UNIVERSIDADE DE BRASÍLIA
CAMPUS UNIVERSITÁRIO DARCY RIBEIRO
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA MOLECULAR

VICTOR LUNA PICOLO

Impacto da dieta rica em lipídios nas funções mitocondriais cerebrais e no comportamento de peixe-zebra: papel do envelhecimento

BRASÍLIA

2025

UNIVERSIDADE DE BRASÍLIA
CAMPUS UNIVERSITÁRIO DARCY RIBEIRO
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA MOLECULAR

VICTOR LUNA PICOLO

Impacto da dieta rica em lipídios nas funções mitocondriais cerebrais e no comportamento de peixe-zebra: papel do envelhecimento

Tese de doutorado apresentada à Faculdade de Medicina da Universidade de Brasília como parte dos requisitos para obtenção do título de doutor em Patologia Molecular

Área de Concentração: Bioquímica.

Orientadora: Profª. Drª. Andreza Fabro de Bem

Co-orientador: Prof. Dr. Daniel Mendes Pereira Ardisson de Araújo

BRASÍLIA

2025

Ficha catalográfica elaborada automaticamente,
com os dados fornecidos pelo(a) autor(a)

Li Luna Picolo, Victor
Impacto da dieta rica em lipídios nas funções mitocondriais cerebrais e no comportamento de peixe-zebra: papel do envelhecimento / Victor Luna Picolo; orientador Andreza Fabro de Bem; co-orientador Daniel Ardisson-Araújo. Brasília, 2025.
86 p.

Tese(Doutorado em Patologia Molecular) Universidade de Brasília, 2025.

1. Neurociência. 2. Metabolismo. 3. Envelhecimento. 4. Peixe-zebra. 5. Comportamento. I. Fabro de Bem, Andreza, orient. II. Ardisson-Araújo, Daniel, co-orient. III. Título.

VICTOR LUNA PICOLO

Impacto da dieta rica em lipídios nas funções mitocondriais cerebrais e no comportamento de peixe-zebra: papel do envelhecimento

Tese de doutorado apresentada à Faculdade de Medicina da Universidade de Brasília como parte dos requisitos para obtenção do título de doutor em Patologia Molecular

Área de Concentração: Bioquímica.

BANCA EXAMINADORA

Prof Dra. Andreza Fabro de Bem, Universidade de Brasília – Presidente

Prof Dr. Felipe von Glehn Silva, Universidade de Brasília

Prof Dra. Ana Carolina Luchiari, Universidade Federal do Rio Grande do Norte

Dr. Mauro Eugênio Medina Nunes, Universidade Federal do Pampa

Prof. Dr. Rafael Barrera Salgueiro, Universidade de Brasília - Suplente

BRASÍLIA

2025

Dedico esta tese ao meu pai e minha mãe com quem compartilho o mérito de todas as minhas conquistas.

AGRADECIMENTOS

Aos meus pais, minha eterna gratidão. Vocês são o alicerce do meu ser, oferecendo apoio e incentivo em cada passo, tanto na minha trajetória pessoal quanto profissional.

À professora Andreza Fabro de Bem, deixo meu sincero agradecimento por cada oportunidades geradas, por sua paciência e compreensão. Seu apoio foi imprescindível para que eu pudesse crescer e me desenvolver ao longo destes anos.

Agradeço também a todos os professores e colegas que, com dedicação e generosidade, contribuíram para o sucesso deste trabalho. Em especial, as companheiras de bancada, Whitney Ribeiro Santos e Letícia Alves Tavares, ao professor Jair Trapé Goulart, pelos ensinamentos e apoio constantes; e ao professor Cesar Koppe Grisolia, cuja acolhida abriu as portas do seu laboratório e cujo apoio constante serviu como exemplo e inspiração para boas práticas cientificas.

Minha gratidão ao meu colega e amigo Wembley Vilela, que esteve presente tanto nos momentos de alegria quanto nos difíceis, oferecendo conselhos e ouvindo atento todas minhas milhões de reclamações.

Aos funcionários da Universidade de Brasília, agradeço por tornarem possível a realização deste trabalho, direta ou indiretamente, com sua dedicação e eficiência.

E, finalmente, agradeço às agências de fomento CNPq, CAPES e FAPDF pelo suporte financeiro.

Sumário

RESUMO

Envelhecimento e hábitos alimentares não saudáveis prejudicam, de forma independente e sinérgica, a homeostase do sistema nervoso central (SNC), aumentando a suscetibilidade a distúrbios neurológicos e comportamentais. A mitocôndria desempenha um papel crítico na manutenção da sobrevivência e atividade neuronal, representando um fator central na patogênese das doenças neurodegenerativas. Aqui, utilizamos o peixe-zebra como modelo para investigar como o envelhecimento e a dieta hiperlipídica (DH) afetam a bioenergética cerebral e o comportamento. Peixes-zebra machos jovens (4–6 meses) e envelhecidos (17–22 meses) foram alimentados por 14 dias com uma dieta padrão ou uma DH à base de gema de ovo de galinha cozida. As mitocôndrias do cérebro foram avaliadas por meio de respirometria de alta resolução, microscopia eletrônica de transmissão (MET) e qRT-PCR. A DH prejudicou a saúde metabólica de ambos os grupos, promovendo ganho de peso, aumento do comprimento abdominal e elevação dos níveis de glicemia em jejum. O envelhecimento intensificou os efeitos deletérios da DH sobre o comportamento: peixes-zebra envelhecidos e alimentados com DH exibiram comportamento tipo ansioso no teste do tanque novo e comprometimento cognitivo no teste do labirinto em T. Notavelmente, a DH não teve efeito significativo sobre o comportamento agressivo, independentemente da idade. Os efeitos da DH sobre a funcionamento mitocondrial variou conforme a idade: enquanto a função bioenergética cerebral declinou nos peixes jovens, os animais envelhecidos apresentaram uma tendência oposta. A análise por MET revelou um acúmulo aumentado de mitocôndrias fragmentadas no grupo DH, indicando possível disfunção mitocondrial. O qRT-PCR demonstrou aumento na expressão de genes envolvidos na cadeia de transporte de elétrons, especialmente nos peixes envelhecidos. Concluindo, nossos achados demonstram uma vulnerabilidade promovida pelo envelhecimento aos efeitos da DH sobre parâmetros neurocomportamentais e mitocondriais.

Palavras-chaves: Dieta hiperlipídica, Envelhecimento, Peixe-zebra, Metabolismo Cerebral, Função Mitocondrial, Cognição

ABSTRACT

Aging and unhealthy eating habits independently and synergistically disrupt central nervous system (CNS) homeostasis, increasing susceptibility to neurological and behavioral disorders. Mitochondria play a critical role in maintaining neuronal survival and activity, representing a central player in the pathogenesