, indicating fumarate may be elevated, and potentially contribute to a shift towards ketogenic metabolism that is basically fueled by fatty acids in the liver (Mentzelou *et al.*, 2023). Therefore, a decrease in FH levels and an increase in  $\beta$ -oxidation can eventually lead to increased itaconate synthesis, a plausible mechanism that could contribute to the promotion of metabolic remodeling by CMS121. However, additional experiments are needed to clarify this possibility.

Despite limited succinyl-CoA-3-oxoacid CoA transferase activity in hepatocytes and the observation that certain ketone supplements can limit the availability of mevalonate for cholesterol synthesis (Kemper *et al.*, 2015), increased cholesterol is frequently found in studies using a ketogenic diet (Dyńska *et al.*, 2023). The readily available acetyl-CoA resulting from a ketogenic diet can be diverted to the mevalonate pathway, leading to cholesterol synthesis (Faulkner; Jo, 2022). The elevated levels of plasma cholesterol in mice treated with CMS121 suggests an increase in synthesis, which is consistent with the increased plasma levels of 7-HOCA, a cholesterol metabolite (Saeed *et al.*, 2017). In addition, limitation of hepatic FASN activity impairs the retention of cholesterol in the plasma membrane (Wei *et al.*, 2016), which may constitute another possible explanation for the elevated cholesterol levels in the plasma of mice receiving the CMS121 diet. Interestingly, the limited cholesterol availability in the plasma membrane of FASN-deficient cells was associated with a lower inflammatory status, since cholesterol is required for propagating inflammatory signals (Wei *et al.*, 2016). The lower hepatic levels of FASN may also account for the decreased inflammatory status found in the livers of mice fed a CMS121 diet. The results showed a clear decrease in hepatic caspase 1, caspase 3 and mitochondrial NOX4, all known to promote hepatic inflammation (Asadi *et al.*, 2022; Lan; Kisseleva; Brenner, 2015; Sun; Scott, 2016). The obese db/db mice fed a CMS121 diet also showed a clear decrease in hepatic inflammatory markers such as NF- $\kappa$ B, IL-18, and caspase 3 (Zahid *et al.*, 2023), reinforcing the liver anti-inflammatory response induced by CMS121 in mice. The precise mechanisms underlying these anti-inflammatory effects of CMS121 in the liver remain to be determined.

#### 4.1.5 Supplementary material



Capitulo 1 - Figure 7 - Supplementary: Locomotor activity  
 Locomotor activity in animals freely exploring an open field two weeks after treatment began. Animals were exposed to the open field for 5 min and their activity recorded and analyzed with the Anymaze software. (A) Total distance traveled; (B) ambulatory time, (C) average velocity, and (D) stereotypic movements. Data are presented as mean + SD (n = 12). Bold underlined p-values indicate statistical significance.

## 4.2 CAPÍTULO 2

### 4.2.1 Objetivos específicos

- Analisar os efeitos do CMS121 em modelo de obesidade e disfunção metabólica induzida por dieta hiperlipídica contendo frutose, com foco na variação de peso, perfil lipídico, parâmetros hepáticos, histologia tecidual e tolerância à glicose.

### 4.2.2 Material and Methods

#### 4.2.2.1 *Animals*

This study was approved by the Ethics Committee on Animal Use of the Universidade Federal de Santa Catarina (UFSC; #1058210524). The male Swiss mice, 6 weeks old, 3 mice per cage, were maintained under controlled conditions of temperature (21°C-23°C) and luminosity (12h light cycle/12h dark), in the animal maintenance facility of the institution.

#### 4.2.2.2 *Experimental protocol*

Sixty Swiss mice of the age of 6 weeks received the experimental standard rodent diet for seven days for acclimation. After this period, the mice were randomly assigned to five groups (n:12): 1) Control (CTL), which received the experimental standard rodent diet; 2) CMS121, animals that received the experimental standard rodent diet with CMS121 (400 ppm/kg); 3) HFFD: animals that received a rodent diet with 30% fat and fructose in drinking water 10% was offered to the animal during the entire experimental period; 4) HFFD+CMS Full: animals that received the same HFFD diet with CMS121 the entire treatment period; 5) HFFD+CMS Half: animals that received the same HFFD diet for the first 8 weeks of treatment, and the diet was replaced for the HFFD+CMS during the last 8 weeks. The experimental diet was provided ad libitum and was prepared by Pragsoluções Biociências (Jaú, São Paulo, Brazil). The dose of CMS121 was previously tested in models of aging and Alzheimer's disease, and diabetes (Ates *et al.*, 2020; Currais *et al.*, 2019; Kepchia *et al.*, 2021; Zahid *et al.*, 2023).

Body mass, water, and food consumption were analyzed twice a week. After 16 weeks of treatment, mice were anesthetized, and their blood was collected by cardiac puncture. After perfusion with PBS, the liver was removed and stored at -80°C until further use. The Open Field Test was applied to analyze the animals' exploratory loco-motor behavior at week 14. At week 11, blood glucose levels were evaluated by tail puncture, and at week 15, the glucose tolerance test (GTT) was performed.

#### 4.2.2.3 *Open field*

Each animal was placed separately in a box (40x40 cm), its behavior was recorded and subsequently analyzed for 5 minutes. Image analysis was performed using the Any-maze software, measuring the distance covered (cm), time in the center and corners of the arena (seconds), as well as the number of rearing's (normal exploratory movements of mice, in which the animal stands up or stands on its hind legs) (Davis; Stevens; Thomas Curtis, 2017). The reduction in distance traveled and the increase in time spent near the corners or walls of the box are reliable indicators of behavior arising from both the presence and severity of the disease. Spending time near the corners or walls of the box is also related to anxiety-like behavior (Bailey, K.R.; Crawley, J.N, 2009).

#### 4.2.2.4 *Blood borne biochemical markers*

At week eleven, the animals were fasted overnight, and the following morning, blood glucose levels were evaluated by tail puncture using reagent strips and a portable glucose meter (G-Tech Lite®, AccuMed Produtos Médicos Hospitalares Ltda., Duque de Caxias, RJ, Brazil).

At week fifteen, the mice were deprived of food and fructose in water overnight to perform the glucose tolerance test (GTT) the next morning. The animals received a glucose solution (2g/kg) intraperitoneally. Blood glucose levels were measured immediately before and at 30, 60, 90, and 120 minutes after glucose administration. Blood collection was performed by tail puncture using reagent strips and a portable glucose meter.

At the end of the experiment, the mice were anesthetized with a solution of xylazine and ketamine (10 mg/kg and 100 mg/kg, respectively) in PBS, and blood was

collected via cardiac puncture. Red blood cells and plasma were separated by centrifugation (15 minutes, 2000g, 10°C). Commercial kits from Labtest® (Lagoa Santa, MG, Brazil) were used to analyze plasma levels of glucose (Ref 84), AST-GOT (Ref 109), triglycerides (Ref. 87), cholesterol (Ref. 76), high-density lipoprotein (HDL; Ref. 145), low-density lipoprotein (LDL; Ref. 146), lactate de-hydrogenase (LDH; Ref. 86), urea (Ref. 104), and lactate (Ref. 138).

Cholesterol and triglycerides extraction from the liver was performed using the method described previously (Folch, J; Lees, M; Sloane Stanley, G.H, 1957). A total of 100 mg of liver tissue was homogenized with a pestle in a 1:15 ratio in a chloroform:methanol (2:1) solution. After 30 minutes at 25°C for phase separation, the liquid phase was collected and kept at room temperature overnight to dry. An equal volume of DMSO to that of the original sample was added, and the sample was analyzed according to the instructions provided by the commercial kits.

#### 4.2.2.5 *Histology*

To evaluate the effectiveness of the high-fat and fructose diet in inducing hepatic steatosis, histological analyses of the liver were also conducted.

Approximately 300 mg of liver tissue was stored in 10% formalin at room temperature immediately after collection. The samples were prepared and stained with Hematoxylin and Eosin and analyzed by two blinded to evaluators.

The samples were assessed based on the presence of three patterns: 1) absence of or rare foci of microvesicular steatosis; 2) microvesicular steatosis; 3) macrovesicular and microvesicular steatosis. Microvesicular steatosis was defined by the presence of multiple tiny droplets (1–2 micrometers in diameter), giving a foamy appearance to the cytoplasm that does not displace but may sometimes indent the nucleus. In contrast, macrovesicular steatosis was characterized by the presence of large fat droplets observed in hepatocytes, typically one per cell, pushing the nucleus to the periphery and creating a signet-ring or adipocyte appearance, which could be classified as either small or large droplet type (Chowdhury; Mehta, 2022).

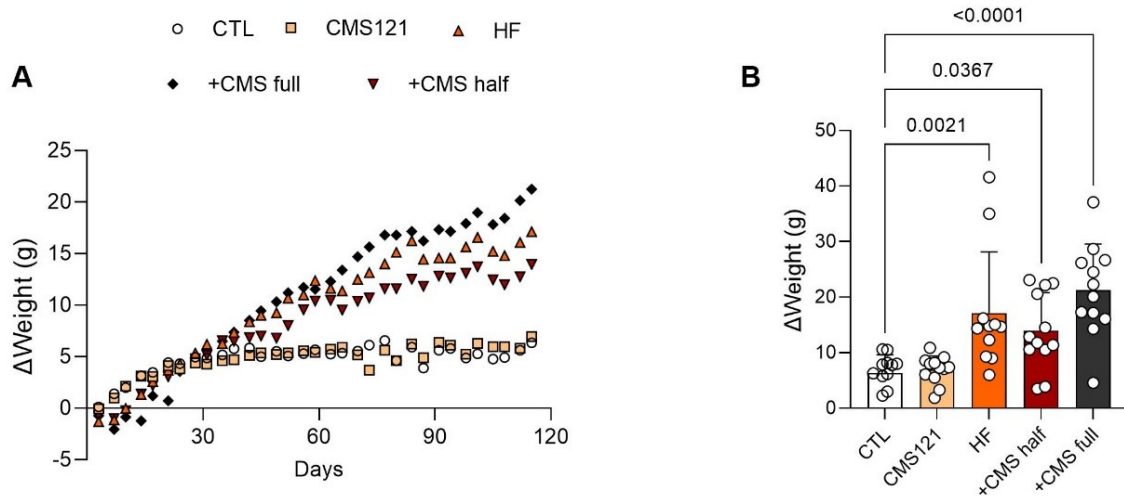
#### 4.2.2.6 Statistical analysis

Data normality was assessed using the Shapiro-Wilk test. Comparisons between groups were performed using one-way analysis of variance (One-way ANOVA) followed by Tukey's post-hoc test for parametric data or Dunnett's multiple comparisons test, or the Kruskal-Wallis' test followed by Dunn's post-hoc test for non-parametric data. Body weight variation was analyzed using two-way analysis of variance (Two-way ANOVA) with Tukey's post-hoc test when statistically significant differences were observed. Statistical comparisons between the CMS121-treated and control groups were performed using the T-test. Statistical significance was set at 5% ( $p < 0.05$ ).

#### 4.2.3 Results

Since treatment with CMS121 was able to reverse weight loss in acute LPS-induced inflammation, and previous studies demonstrated that a CMS121 containing diet induced a reduction in body weight gain and improved glycemic and lipid parameters in obese mice (Zahid *et al.*, 2023), as well as in health mice (Chapter 1), we evaluated the effects of CMS121 supplementation in a diet-induced obesity model using a high-fat and fructose diet (HF).

We monitored changes through body weight variation, food and water intake, and caloric consumption. One-way ANOVA showed a statistically significant difference in weight variation between day zero (D0) and day 115 (D115) ( $F(4,54) = 10.01$ ,  $p < 0.0001$ ), and the post-hoc test indicated an increase in HF group compared to control group ( $p = 0.0021$ ) (Fig. 1B). The groups that received the high fat and fructose diet with CMS121 treatment, whether in part or in full protocol, also showed an increase in body weight when compared to the control group ( $p = 0.036$  for +CMS half and  $p < 0.001$  for +CMS full) (Fig. 1B).

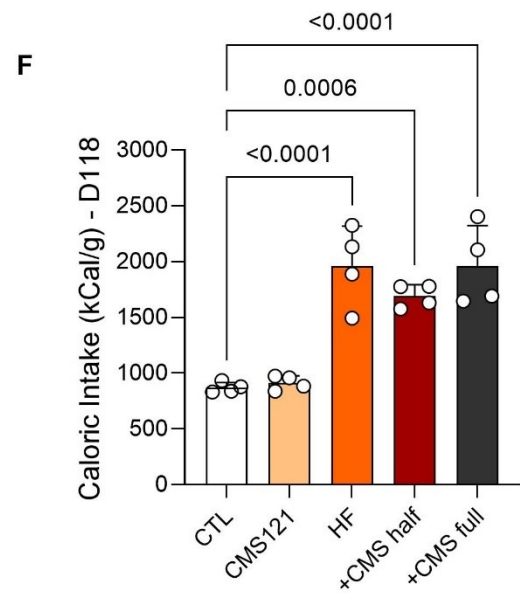
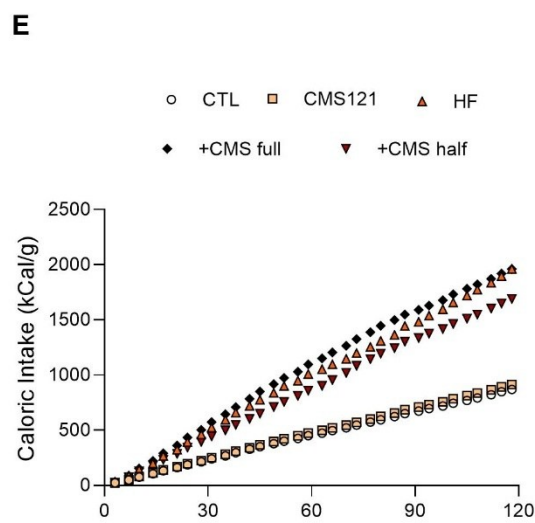
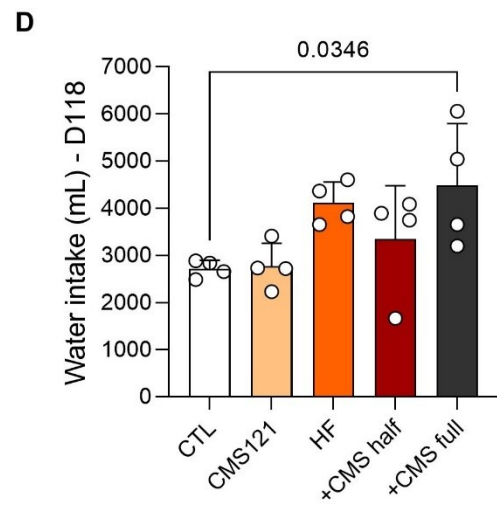
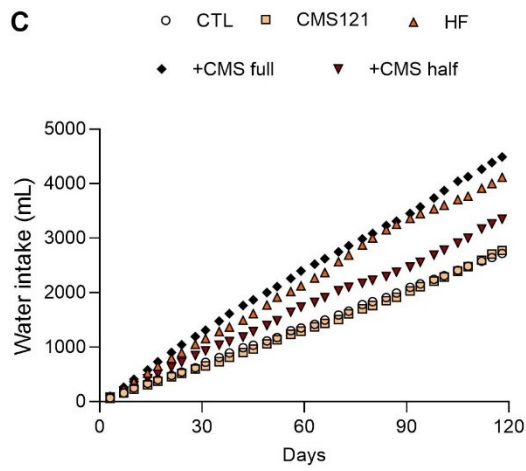
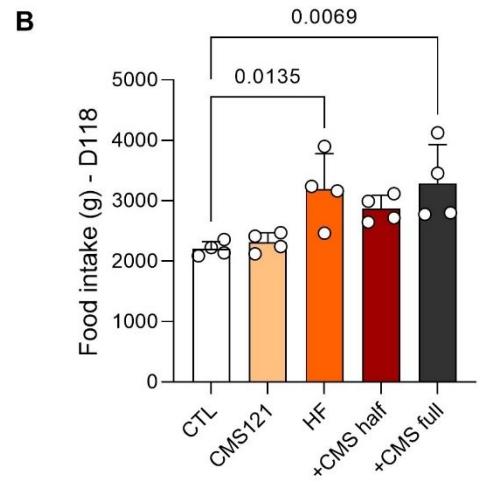
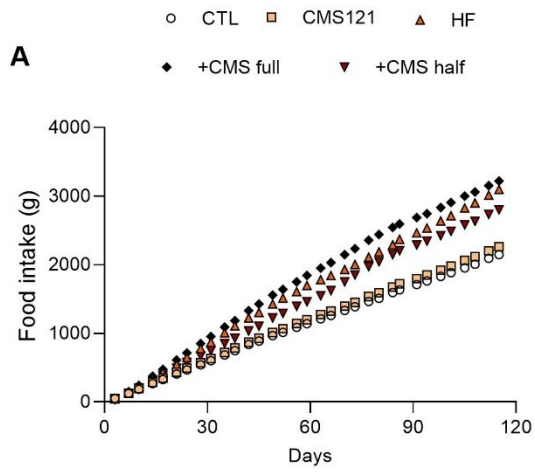


Capítulo 2 - Figure 1 - Body weight variation in HF diet

(A) Body weight variation over the course of the treatment. (B) Body weight changes between D0 and D118. N=9-12. Values expressed as mean or mean + SD.

There is also a statistically significant difference in food intake between D0 and D118 ( $F(4,15) = 5.839$ ;  $p = 0.004$ ), and the post-hoc test indicated an increased intake in the HF and +CMS full groups compared to the control group ( $p = 0.013$  and  $p = 0.006$ , respectively) (Fig. 2B). Treatment with CMS121 did not affect food consumption (Fig. 2A-B). In water consumption there is a statistically significant difference ( $F(4,15) = 3.638$ ;  $p = 0.029$ ), and the post-hoc tests indicated an increase in +CMS full group compared to control ( $p = 0.0346$ ) (Fig. 2D).

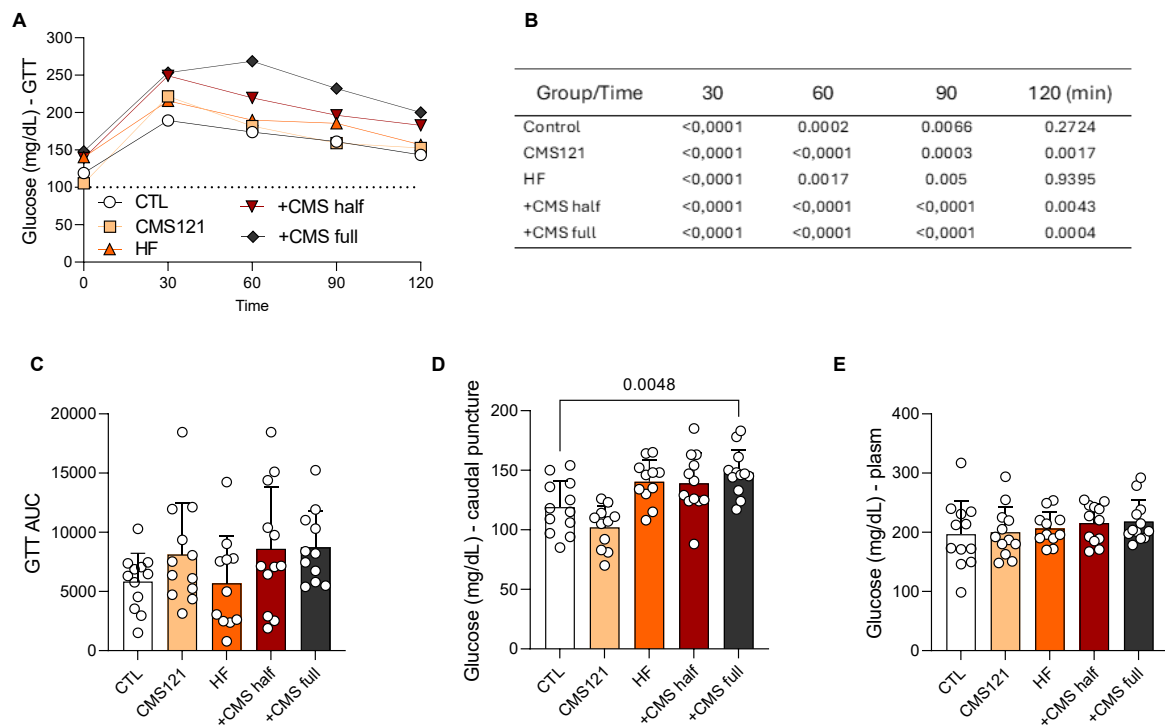
One-way ANOVA showed a statistically significant difference in caloric intake between D0 and D118 ( $F(4,15) = 21.77$ ;  $p < 0.0001$ ), and the post-hoc test indicated an increased intake in the HF group compared to the CTL group ( $p < 0.0001$ ), and also in groups that received the high fat diet with CMS121 treatment, whether in part or in full protocol ( $p = 0.0006$  for +CMS half;  $p < 0.0001$ , for +CMS full) (Fig. 2F). Treatment with CMS121 did not influence caloric intake (Fig. 2F).



Capítulo 2 - Figure 2 - Food consumption and caloric intake in HF diet  
 (A and B) Total food consumption per animal, values expressed in grams; (C and D) Total water consumption per animal, values expressed in mL; (E and F) Total caloric intake per animal, values expressed in Kcal/g. N=4 cages per group with 3 animals each. Values expressed as mean or mean + SD.

The profile of the GTT presented the typical profile of increasing values (30-60 min) of glycemia, following for a period of decay in the glycemic levels (90-120 min), as depicted in Figure 3A. As can be seen, the statistical increase in the glycemic levels was observed at 30-90 min in all groups and values tended to return to control values at 120 min in all groups (Fig. 3B). However, no difference between groups was observed, as the one-way ANOVA showed no statistically significant difference in the area under the curve (AUC) during the glucose tolerance test ( $F(3,53) = 1.667$ ;  $p = 0.1713$ ) (Fig. 3C).

We evaluated the glycemic profile at different time points: at week 11 via tail puncture and glucometer analysis, at week 15 through the glucose tolerance test, and at the end of the protocol using plasma glucose levels measured by colorimetric methods. One-way ANOVA revealed a statistically significant difference in glycemia at week 11 ( $F(4,54) = 8.164$ ;  $p < 0.0001$ ), and post-hoc testing indicated an increase in glycemia in the HF +CMS full group compared to the CTL group ( $p = 0.01$ ) (Fig. 3D). No other statistical difference was found at 11 weeks. Kruskal-Wallis test was performed to assess plasma glycemia at the end of the protocol ( $H = 3.092$ ;  $p = 0.542$ ), showed also no significant difference in median values between groups (Fig. 3E).



Capítulo 2 - Figure 3 - Glycemic profile in HF diet

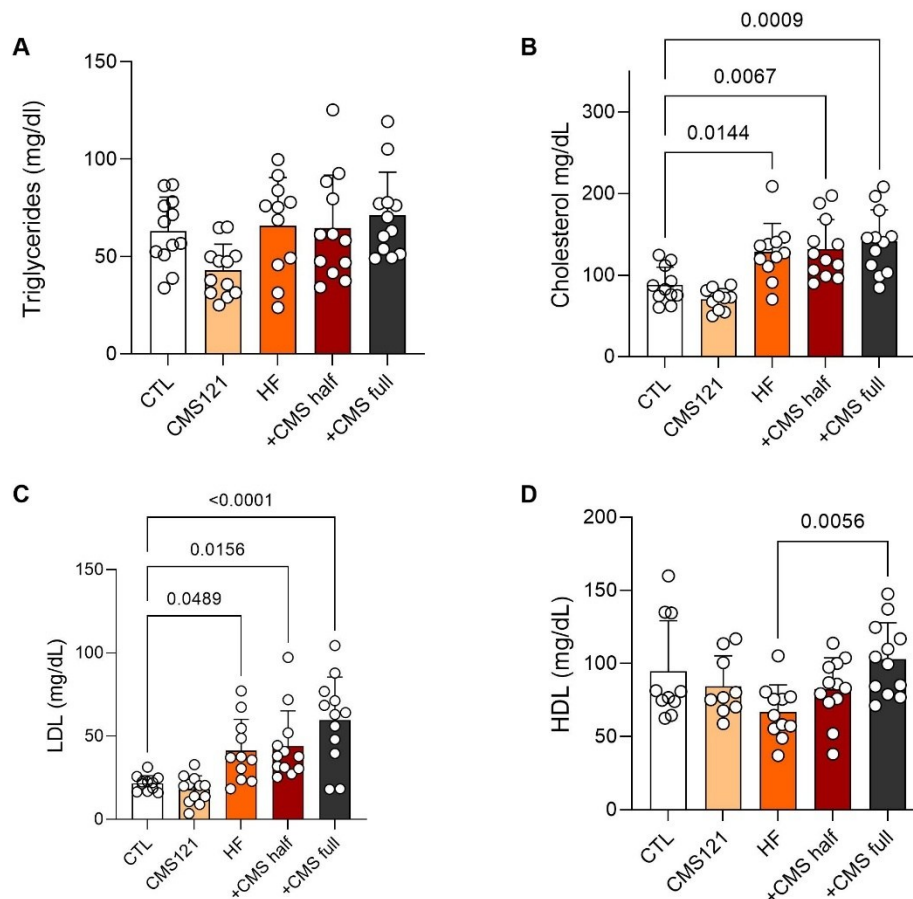
(A) Glucose tolerance test performed at week 15 of the protocol; (B) Statistical results presented as “p” values at different time points in the GTT, as compared to the time zero from the same group. (C) Area under the curve of the glucose tolerance test; (D) Glucose values measured by tail prick using a glucometer at week 11 of the protocol; (E) Plasma glucose values assessed at the end of the protocol using a commercial kit. N = 11-12 animals per group. Values expressed as mean  $\pm$  SD.

In the lipidic profile, one-way ANOVA ( $F(4,54) = 3.003$ ;  $p = 0.0261$ ) showed no statistically significant difference in triglyceride levels (Fig. 4A). One-way Anova test was conducted to evaluate cholesterol levels, showing statistical differences exist among groups ( $F(4,54) = 10.69$ ;  $p < 0.0001$ ) (Fig. 4B). The CMS121 treatment did not significantly influence this variable. The post-hoc analysis indicated an increase in the HF ( $p=0.014$ ), +CMS half ( $p=0.006$ ), and +CMS full ( $p=0.0009$ ) groups as compared to the CTL group. There is no effect of CMS121 treatment, as compared to the effect of HF.

One-way ANOVA showed a statistically significant difference in LDL values ( $F(4,53) = 11.01$ ;  $p < 0.0001$ ) (Fig. 4C). The post-hoc tests failed to find an effect of CMS121 per se. However, an increase in the HF ( $p=0.048$ ), +CMS half ( $p=0.015$ ), and

+CMS full ( $p < 0.001$ ) groups were observed, as compared to the CTL group. There is no effect of CMS121 treatment, as compared to the effect of HF.

One-way ANOVA revealed a statistically significant difference in HDL levels ( $F(4,49) = 3.471$ ;  $p = 0.014$ ). The post-hoc analysis failed to find any significant difference, as compared to the CTL group, however, the group +CMS full group presented higher values ( $p = 0.005$ ), as compared to the HF group (Fig. 4D).

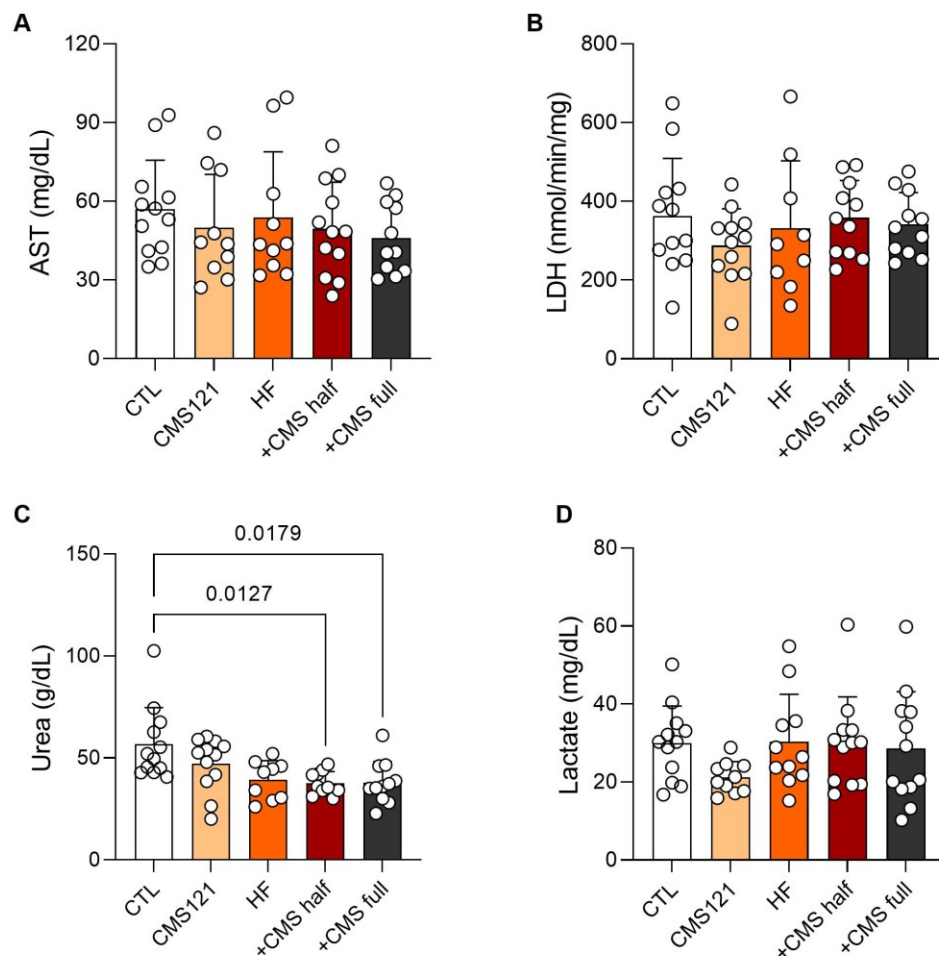


Capítulo 2 - Figure 4 - Lipid profile in HF diet

(A) Plasma triglyceride levels; (B) Plasma cholesterol levels; (C) Plasma low-density lipoprotein (LDL) levels; (D) Plasma high-density lipoprotein (HDL) levels. Values expressed in mg/dL. N=11-12 animals per group. Values expressed as mean + SD.

We also evaluated renal function through plasma urea levels, hepatic damage via plasma AST levels and LDH, as well as glucose metabolism by measuring lactate levels. The Kruskal-Wallis test was performed to assess AST levels revealing no significant differences in group medians ( $H = 2.292$ ;  $p = 0.682$ ) (Fig. 5A). One-way

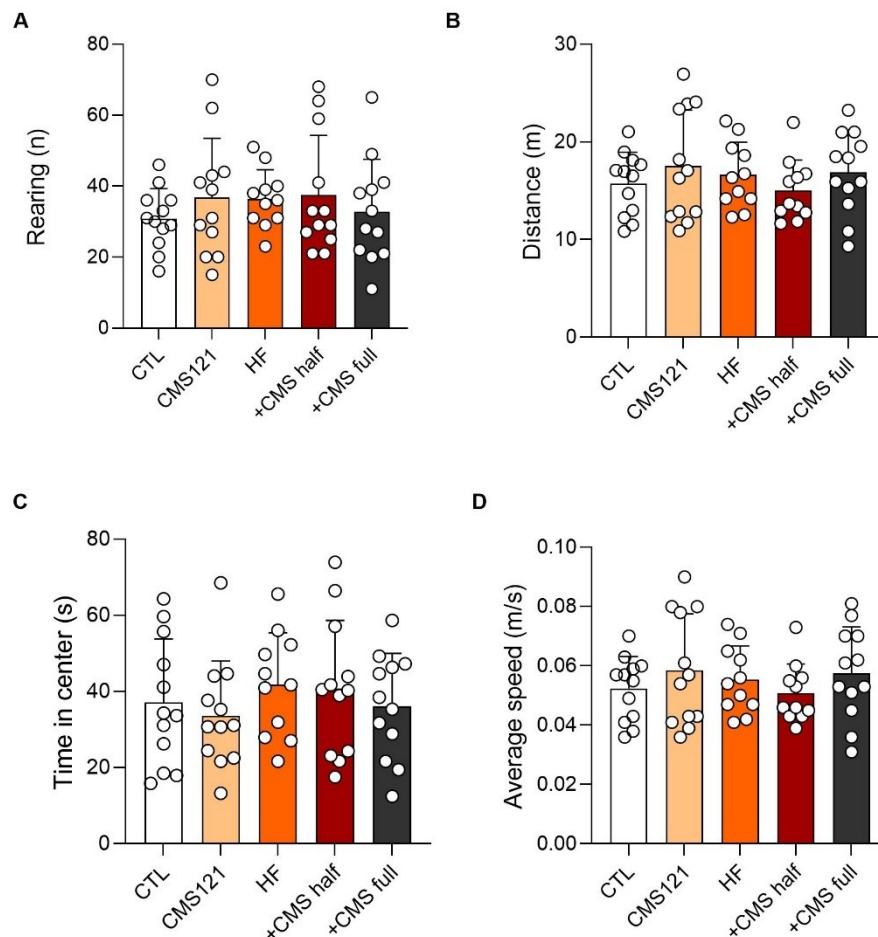
ANOVA showed no statistically significant differences in LDH levels ( $F(4,50) = 0.727$ ;  $p = 0.577$ ) (Fig. 5B). Plasma urea levels were analyzed using the Kruskal-Wallis's test ( $H = 15.30$ ;  $p = 0.012$ ). The post-hoc tests found significant differences observed among +CMS half ( $p = 0.012$ ) and +CMS full ( $P = 0.012$ ) when compared to the CTL group medians (Fig. 5C). Lactate levels presented no significant difference in the Kruskal-Wallis's test when compared to the control group ( $H = 6.245$ ;  $p = 0.181$ ) (Fig. 5D).



Capítulo 2 - Figure 5 - Markers of hepatic, renal and glucose metabolism in HF diet  
(A) Plasma levels of aspartate aminotransferase (AST); (B) Plasma levels of lactate dehydrogenase (LDH); (C) Plasma urea levels; (D) Plasma lactate levels. Values are expressed in mg/dL. N=11-12 animals per group. Values expressed as mean  $\pm$  SD.

To assess the exploratory activity of the animals, the open field test was conducted during the 14th week of the protocol. The Kruskal-Wallis test was performed to evaluate the number of rearing, revealing no significant difference between group

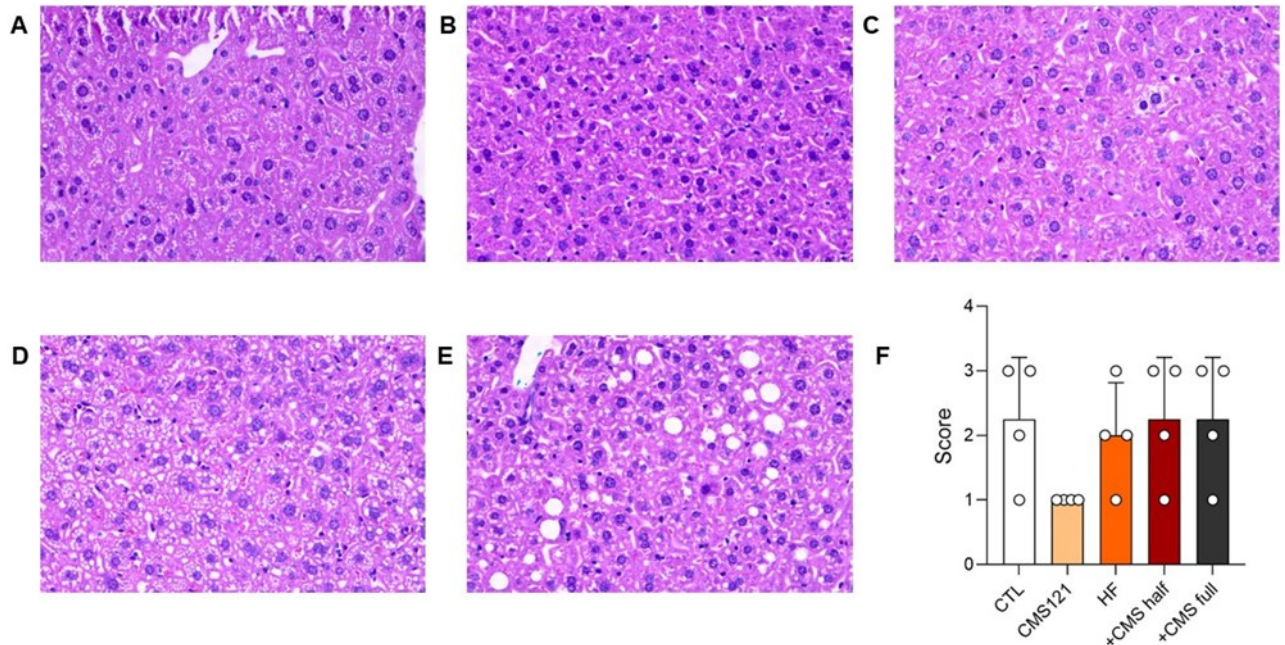
medians ( $H=1.974$ ;  $p=0.74$ ) (Fig. 6A). One-way ANOVA showed no statistically significant difference in the distance traveled ( $F(4,53)=0.683$ ;  $p=0.606$ ) (Fig. 6B), nor in the time spent in the center of the apparatus or in the average speed achieved ( $F(4,54)=0.54$ ;  $p=0.701$ ;  $F(4,53)=0.65$ ;  $p=0.629$ , respectively) (Fig. 6C and D).



Capítulo 2 - Figure 6 - Open field test at 11th week in HF diet  
(A) Number of rearing; (B) Distance traveled (cm); (C) Average speed (m/s); (D) Time spent in the central zone of the apparatus (seconds).  $N=11$  for the HF group;  $n=12$  for the other groups. Values are expressed as mean + SD.

The histology of liver tissue revealed that the high-fat and fructose diet was not able to induce hepatic steatosis (macro- or microvesicular), as the groups receiving this diet showed scores like the control group, mostly displaying a pattern score of level 2, indicating signs of microvesicular steatosis (Fig. 7A, C–F). Supplementation with CMS121 in the diet improved the liver appearance in mice, with all animals presenting

a pattern score of level 1, characterized by absent or rare foci of microvesicular steatosis (Fig. 7B, F).

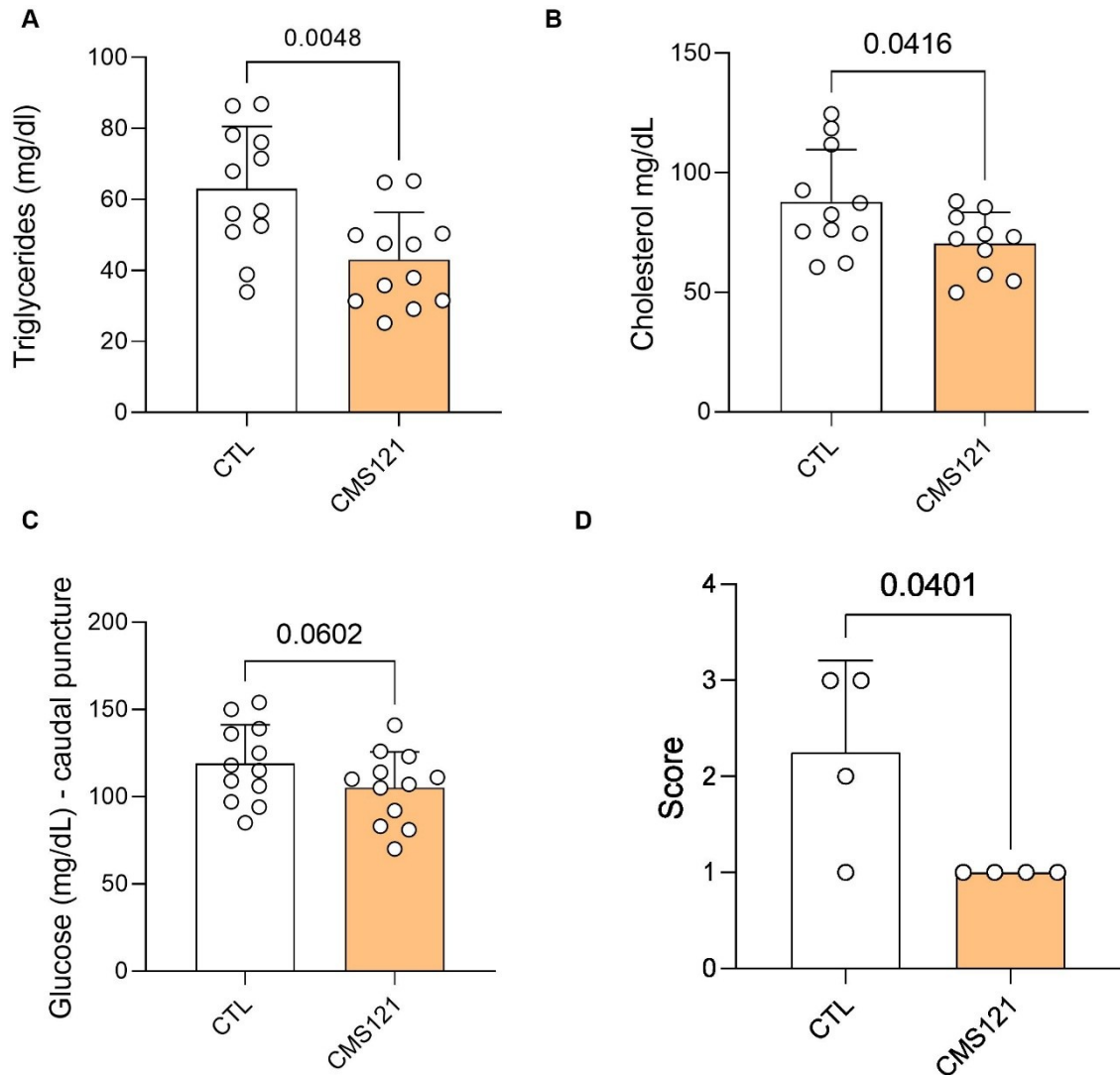


Capítulo 2 - Figure 7 - Histological analyses of liver in HF diet  
 A) Control group; (B) CMS121 group; (C) HF group; (D) +CMS Half group; (E) +CMS Full group; (F) Clinical score - 1: none or rare foci of microvesicular steatosis; 2: microvesicular steatosis; 3: macro- and microvesicular steatosis. N=4 per group. Representative images from each group.

The proposed high-fat and fructose diet protocol induces an increase in body weight and in eating behavior, with increased food and caloric consumption, as well an increase levels of LDL in plasm. Meanwhile it did not induce alterations in glycemic profile, glucose metabolism, markers of hepatic and renal function, or locomotor activity in the animals.

Supplementation with CMS121 per se can reduce levels of plasma TG ( $p=0.0048$ ) (Fig. 8A) in comparison with CTL group, as well cholesterol ( $p=0.0416$ ) (Fig. 8B), glucose by caudal puncture presented the same tendency ( $p=0.0602$ ) (Fig. 8C). Regarding the histopathology results, CMS121-treated mice presented only normal scores (score=1), which is considered normal for the four animals analyzed, while control animals presented worse scores (1, 2, 3, 3), indicating CMS121 can improve the liver appearance (Fig. 8D). Interestingly, CMS121 supplementation

throughout the period that the animals received a high-fat and fructose diet resulted in an approximately 70% increase in HDL levels, if compared to the HF group.



Capítulo 2 - Figure 8 – Comparison CTL with CMS121 group  
 (A) Triglycerides; (B) Cholesterol; (C) Glucose in caudal puncture; (D) Liver histopathology. Values are expressed in mg/dL. Values expressed as mean  $\pm$  SD

#### 4.2.4 Discussion

The effects of CMS121 failed to be observed in the high-fat and fructose diet-induced metabolic disturbance model. As expected, the high-fat diet increased body weight, caloric intake, and circulating LDL and cholesterol levels, as well as increased

food intake, consistent with diet-induced obesity literature (Buettner; Schölmerich; Bollheimer, 2007; Petronek *et al.*, 2019). These alterations induced by HF diet were not modulated by the inclusion of CMS121 in the diet, independent if it was added since the beginning of the treatment (+CMS full) or only for 2 months (+CMS half) after the beginning of the treatment. The only possible improvement was an increase in the HDL levels, when compared to the HF diet. However, this isolated effect cannot be accounted for as a beneficial effect and may represent an unrelated event.

This lipid-modulating effect of CMS121 were not observed in the current experiment, however the literature has documented inhibition of fatty acid synthase and activation of AMPK, regulating lipid metabolism and reducing lipotoxicity, effects that were observed in the genetic in a model of obesity, db/db mice lacking the leptin receptor (Zahid *et al.*, 2023) and in models of aging (Ates *et al.*, 2020; Currais; Raschke; Maher, 2024).

Despite the lack of effectiveness in limiting the HF diet-induced alterations, CMS121 produced a clear response in the control mice, like those observed in the healthy mice described in Chapter 1. CMS121 supplementation produced favorable metabolic effects, including reduced plasma levels of TG and cholesterol. Along with these lipid-lowering effects, a tendency to decreased glucose levels was also observed at 11 weeks of CMS121 diet. Despite HF did no increase histopathological index, preventing the observation of any possible effect of CMS121, as compared to control animal, CMS121 treatment improved liver histology with reduced microvesicular steatosis severity. This result suggests that CMS121 primary action under this protocol was lipid metabolism modulation and hepatic protection in healthy animals, but not in HF-treated animals. These observations are consistent with prior studies demonstrating CMS121's efficacy in improving mitochondrial function and reducing lipid accumulation in metabolic diseases (Ates *et al.*, 2020; Currais *et al.*, 2019; Zahid *et al.*, 2023). Additionally, the increase in HDL following CMS121 treatment aligns with findings that interventions targeting lipid biosynthesis pathways can shift lipoprotein profiles towards anti-atherogenic patterns (Ates *et al.*, 2020; Currais *et al.*, 2019; Zahid *et al.*, 2023).

The publications that showed CMS121 can modulate lipid metabolism used C57/BL6 background mice (Chapter 1 and (Zahid *et al.*, 2023)), and the strain used in this work was Swiss mice, which is a potential factor to be further tested.

In consonance with data presented in Chapter 1, CMS121 can lower circulating lipids in control animals, probably by inhibiting lipid synthesis that is dependent on the fatty acid synthase and ACC1 activity (Ates *et al.*, 2020). CMS121 also activates AMPK (Currais; Raschke; Maher, 2024), which can result in ACC1 phosphorylation and inhibition, thus limiting lipid synthesis. Despite its' lipid lowering properties, CMS121 was ineffective in preventing the increase in the HF diet-induced lipid increase. We hypothesized that that diet formulation would have affected CMS121 absorption or activity. Alternatively, CMS121 may need to be given in a higher dose, as it passed toxicity in both animals and humans up to 900 mg/kg (Maher *et al.*, 2025). Since the lipid lowering effects were observed only in control animals, the dose employed may have been insufficient to counteract the diet rising effect on circulating lipids.

The differential effects observed across the tested models highlight the importance of disease context in determining CMS121 efficacy. While CMS121 did not exhibit antidepressant-like or strong anti-inflammatory effects in acute sepsis and LPS models, or in the chronic high-fat diet, it demonstrated clear metabolic benefits in control mice. This context-dependent pharmacological profile suggests that CMS121's primary therapeutic window may reside in chronic metabolic or neurodegenerative diseases rather than acute inflammatory conditions.

One limitation of this study is the exclusive use of male Swiss mice, which may not capture sex-dependent responses reported for both depression and metabolic diseases (Maris *et al.*, 2010; Pereira-Silva *et al.*, 2019; Won *et al.*, 2013). Additionally, the absence of molecular analyses (e.g., inflammatory cytokines, mitochondrial markers) limits mechanistic interpretation. Future studies incorporating molecular endpoints and chronic stress-based models of depression or sepsis could better clarify the therapeutic scope of CMS121.

## 4.3 CAPÍTULO 3

### 4.3.1 Objetivos específicos

- Investigar os efeitos da administração de CMS121 em camundongos saudáveis sobre o comportamento locomotor, tipo depressivo e ansioso, por meio de testes comportamentais padronizados
- Avaliar o impacto do CMS121 em modelo de sepse pulmonar induzida por *Klebsiella pneumoniae*, considerando taxa de sobrevivência, parâmetros clínicos, consumo alimentar, e variação de peso.
- Determinar os efeitos do CMS121 em modelo de inflamação sistêmica induzida por lipopolissacarídeo (LPS), analisando sinais clínicos, alterações comportamentais, consumo alimentar e recuperação corporal durante o período pós-insulto.

### 4.3.2 Material and Methods

#### 4.3.2.1 *Animals*

This study was approved by the Ethics Committee on Animal Use of the Universidade Federal de Santa Catarina (UFSC; #1058210524). The male Swiss mice, 6 weeks old, 3 mice per cage, were maintained under controlled conditions of temperature (21°C-23°C) and luminosity (12h light cycle/12h dark), in the animal maintenance facility of the institution.

#### 4.3.2.2 *Experimental protocols*

##### 4.3.2.2.1 Effects of CMS121 on anxiety-like and depressive-like behavior in healthy animals

Forty animals were sorted into four experimental groups (n=10): control group (CTL) receiving the vehicle solution or the CMS121 groups receiving the concentration of 1 (CMS1), 3 (CMS3), or 10 mg/kg (CMS10). CMS121 was initially diluted in 5% DMSO and then in 50% propylene glycol to adjust the dose. Each animal received an i.p. injection at proportion of 3 ml/kg for seven consecutive days.

Behavioral tests were conducted on these animals: the open field test and the tail suspension test were performed 2 hours after the first dose to detect any possible

acute effect. The elevated plus maze and the splash tests were conducted on the seventh and ninth day of administration after the last injection, respectively.

#### 4.3.2.2.2 The potential effects of CMS121 in alleviating the deleterious effects observed in experimental Sepsis

Thirty animals were grouped into three experimental groups (n:10): pneumosepsis (PS), pneumosepsis treated with CMS121 3 mg/kg (PS+CMS3), and pneumosepsis treated with CMS121 10 mg/kg (PS+CMS10). All groups initially received an intratracheal saline solution (10 ml/kg) containing 10<sup>9</sup> colony-forming units (CFU) of *Klebsiella pneumoniae* (*K.pneumoniae*).

As described previously [63], the contents of a microtube of lyophilized bacteria were suspended in 45 mL of Brain Heart Infusion (BHI), broth in a Falcon tube and incubated for 18 hours at 37°C. The bacteria were washed, and the resulting sediment was suspended in 0.5 mL of sterile phosphate-buffered saline (PBS) and aliquoted into microtubes. Serial dilutions were performed, and optical densities (OD) were determined at 630 nm using a microplate reader. In parallel, the OD of the McFarland scale, a standard turbidity scale commonly used in microbiology to estimate bacterial concentration was assessed. A linear regression calculation was then performed, correlating the ODs to estimate the CFU of the aliquots used in this experiment.

The bacterial suspension was diluted in sterile PBS to a concentration of 1.22 x 10<sup>5</sup> CFU/mL and was injected into the animals. The animals were anesthetized with a solution composed of ketamine (3 mL), xylazine (1.8 mL), and PBS (25.2 mL), positioned dorsally at a 45° angle. In an aseptic manner, a 5mm incision was made in the skin of the ventral neck region, the muscles were gently separated to expose the trachea for the intratracheal injection of 0.05 mL of the bacterial suspension (Probst *et al.*, 2019; Sordi *et al.*, 2013). After the procedure, the skin was sutured, the animals received subcutaneous PBS (30 mL/kg) for fluid replacement and were kept in a heated environment until recovery from the surgery.

Four hours after recovering from the bacterial inoculation procedure, the animals received an i.p. injection of 3 mL/kg of the solution with CMS121 at concentrations of 3 mg/kg (PS+CMS3) and 10 mg/kg (PS+CMS10). The

pneumosepsis group (PS) received the vehicle (DMSO and propylene glycol) without CMS121, as described above.

Over the course of 15 days, body weight, water and food consumption, and clinical scores were evaluated to assess the morbidity of the animals, as shown in the figure below. In the clinical score, the animals were assessed based on fur appearance, eye condition, level of consciousness, respiration, and exploratory activity. Each item was scored from 0 to 2, with the total clinical score ranging from 0 to 6, where higher scores indicate a decline in the animal's overall wellbeing (Gonçalves *et al.*, 2017).

#### 4.3.2.2.3 Effects of CMS121 on an inflammation model induced by LPS

Thirty-eight animals were grouped into four experimental groups: control (CTL, n:8), CMS121 (n:10), LPS (n:10), and LPS+CMS121 (n:10). Initially, all animals underwent a voluntary intake adaptation period with peanut oil (100 µL) for 4 days. CMS121 was added to the peanut oil at a concentration of 30 mg/kg, and the treatment followed for 3 days. After the CMS121 treatment the animals receiving LPS (LPS and LPS+CMS121 groups) received an i.p. injection of 5 mg/kg of LPS diluted in PBS, while the other groups received only PBS. The animals were monitored for 7 days after the LPS injection, and body weight, water, and food consumption were evaluated every other day.

To evaluate the sickness behavior, the animals were exposed to the open field test twenty-four hours after the administration of LPS. It is known that sepsis is associated to depression in humans and animals (Ackermann *et al.*, 2025; Yang, Xin *et al.*, 2024). To assess any potential antidepressant-like effect of CMS121 the tail suspension test was performed 7 days after the LPS injection.

#### 4.3.2.3 Behavior tests

##### 4.3.2.3.1 Open field

Each animal was placed separately in a box (40x40 cm), its behavior was recorded and subsequently analyzed for 5 minutes. Image analysis was performed using the Any-maze software, measuring the distance covered (cm), time in the center and corners of the arena (seconds), as well as the number of rearing's (normal

exploratory movements of mice, in which the animal stands up or stands on its hind legs) (Davis; Stevens; Thomas Curtis, 2017). The reduction in distance traveled and the increase in time spent near the corners or walls of the box are reliable indicators of behavior arising from both the presence and severity of the disease. Spending time near the corners or walls of the box is also related to anxiety-like behavior (Bailey, K.R.; Crawley, J.N, 2009).

#### 4.3.2.3.2 Tail suspension test

The distal end of the animal's tail was attached to a horizontal wooden bar 60 cm above the ground. The animal was fixed to the wooden bar with adhesive tape at a 90° angle to the longitudinal axis of the tail, keeping it in a vertical position, head down. In this position, animal control typically exhibits struggling behavior or maintains prolonged sequences of continuous body movements, seeking support or attempting to escape. A significant reduction in the duration of this behavior, considered presumably distressing and inescapable for the animal, thus increasing its immobility, is associated with depressive-like behaviors (Can *et al.*, 2011). Therefore, each animal was suspended by the tail for 6 minutes, and the total time of immobility (in seconds) was quantified. Immobility was defined as the absence of initiated movements, including passive body swaying (Steru *et al.*, 1985).

#### 4.3.2.3.3 Elevated plus maze

To investigate anxiety-like behavior, mice were evaluated in the elevated plus maze apparatus, made of gray acrylic, with four arms (two open, without walls, and two enclosed with 15 cm high walls), each measuring 30 cm in length and 5 cm in width, elevated 50 cm from the ground. In this test, the animals were placed individually in the center of the apparatus, and their behavior was recorded for 5 minutes. The number of entries and the time spent in the open and closed arms were measured. Entries were counted when the animal entered the arm with all four paws. Variables such as the time spent in the neutral zone (the area between the four arms) and the number of attempts in the open arms (where the animal places only part of its body in the open arms) were also measured. This test is based on rodents' natural tendency to seek dark, enclosed spaces and their unconditioned fear of heights/open spaces.

Thus, anxiety-like behavior is characterized by increased time spent and/or an increase in the number of entries into the closed arms (Walf; Frye, 2007).

#### 4.3.2.3.4 Splash test

In this test, 1 mL of a solution containing 10% sucrose was sprayed on the dorsal coat of mice placed individually in an acrylic box (9 x 7 x 11 cm). Due to its viscosity, the sucrose solution "soiled" the animal's fur, inducing grooming behavior. After applying the sucrose solution, the frequency (duration) and the latency to the onset of grooming behavior were observed for 5 minutes, and these times were recorded. Signs of poor self-care and motivational behavior are considered parallel to some symptoms of depression, such as anhedonia (Willner, 2005).

#### 4.3.2.4 Statistical analysis

Data normality was assessed using the Shapiro-Wilk test. Comparisons between groups were performed using one-way analysis of variance (One-way ANOVA) followed by Tukey's post-hoc test for parametric data or Dunnett's multiple comparisons test, or the Kruskal-Wallis' test followed by Dunn's post-hoc test for non-parametric data. Body weight variation and clinical scores were analyzed using two-way analysis of variance (Two-way ANOVA) with Tukey's post-hoc test when statistically significant differences were observed. Survival curves were evaluated using the Log-rank (Mantel-Cox) test. Statistical significance was set at 5% ( $p < 0.05$ ).

### 4.3.3 Results

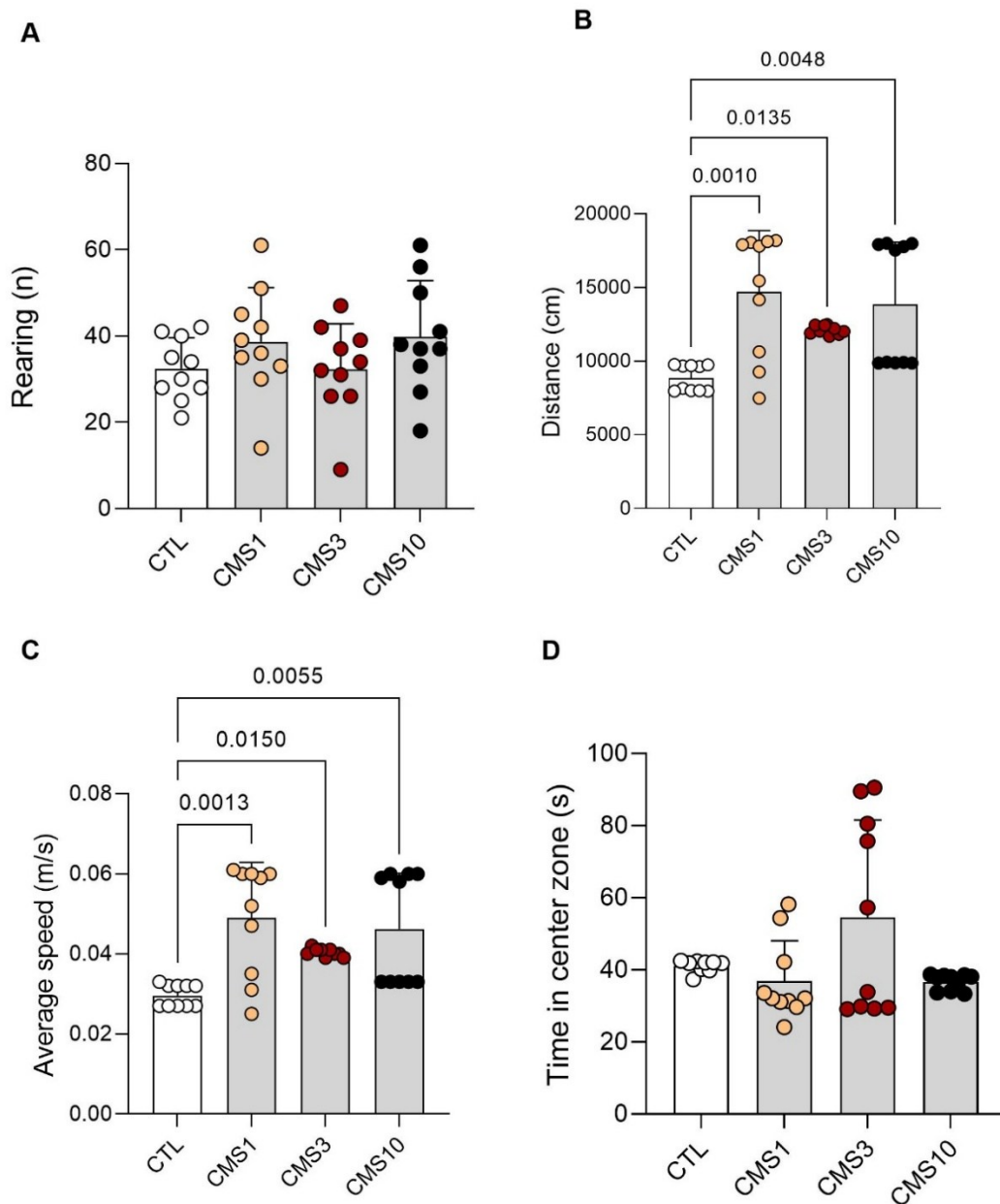
#### 4.3.3.1 *CMS121 treatment promotes locomotor activity but fails to induce an antidepressant-like effect neither alter the anxiety status*

In the first experimental protocol, healthy male Swiss mice received daily doses of CMS121 via intraperitoneal (i.p.) injection for seven days at different concentrations according to their allocated group: 1 mg/kg (CMS1), 3 mg/kg (CMS3), and 10 mg/kg (CMS10). The control group (CTL) received the vehicle solution. Knowing the time for CMS121 to reach its maximum concentration of approximately two hours, the animals were evaluated two hours after the first dose.

To evaluate whether CMS121 influenced the exploratory activity of the animals, they were exposed to the open field test. One-way ANOVA showed no significant differences for CMS121 treatment in the number of rearing in the open field test ( $F(3,36) = 1.292$ ;  $p = 0.2921$ ) (Fig. 1A).

The Kruskal-Wallis test was performed to compare the distance traveled ( $H = 17.9$ ;  $p < 0.001$ ) and the average speed ( $H = 17.3$ ;  $p < 0.001$ ). Dunn's post-hoc test indicated that the distance traveled by the groups that received CMS121 injections was greater than that of the control group at concentrations of 1 mg/kg ( $p = 0.001$ ), 3 mg/kg ( $p = 0.0135$ ), and 10 mg/kg ( $p = 0.0048$ ) (Fig. 1B), as well as the average speed reached at concentrations of 1 mg/kg ( $p = 0.0013$ ), 3 mg/kg ( $p = 0.015$ ), and 10 mg/kg ( $p = 0.0055$ ) (Fig. 1C).

The Kruskal-Wallis test did not indicate a significant difference among the different doses of CMS121 for the time spent in the center of the open field test ( $H = 5.402$ ;  $p = 0.1446$ ) (Fig. 1D). The graph shows that data dispersion seems to separate the animals into two groups: those with higher locomotion and speed, and others that were less active. This same was observed in the time spent in the center of the open field at the 3 mg/kg concentration.

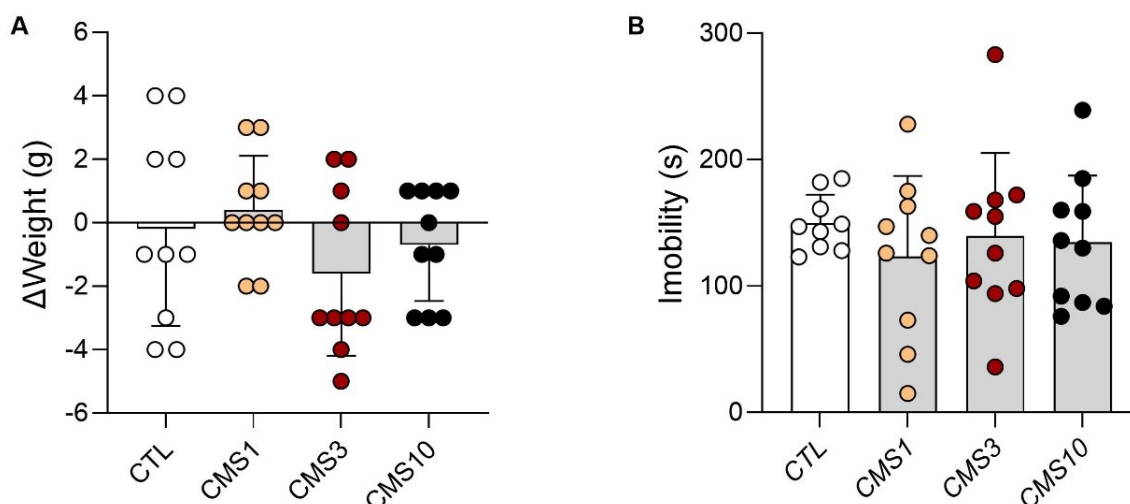


Capítulo 3 - Figure 1 - Locomotor activity

Locomotor activity in the open field 2 hours after administration of different i.p. doses of CMS121 in healthy animals: (A) Number of rearing; (B) Distance traveled; (C) Average speed; and (D) Time spent in the center of the apparatus; (E) Immobility time in the tail suspension test performed 2 hours after i.p. administration of CMS121; (F) Body weight variation prior to CMS121 injection and 8 days after administration at different doses. N = 10 animals per group. Values expressed as mean + SD.

The tail suspension test was performed 2 hours after administration of different doses CMS121 to evaluate depression-like behavior. One-way ANOVA showed no significant differences for CMS121 treatment in immobility time in the tail suspension

test ( $F_{(3,36)} = 0.377$ ;  $p = 0.7697$ ) (Fig. 2B). No changes in body weight variation were observed; the Kruskal-Wallis test showed no significant differences among the different doses of CMS121 ( $H = 3.217$ ;  $p = 0.359$ ) (Fig. 2A).



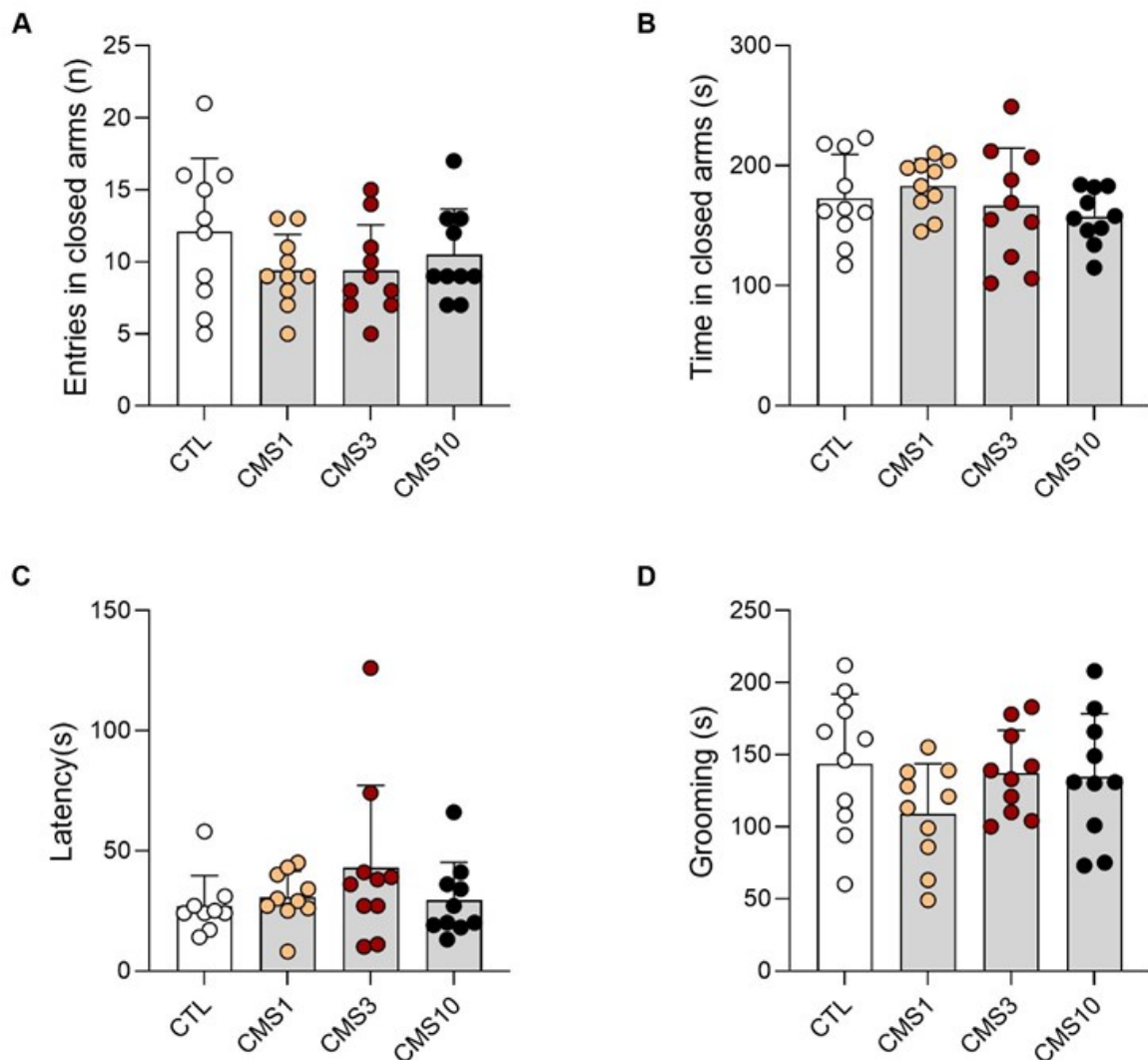
Capítulo 3 - Figure 2 - Body weight variation

Body weight variation across 8 days and tail test in healthy animals: (A) Body weight variation prior to CMS121 injection and 8 days after administration at different doses; (B) Imobility time in the tail suspension test performed 2 hours after i.p. administration of CMS121.  $N = 10$  animals per group. Values expressed as mean + SD.

In conclusion, the behavioral analyses in the open field test revealed that animals treated with CMS121 at different doses showed an increase of approximately 50% in both the distance traveled and the average speed reached 2 hours after receiving the CMS121 dose of 1, 3, and 10 mg/kg. The same doses did not affect the time spent in the center of the apparatus, nor did they alter the number of rearing, or the depression-like behavior.

The animals were also evaluated after 7 days of treatment in the elevated plus maze test to evaluate anxiety levels and after 9 days, by the sucrose splash test to assess anhedonia-like behavior. One-way ANOVA revealed no significant differences for CMS121 treatment in the number of entries ( $F_{(3,36)} = 1.248$ ;  $p = 0.303$ ) and the time spent in the closed arms of the elevated plus maze ( $F_{(3,36)} = 0.979$ ;  $p = 0.413$ ) (Fig. 3A and B), as well as grooming activity in the sucrose splash test ( $F_{(3,36)} = 1.447$ ;  $p = 0.237$ ) (Fig. 3D). The Kruskal-Wallis test also did not indicate significant differences

among the different doses of CMS121 in latency in the sucrose splash test ( $H = 3.17$ ;  $p = 0.365$ ) (Fig. 3C).



Capítulo 3 - Figure 3 -Analyses of anxiety-like states, and assessment of anhedonia behavior  
Analysis of anxiety-like states, and assessment of anhedonia behavior. (A) Total number of entries into the closed arms in the elevated plus maze, 7 days after CMS121 administration; (B) Total time spent in the closed arms; (C) Latency to the first grooming behavior in the sucrose splash test, conducted 9 days after CMS121 administration; (D) Total grooming time during the test.  $N = 10$  animals per group. Values expressed as mean + SD.

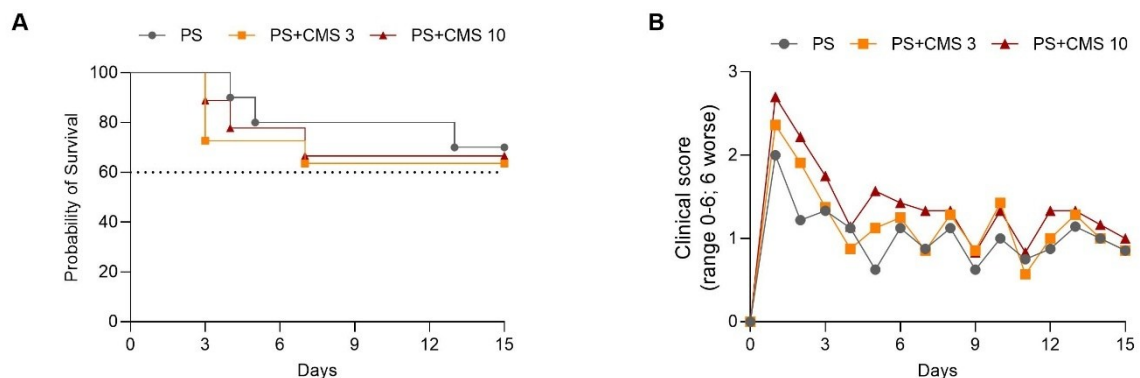
These results further demonstrate the safety of the different doses of CMS121, as no behavioral alterations or changes in body weight were observed in the evaluated animals. Although CMS121 treatment increased the exploratory activity of the animals in the first hours evaluated, with an increase in distance traveled and average speed,

it was not able to influence depression-like behavior during the same evaluation period. Anxiety-like and anhedonia-related behavioral analyses performed days after the last CMS121 administration also showed no influence on the behavior of these animals.

#### 4.3.3.2 CMS121 was unable to attenuate the effects of pneumosepsis

Since the tested doses of CMS121 proved to be safe, given that they did not alter the animals' behavior or cause adverse reactions, we tested doses of 3 and 10 mg/kg of CMS121 in animals subjected to an acute bacterial infection. The animals received an intratracheal instillation of *Klebsiella pneumoniae* and, 4 hours later, a single i.p. injection of CMS121 at the indicated doses. The animals were monitored for 15 days.

Previous studies of pneumosepsis models induced by *K. pneumoniae* report approximately 40% mortality between the third and fifth day (Bonorino *et al.*, 2024; Probst *et al.*, 2019). In our study, the same mortality rate was observed; however, the deaths occurred later, between 3 and 12 days after the insult (Fig. 4A). CMS121 showed no effect on the animals' mortality.

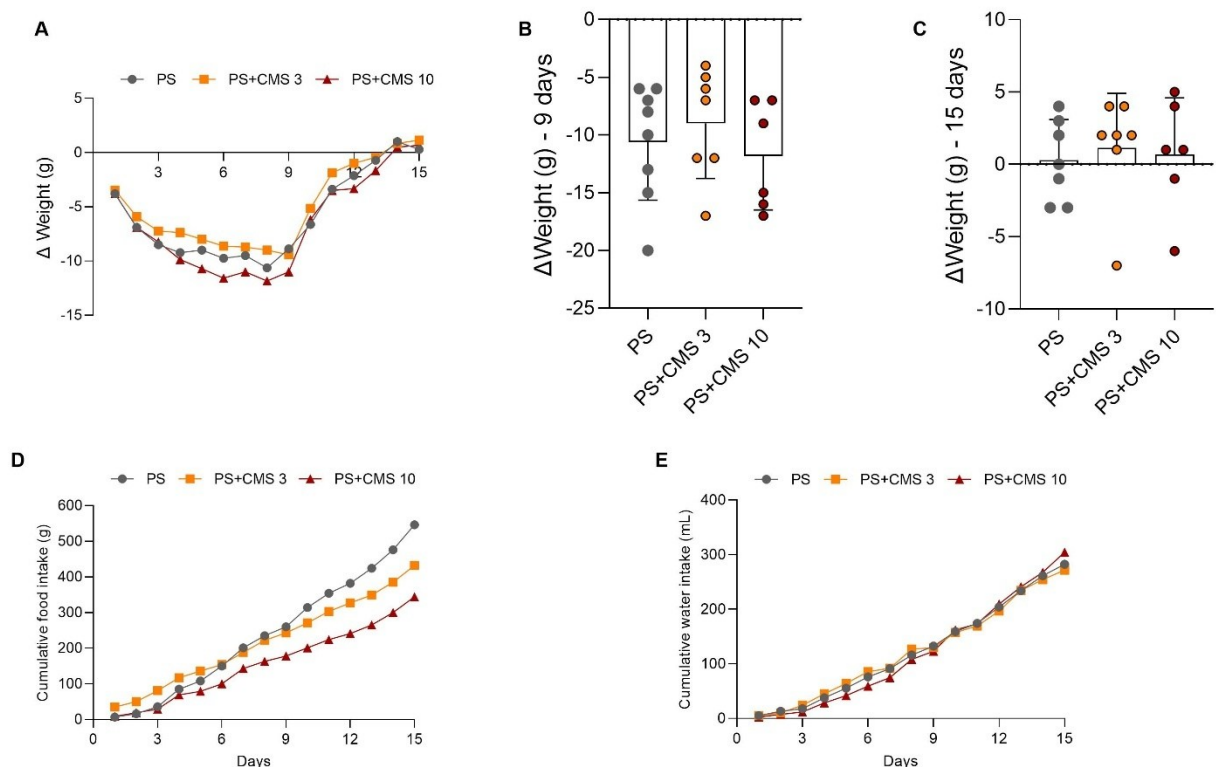


Capítulo 3 - Figure 4 -Survival and clinical score after pneumosepsis  
Survival curve and clinical score after induction of pneumosepsis with *K. pneumoniae* and treatment with CMS121 at different doses 4 hours after the insult. (A) Survival curve, results expressed as percentage survival; (B) Clinical score, scale ranging from 0 to 6 (indicating best and worst clinical conditions, respectively) over a 15-day period. Sample size was 10-11 per group.

Twenty-four hours after the bacterial insult, the animals showed clinical worsening as observed by the clinical score, which increased from 0 (healthy) to 2–3 (worse health condition). The score gradually returned to a lower level (0.5–1.5) around

4 days after pulmonary bacterial instillation. This score level remained stable throughout the observation period. Treatment with CMS121 (3 and 10 mg/kg) did not influence the clinical score values. One-way ANOVA revealed a significant effect on the clinical score ( $F(6.24, 122.3) = 16.77$ ;  $p < 0.0001$ ), but no effect was observed between groups ( $F(2, 27) = 1.244$ ;  $p = 0.304$ ). There was no interaction between days and groups ( $F(30, 294) = 0.568$ ;  $p = 0.968$ ) (Fig. 4B).

The animals experienced a weight loss of approximately 10 grams up to 9 days after the infectious insult, with full weight recovery by the end of the 15-day monitoring period (Fig. 5A). Accordingly, one-way ANOVA showed no significant differences in body weight variation in day 9 ( $F(2, 18) = 0.5655$ ;  $p = 0.5778$ ) (Fig. 5B), and the Kruskal-Wallis test at the end of the experiment ( $H = 0.674$ ;  $p = 0.728$ ) (Fig. 5C). One-way ANOVA showed no significant differences for CMS121 treatment in food consumption ( $F(2, 42) = 1.456$ ;  $p = 0.2448$ ) (Fig. 5D) or water consumption ( $F(2, 42) = 0.005$ ;  $p = 0.994$ ) (Fig. 5E). Once again, CMS121 had no effect on these parameters analyzed.



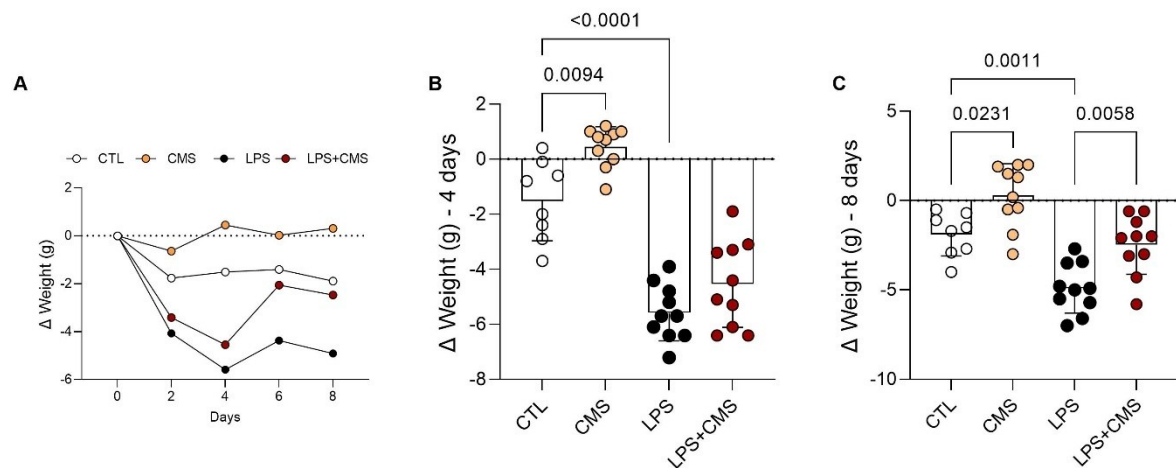
Capítulo 3 - Figure 5 - Weight variation and consumption after pneumosepsis  
Weight variation and water and food consumption after pneumosepsis with *K. pneumoniae* and treatment with CMS121 at different doses 4 hours after the insult. (A) Body weight variation from prior

to *K. pneumoniae* induction up to 15 days after, values expressed in grams; Body weight variation on day 8 (B) and 15 (C) after *K. pneumoniae* induction and CMS121 administration (n = 6-7 mice per group); (D) Cumulative food consumption over 15 days; (E) Cumulative water consumption over 15 days. Values expressed as mean or mean + SD.

#### 4.3.3.3 *CMS121 attenuates body weight loss after LPS treatment and mitigated the post-treatment hyperactivity*

Since CMS121 could not mitigate the alterations caused by sepsis, and, considering that the septic insult with *K. pneumoniae* may have triggered a strong inflammatory response, we tested the effects of CMS121 on a more controlled and well-known inflammatory response induced by LPS (5 mg/kg; i.p.). In this phase, CMS121 treatment was administered orally, diluted in peanut oil, at a concentration of 30 mg/kg per animal, starting 3 days before the LPS insult and continuing for up to 7 days after the start of treatment.

The body weight, food consumption, and the clinical score of the animals were evaluated. One-way ANOVA showed statistically significant differences in body weight variation in 4 days after de LPS injection ( $F(3,34) = 50.43$ ;  $p < 0.0001$ ) (Fig. 6B). Post-hoc testing indicated that CMS121 treatment prevented weight loss observed in the control group ( $p = 0.0094$ ) and the LPS insult group experienced greater weight loss compared to control ( $p < 0.0001$ ), but CMS121 treatment in the LPS group could not mitigate this weight loss ( $p = 0.2465$ ) (Fig. 6B). These body weight changes were maintained up to 8 days after LPS injection, one-way ANOVA showed statistically significant differences ( $F(3,34) = 19.62$ ;  $p < 0.0001$ ) (Fig. 6C). Post-hoc testing indicated that CMS121 treatment maintained the prevention weight loss observed in the control group ( $p = 0.0231$ ) and the LPS insult group also showed greater weight loss compared to control ( $p = 0.0011$ ) (Fig. 6C). However, 8 days after the LPS injection the CMS121 treatment in the LPS group was able to mitigate this weight loss ( $p = 0.0058$ ) (Fig. 6C).

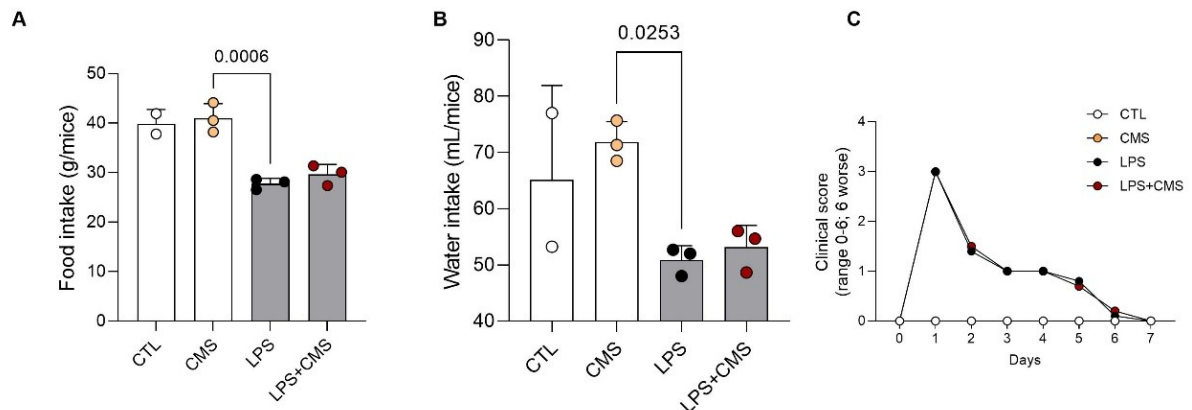


Capítulo 3 - Figure 6 - Body weight variation after LPS

Body weight variation in an LPS-induced inflammation model with oral CMS121 treatment. (A) Body weight variation from before LPS injection up to 8 days after. (B) Body weight variation on day 4 after i.p. LPS administration; (C) Body weight variation on day 8 after i.p. LPS administration. N=8-10 per group. Values are expressed as mean or mean +SD. Statistical differences (p values) are indicated in B and C.

One-way ANOVA showed statistically significant differences in food and water consumption among the animals ( $F(3,7) = 24.69$ ,  $p = 0.0004$ ;  $F(3,7) = 5.733$ ,  $p = 0.026$ , respectively) (Fig. 7A-B). Sidak post-hoc testing indicated that the LPS insult group had lower consumption compared to the CMS group ( $p = 0.0006$  for food intake and  $p = 0.025$  for water intake) (Fig. 7A-B). Due to the low sample size of the control group ( $n=2$ ), these comparisons of water and feed consumption were carried out with the CMS group, once it was analyzed that there is no difference between the consumption of the control group and the CMS.

Like the *K.pneumoniae* insult, 24 hours after the LPS insult the animals also showed clinical worsening, reflected in an increase in clinical score from 0 to 3 (Fig. 7C). The clinical score progressively decreased until reaching zero (normal) by the end of the 7-day treatment period. CMS121 treatment had no effect on the clinical score profile. One-way ANOVA revealed significant effects for the factors: days ( $F(2,633, 89.52) = 232.7$ ;  $p < 0.0001$ ), groups ( $F(3, 34) = 464.1$ ;  $p < 0.0001$ ), and the interaction between days  $\times$  groups ( $F(21, 238) = 78.24$ ;  $p < 0.0001$ ) (Fig. 7C). Post-hoc testing indicated worsening of the clinical score in the LPS group compared to control from day to day 4 ( $p < 0.005$ ), with recovery by the sixth day ( $p = 0.753$ ).



Capítulo 3 - Figure 7 - Food consumption and clinical score after LPS

Food and water consumption, and clinical score in an LPS-induced inflammation model with oral CMS121 treatment. (A) Total food consumption per animal per cage; (B) Total water consumption per animal per cage, values expressed in mL; (C) Total clinical score, assessed daily with scores ranging from 0 to 6 (n=8-10). Values are expressed as mean or mean + SD.

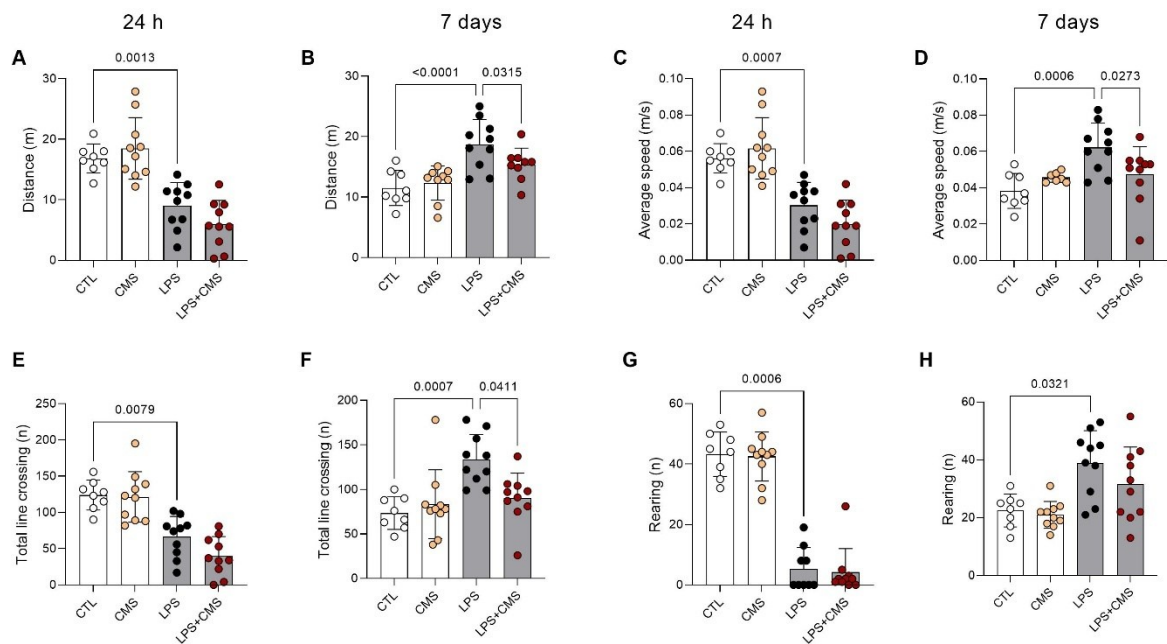
Behavior was assessed using the open field test 24 hours and 7 days after the LPS insult. Within the first 24 hours, One-way ANOVA showed a statistically significant difference in the distance traveled ( $F(3,34) = 22.03$ ;  $p < 0.0001$ ), and the post-hoc test indicated a reduction in distance traveled in the LPS group compared to the control ( $p = 0.0013$ ), again with no effect of CMS121 treatment (Fig. 8A). One-way ANOVA test was also performed to compare the distance traveled in the open field in 7 days ( $F(3,32) = 9.54$ ;  $p < 0.0001$ ). Given the significant difference in groups medians, the Holms Sidak post-hoc test indicated that, unlike the decrease at 24 hours after the insult, at 7 days post-insult there was an increase in the distance traveled by the LPS group compared to the control ( $p < 0.0001$ ), where CMS121 treatment reverted this effect ( $p = 0.031$ ) (Fig. 8B).

There was a statistically significant difference in the average speed reached during the 24 h test ( $F(3,34) = 21.87$ ;  $p < 0.0001$ ), and the post-hoc test indicated a decrease in average speed in the LPS group compared to the control ( $p = 0.0007$ ), with no effect of CMS121 treatment (Fig. 8C). 7 days after LPS injection, One-way ANOVA showed a statistically significant difference in the average speed reached ( $F(3,31) = 6.421$ ;  $p = 0.0016$ ), and the post-hoc test indicated an increase in the group that received the LPS insult compared to the control ( $p = 0.0006$ ) (Fig. 8D). Additionally, in

this timepoint, CMS121 treatment reduced the average speed of the animals exposed to LPS ( $p=0.027$ ) (Fig. 8D).

There was a statistically significant difference in the total line crossings reached during the 24 h test ( $H=26.29$ ;  $p<0.0001$ ) as revealed by the Kruskal-Wallis test. The post-hoc comparison of means indicated a decrease in total line crossings in the LPS group compared to the control ( $p=0.007$ ), without effect of CMS121 treatment (Fig. 8E). 7 days after LPS injection, Kruskal-Wallis test showed a statistically significant difference in the total line crossings reached ( $H=17.1$ ;  $p=0.007$ ). The post-hoc test indicated an increase in the group that received the LPS insult compared to the control ( $p=0.007$ ) (Fig. 8F). Additionally, CMS121 treatment reduced the average speed of the animals exposed to LPS ( $p=0.041$ ) (Fig. 8F).

The Kruskal-Wallis test was performed to compare the number of rearing 24 h after LPS treatment ( $H=27.3$ ;  $p<0.0001$ ). As there was a significant difference in group medians, Dunn's post-hoc test indicated a reduction in the number of rearing in the LPS group compared to the control ( $p=0.0006$ ) (Fig. 8G), with no influence from CMS121 treatment. The Kruskal-Wallis test showed a statistically significant difference in the number of rearing performed by the animals 7 days after LPS injection ( $H=13.08$ ;  $p=0.004$ ). The post-hoc test indicated that, unlike the decrease at 24 hours, there was an increase in the number of rearing in the LPS group compared to the control ( $p=0.032$ ), with no effect of CMS121 treatment (Fig. 8H).



Capítulo 3 - Figure 8 - Locomotor activity and rearing after LPS

Locomotor activity and rearing in the open field conducted at 24 hours (A, C, E and G) and 7 days (B, D, F and H) after LPS i.p. administration: (A-B) Distance traveled (cm); (C-D) Average speed (m/s); (E-F) Total line crossing, and (G-H) Number of rearing; N = 8-10 animals per group. Values are expressed as mean + SD. Statistical differences are indicated in the graphs, which were computed for the effect of CMS, LPS and ability of CMS121 to revert the effects induced by LPS (LPS vs LPS+CMS).

The inflammation induced by LPS resulted in alterations in functional activity. Within the first 24 hours after the LPS challenge distance traveled, average speed, total line crossings, and a reduction in the number of rearing. CMS121 was unable to revert the alterations in locomotor activity induced by LPS. Seven days after LPS injection, all these behaviors presented the opposite effects, that is an increase in the locomotor activity (distance, velocity and total lone crossings), as well as in the rearing behavior. Interestingly, CMS121 treatment was able to reverse the LPS effect, where the increased activity returned to normal, such as locomotor distance, distance and line crossings. The rearing behavior presented a tendency to return to normal, but without statistical significance.

The weight loss observed up to the 4th day following LPS administration was reversed by CMS121 on the 7th day after treatment, since by the end of the treatment period, the body weight of the CMS121 treated group was comparable to that of the control animals, while the LPS group still present weight loss.

On day 7 after treatment, instead of decreasing, the LPS group exhibited higher locomotor activity than the control group. CMS121 treatment reduced locomotor activity relative to the LPS group, resulting in values like the control group. Although not statistically significant, the change in distance travel followed a pattern like that observed for average speed.

#### 4.3.4 Discussion

##### 4.3.4.1 *CMS121 Effects on Locomotor Activity and Depression-like Behavior*

CMS121 administration increased locomotor activity in healthy animals, as indicated by enhanced distance traveled and average speed in the open field test. These results are in line with the increased metabolic activity found in healthy mice receiving CMS121 in the diet for 6 months (Chapter 1). This result also aligns with previous studies demonstrating that fisetin and its derivatives can modulate energy metabolism and improve age-associated metabolic changes (Ates *et al.*, 2020; Currais *et al.*, 2019; Currais; Raschke; Maher, 2024). However, despite this stimulatory effect on locomotion, CMS121 failed to induce antidepressant-like effects in the tail suspension test. Moreover, repeated CMS121 treatment over seven days did not influence anxiety-related behavior in the elevated plus maze or anhedonia-like behavior in the sucrose splash test. These findings suggest that although CMS121 influences motor activity acutely, it does not produce detectable changes in affective-like behaviors in non-stressed, healthy mice at the tested concentrations. This outcome contrasts with reports of flavonoids such as fisetin exhibiting antidepressant-like effects under chronic stress models, suggesting that the presence of a stress-induced pathological background may be necessary to unmask potential antidepressant properties (Wang, Yamin *et al.*, 2017; Zhen *et al.*, 2012). Testing other doses and other treatment routes and regimens are necessary to reject the hypothesis that CMS121 has an anti-depressant-like effect. We used a predictive model (Lobato *et al.*, 2010), where response of healthy animals are likely to be reproduced in models using “depressed” animals, such the mild unpredictable stress (CUMS)(Jiao *et al.*, 2021). The use of models of depression, such as CUMS, can contribute to further test the potential of CMS121 as an antidepressant drug candidate.

#### 4.3.4.2 *Lack of Effect of CMS121 in a Pneumosepsis Model*

Given the safety profile observed in healthy animals, we next evaluated CMS121 in a pneumosepsis model induced by *K. pneumoniae*. CMS121 treatment, administered four hours post-infection, failed to reduce mortality, clinical scores, or body weight loss in this model. Sepsis induces an overwhelming inflammatory response and mitochondrial dysfunction, processes theoretically targeted by CMS121 due to its previously demonstrated anti-inflammatory and mitochondrial-protective properties (Zahid *et al.*, 2023), as well as presented in Chapter 1. However, the acute nature and high severity of the bacterial insult likely limited the window for therapeutic intervention. A similar lack of efficacy has been reported for other antioxidant-based interventions in severe bacterial sepsis models when treatment was not initiated at earlier time points (Venet; Monneret, 2018). Moreover, while CMS121 inhibits fatty acid synthase and activates AMPK, these pathways may not play a central role in the acute immune response necessary for survival during bacterial sepsis (Currais; Raschke; Maher, 2024).

#### 4.3.4.3 *Partial Efficacy in LPS-Induced Inflammation*

In contrast to the bacterial sepsis model, CMS121 showed partial efficacy in the LPS-induced inflammation model. LPS exposure caused acute weight loss, reduced food and water intake, and impaired locomotor activity. While CMS121 did not prevent the initial weight loss following LPS injection, it significantly improved weight recovery by day 7, suggesting a delayed metabolic benefit. This effect may be linked to CMS121's known capacity to improve mitochondrial function and energy metabolism.

The sickness behavior was clearly demonstrated 24 h after the LPS injection. Alterations were observed in the open field, such as decreased locomotor activity (distance, velocity and total line crossings), as well as decreased rearing behavior. None of these behavioral alterations induced by LPS were modified by the CMS121 treatment. At seven days after LPS treatment, clinical scores had returned to basal levels, indicating the full clinical recovery. Nevertheless, the locomotor activity changed toward a contrary direction, LPS treatment induced a marked increase in the locomotor

activity (distance, velocity and total line crossings), as well as an increased rearing behavior. Interestingly, CMS reversed all these late behavioral changes in the locomotor activity, and rearing presented a tendency to return to control levels.

Septic individuals experience the worst clinical outcome, particularly in physical and cognitive domains, when compared to other hospitalized patients (Comim *et al.*, 2009; Iwashyna *et al.*, 2010). Additionally, studies suggest that various disease mechanisms are associated with diffuse brain dysfunction, known as sepsis-associated encephalopathy (SAE), in which patients may present with symptoms ranging from mild inattention or disorientation to more severe consciousness disturbances, such as coma (Chaudhry; Duggal, 2014). Survivors of sepsis may experience long-term cognitive and functional limitations, including learning and memory disorders, attention and mood disturbances, dysfunctions such as fatigue, dyspnea, muscle weakness, reduced physical capacity, and impaired quality of life, with significant interactive repercussions on family and social life (Borges *et al.*, 2015). Despite CMS121 cannot prevent the sickness behavior observed 24h after LPS injection, it reversed the locomotor alterations 7 days after LPS injection. It would be interesting to test if CMS121 can modulate long-lasting alterations observed after a sepsis episode.

The observed effect by lowering circulating lipid was observed with the use of C57/BL6 mice in a diet of 200/400 mg/kg during 6 months (Chapter 1 and (Zahid *et al.*, 2023)), therefore, longer periods of treatment may be required for the effects to take place. This hypothesis is relevant for the short term experiments when the antidepressant and anti-inflammatory effects were tested.

#### 4.4 DISCUSSÃO INTEGRADA

O presente trabalho investigou os efeitos do composto CMS121, um derivado sintético da fisetina, sobre alterações metabólicas, inflamatórias e comportamentais em diferentes modelos murinos, incluindo contextos fisiológicos (animais saudáveis), alterações metabólicas crônicas (obesidade e envelhecimento) e processos inflamatórios agudos (LPS e sepse). A estratégia adotada permitiu avaliar a ação do CMS121 revelando um perfil dependente do contexto fisiopatológico.

Nos experimentos de longo prazo com camundongos C57BL/6 saudáveis, submetidos à suplementação dietética com CMS121 por seis meses, foi observada uma melhora substancial no perfil metabólico dos animais. Esses efeitos incluíram redução significativa no ganho de peso, aumento da massa muscular, menor acúmulo de gordura e aumento na taxa metabólica basal. A redução nos níveis de insulina, glicose e HbA1c, bem como a diminuição dos ácidos graxos livres e triglicerídeos, ressaltam diversas melhorias metabólicas proporcionadas pelo CMS121, similares às observadas nos camundongos *db/db* tratados com a mesma dieta (Zahid *et al.*, 2023).

A análise de tecidos revelou aumento da biogênese mitocondrial no tecido adiposo, com elevação dos níveis de Nrf1 e TFAM, e inibição da lipogênese hepática, (Zahid *et al.*, 2023). Sabendo-se que o CMS121 tem um efeito inibitório sobre a atividade da FASN no fígado e que há aumento da fosforilação da ACC1 no tecido adiposo, este parece ser o mecanismo de ação mais provável do CMS121. A inibição da síntese de ácidos graxos já havia sido associada à redução de ácidos graxos poli-insaturados livres em neurônios primários e em cérebros de camundongos transgênicos com doença de Alzheimer (Ates *et al.*, 2020), bem como à redução de ácidos graxos livres plasmáticos em camundongos obesos *db/db* (Zahid *et al.*, 2023), e no córtex cerebral e plasma de camundongos envelhecidos SAMP8 (Currais *et al.*, 2019). Interessantemente, nestes modelos de doenças neurodegenerativas o aumento dos níveis de acetil-CoA pare estar intimamente ligado aos efeitos benéficos do CMS121 (Currais *et al.*, 2019).

Essas alterações são compatíveis com a indução de um estado metabólico cetogênico, refletido também pelo aumento de acilcarnitinas de cadeia curta e metabólitos do butirato no plasma (Hung *et al.*, 2020). O aumento da mobilização lipídica promove a oxidação de ácidos graxos, gerando mais acetil-CoA nos

hepatócitos (Guertin; Wellen, 2023), substrato para formação de corpos cetônicos como acetoacetato e metabólitos do butirato. A dieta cetogênica é conhecida por reduzir drasticamente os níveis hepáticos de triglicerídeos (Luukkonen *et al.*, 2020), o que também foi observado com a dieta CMS121. Além disso, a dieta cetogênica pode aumentar a hidrólise de lipídios hepáticos, associada à redução da insulina sérica e aumento da tolerância à glicose (Mooli; Ramakrishnan, 2022), em concordância com os níveis mais baixos de insulina e a melhora do status glicêmico observados nos animais tratados com CMS121.

O CMS121 promove uma reprogramação metabólica significativa no fígado e tecido adiposo, inibindo a síntese de ácidos graxos por meio da ativação da AMPK e inibição da FASN, promovendo a fosforilação inibitória da ACC1, o que aumenta os níveis de acetil-CoA (Ates *et al.*, 2020; Guertin; Wellen, 2023). Este composto também parece reduzir a gliconeogênese (via redução de PEPCK) e estimular a glicólise (via aumento de PFKFB3 e redução de TXNIP), favorecendo o uso de glicose como fonte energética. Apesar da redução da fosforilação da LDH-A, que inibe sua atividade (Hui *et al.*, 2017), os níveis elevados de lactato e piruvato sugerem produção extra-hepática e possível uso pelo músculo esquelético como fonte de energia quando camundongos obesos foram tratados com CMS121 (Zahid *et al.*, 2023).

A redução da fumarato hidratase (FH) sugere aumento do fumarato e da produção de itaconato, o que pode favorecer a  $\beta$ -oxidação e contribuir para a remodelação metabólica. Foi relatado que uma deficiência de FH protege fortemente contra obesidade, resistência à insulina e esteatose hepática (Yang, Hao *et al.*, 2016). Paralelamente, observou-se aumento da síntese de colesterol, frequentemente encontrado em estudos com dieta cetogênica (Dyńska *et al.*, 2023), possivelmente via acetil-CoA e a via do mevalonato, e aumento do metabólito 7-HOCA (um metabólito do colesterol) como demonstrado anteriormente (Faulkner; Jo, 2022; Saeed *et al.*, 2017).

Além disso, a limitação da atividade hepática da FASN compromete a retenção de colesterol na membrana plasmática (Wei *et al.*, 2016), o que pode constituir outra possível explicação para os níveis elevados de colesterol no plasma dos animais que receberam a dieta com CMS121. A inibição da FASN hepática pode reduzir o conteúdo de colesterol na membrana plasmática, atenuando processos

inflamatórios (Wei *et al.*, 2016). De fato, CMS121 reduziu marcadores inflamatórios como caspase 1, caspase 3, e NOX4 no fígado, efeitos semelhantes aos observados anteriormente em camundongos obesos db/db tratados com o mesmo composto (Zahid *et al.*, 2023).

No modelo de obesidade induzida por dieta rica em gordura e frutose, os efeitos do CMS121 foram mais modestos. Como esperado, a dieta aumentou o peso corporal, a ingestão calórica, os níveis circulantes de LDL e colesterol, além da ingestão alimentar, conforme descrito na literatura (Buettner; Schölmerich; Bollheimer, 2007; Petronek *et al.*, 2019), entretanto essas alterações não foram moduladas pela inclusão do CMS121 na dieta. Apesar da ineficácia em limitar as alterações induzidas pela dieta HF, o CMS121 produziu resposta clara em camundongos controle, semelhante à observada em animais saudáveis no modelo de envelhecimento, como mostrado no Capítulo 1. O CMS121 promoveu a redução dos níveis plasmáticos de triglicerídeos e colesterol em animais jovens, além de melhora histológica hepática, com redução da esteatose microvesicular. Isso sugere que sua ação primária nesse protocolo foi a modulação do metabolismo lipídico e proteção hepática em animais saudáveis, em concordância com estudos anteriores que demonstram a eficácia do CMS121 na melhora da função mitocondrial e redução do acúmulo de lipídios em doenças metabólicas (Ates *et al.*, 2020; Currais *et al.*, 2019; Zahid *et al.*, 2023).

Os resultados negativos na dieta HF não justificou o estudo bioquímico, uma vez que a hipótese primária foi rejeitada pelos dados experimentais, isto é, ausência de benefícios na dieta HF. Estes experimentos adicionais poderiam trazer elementos importantes sobre a bioquímica hepática, especialmente em relação a redução de microvesículas lipídicas. Em contraste com um tratamento que iniciou com animais de 45 dias de que durou 6 meses, os experimentos com HF foi realizado com animais foram de 4 meses e em uma linhagem diferente. Neste estudo com HF, os camundongos Swiss de 3 meses de idade, e não C57/BL6, foram usados. Neste sentido, especulamos que pode haver uma diferença de sensibilidade entre as linhas, o que poderia ser um fator relevante. Adicionalmente, a ração com alto teor de gordura pode ter afetado a disponibilidade de CMS121. Neste sentido, os efeitos observados nos animais controle da dieta HF são compatíveis com os dados obtidos no Capítulo 1, que apontam na mesma direção daqueles observados na dieta HF. Adicionalmente,

houve alguns episódios de falta de luz que podem ter afetado os animais promovendo um estresse indesejado.

Em contraste com os dados do Capítulo 1, onde o CMS121 promoveu um menor ganho de peso, o ganho de peso dos animais na dieta HF não foi diferente entre os animais controle e àqueles que receberam CMS121. Mais uma vez indicando que o efeito nos animais Swiss foi muito mais modesto do que àquele observado nos animais C57/BL6. No caso dos animais Swiss, é conhecido que há um ganho de peso substancial com a idade (Menahan, 1982), entretanto a linhagem C57/BL6 tem uma tendência muito menor de aumento de peso com a idade, como reportado na curva de crescimento disponível em Jackson Laboratory. Diferentemente deste menor ganho de peso com a idade, os animais usados no Capítulo 1 tiveram um enorme ganho de peso, sendo que alguns animais chegaram a 80g, o que é cerca de 3 vezes o peso do animal jovem. A diferença média de peso chega a ser 44% maior nos animais estudados no capítulo 1, se comparado ao esperado (Jackson Laboratory). O tratamento com CMS121 impediu este ganho de peso adicional, uma vez que o peso final foi 40% menor do que os animais controle. Neste contexto, as condições ambientais ou fatores genéticos parecem ter aumentado o ganho de peso nos animais C57/BL6. Sendo isto verdade, esta cepa de camundongos poderia ser usada com um modelo de obesidade ao longo do envelhecimento. Podemos especular que o CMS121 pode ter agido em um mecanismo específico, que poderia ser diferente na linhagem Swiss e, portanto, ter apresentado pouco efeito na dieta lipídica e nos animais controle.

Um achado relevante do Capítulo 1 foi o fato de que o tratamento com CMS121 aumentou a atividade locomotora dos animais aos 15 dias de tratamento, entretanto este achado não pode ser confirmado ao final do tratamento por razões técnicas. Maior atividade física poderia reverter a perda de força e massa muscular (sarcopenia), características do envelhecimento (Distefano; Goodpaster, 2018). Neste sentido, os animais jovens tratados por 7 dias com CMS121 apresentaram um claro aumento na atividade locomotora nos animais nas 3 doses estudadas (1, 3 e 10 mg/kg). Experimentos adicionais são necessários para confirmar esta hipótese.

Paradoxalmente ao aumento na atividade locomotora nos animais jovens, o tratamento com CMS121 atenuou o aumento na atividade locomotora dos animais que

foram tratados com LPS, como indicado pelo aumento na distância percorrida, velocidade e no total de linhas cruzadas. É conhecido que há uma drástica redução da atividade locomotora dos animais 24 horas após a sepse experimental (Catalão *et al.*, 2022), entretanto, alguns estudos encontram aumento na atividade locomotora dos animais sépticos durante o período de recuperação (Catalão *et al.*, 2022), dependendo do regime de tratamento. Associando o efeito do CMS121 aumentando a atividade locomotora em animais jovens com a reversão da hiperatividade encontrada 7 dias após a injeção de LPS, parece que o CMS121 é capaz de modular a atividade locomotora dependendo do contexto.

Os testes comportamentais com animais saudáveis revelaram aumento da atividade locomotora após administração de CMS121, como ocorreu no modelo de envelhecimento com animais C57BL/6, porém sem efeitos significativos sobre parâmetros como anedonia, comportamento tipo-depressivo ou tipo-ansioso. Esses achados indicam que o composto pode exercer ação estimulante leve em condições fisiológicas. Entretanto, seus potenciais efeitos neuromodulatórios podem depender da presença de um contexto patológico induzido por estresse, a exemplo do efeito já relatado da fisetina, que apresenta efeitos antidepressivos em modelos de estresse crônico (Wang, Yamin *et al.*, 2017; Zhen *et al.*, 2012).

Em modelos inflamatórios agudos, o CMS121 teve eficácia variável. No modelo de sepse induzida por *Klebsiella pneumoniae*, não foram observadas alterações na mortalidade, nos escores clínicos ou na perda de peso. Para evitar efeitos potencialmente deletérios da sepse, os tratamentos anti-inflamatórios devem ser administrados por um curto período após o insulto (Venet; Monneret, 2018), o que sugere que a rápida progressão da infecção bacteriana possa ter superado a capacidade moduladora do CMS121. Embora no modelo de inflamação sistêmica induzida pelo LPS, o tratamento com CMS121 também não tenha sido capaz de prevenir efeitos deletérios iniciais, como perda aguda de peso, redução na ingestão de alimentos e água e comprometimento da atividade locomotora, o CMS121 promoveu efeitos tardios benéficos. A recuperação do peso corporal e a reversão da hiperlocomoção sete dias após o insulto, sugerem um papel na modulação metabólica pós-inflamatória e principalmente de sequelas comportamentais. Considerando o fato de que pacientes sobreviventes à sepse podem apresentar limitações cognitivas e

funcionais a longo prazo (Borges *et al.*, 2015), seria interessante investigar se o CMS121 pode modular as sequelas da sepse.

Em conjunto, os resultados destacam o potencial do CMS121 como agente geroneuroprotetor e modulador metabólico, com efeitos mais pronunciados em contextos crônicos e de disfunção mitocondrial. Estudos adicionais são necessários para elucidar melhor os mecanismos moleculares envolvidos, explorar a eficácia em modelos de estresse crônico ou neurodegeneração e avaliar possíveis combinações terapêuticas que otimizem seus efeitos em condições inflamatórias agudas.

A ausência de efeitos mais amplos pode estar relacionada à formulação da dieta, à biodisponibilidade do composto ou à necessidade de doses mais elevadas, especialmente em modelos com resistência metabólica já estabelecida. Além disso, a linhagem murina (Swiss), distinta da C57BL/6 utilizada em estudos prévios, pode ter influenciado os resultados, uma vez que diferenças genéticas e ambientais impactam diretamente a resposta farmacológica.

## 5 CONCLUSÃO

Os resultados obtidos neste trabalho indicam que o CMS121, um derivado sintético da fisetina, exerce efeitos farmacológicos promissores em diferentes modelos murinos que simulam condições associadas ao envelhecimento, incluindo disfunções metabólicas, processos inflamatórios e alterações comportamentais. A atuação do composto foi especialmente relevante em cenários de distúrbios metabólicos crônicos, nos quais promoveu melhora do perfil glicêmico e lipídico, aumento da massa magra, redução da adiposidade e indução de um estado metabólico compatível com a cetogênese. Tais efeitos foram acompanhados por alterações moleculares consistentes, como aumento da biogênese mitocondrial, inibição da lipogênese hepática e redução de marcadores inflamatórios.

Nos animais tratados por 6 meses, os dados demonstraram que o CMS121 é capaz de induzir uma reprogramação metabólica adaptativa, favorecendo a oxidação de ácidos graxos e a preservação da função hepática e do tecido adiposo. Essa ação multifatorial posiciona o composto como potencial candidato a terapias voltadas à prevenção e ao tratamento de doenças metabólicas crônicas associadas à idade, como a esteatose hepática não alcoólica e a obesidade osteossarcopênica. A modulação dos níveis de ácidos graxos poli-insaturados, a inibição da FASN e da ACC1, e o aumento de acetil-CoA nos tecidos periféricos e possivelmente no cérebro indicam também implicações relevantes para condições neurodegenerativas com componente metabólico.

Entretanto, em contextos de inflamação aguda intensa, como na sepse bacteriana, o CMS121 não apresentou efeitos terapêuticos significativos. Por outro lado, em um modelo de inflamação sistêmica subaguda induzida por LPS, o composto demonstrou efeitos benéficos na fase de recuperação, sugerindo um possível papel na modulação de sequelas pós-inflamatórias. Esses achados reforçam a ideia de que a eficácia do CMS121 está condicionada ao tipo, intensidade e cronicidade do insulto fisiopatológico.

Em modelos comportamentais com animais jovens, o CMS121 aumentou a atividade locomotora, mas não exerceu efeitos antidepressivos, ansiolíticos ou de anedonia, indicando que os seus potenciais efeitos neuromodulatórios poderiam ser testados em modelo patológicos ao invés de condições basais. A ausência de

resposta em animais sem alterações comportamentais prévias pode também indicar a necessidade de modelos baseados em estresse crônico para revelar seu potencial terapêutico nessa dimensão.

Apesar dos resultados encorajadores, este estudo apresenta algumas limitações importantes. Primeiramente, a maior parte dos experimentos foi conduzida exclusivamente com camundongos machos, o que limita a extrapolação dos achados para fêmeas, especialmente considerando as diferenças sexuais conhecidas em doenças metabólicas e neuropsiquiátricas. Em segundo lugar, o uso de diferentes linhagens murinas (C57BL/6 e Swiss) pode ter contribuído para a variabilidade nas respostas ao tratamento, dificultando a padronização dos resultados. Além disso, a ausência de análises moleculares mais aprofundadas em alguns modelos, como o de sepse, restringe a interpretação dos mecanismos subjacentes aos efeitos (ou à ausência deles) do CMS121. Outro ponto limitante foi o curto tempo de administração em modelos agudos, o que pode ter sido insuficiente para a manifestação plena dos efeitos terapêuticos do composto. Por fim, a biodisponibilidade real do CMS121 em diferentes formulações e contextos experimentais não foi mensurada, o que impede conclusões definitivas sobre sua farmacocinética e eficácia relativa nas diferentes vias de administração.

Em síntese, os achados desta tese evidenciam que o CMS121 possui um perfil farmacológico contexto-dependente, com maior eficácia em cenários de disfunção metabólica crônica e menor impacto em inflamações agudas de alta gravidade. O composto emerge como um potencial agente terapêutico no manejo de doenças metabólicas e neurodegenerativas associadas à disfunção mitocondrial e inflamação crônica de baixo grau. Futuras investigações devem aprofundar os mecanismos moleculares subjacentes à sua ação, explorar diferentes regimes de administração, avaliar o impacto do sexo e da linhagem animal sobre os efeitos observados, além de testar formulações combinadas com terapias convencionais que ampliem sua eficácia em quadros agudos e complexos.

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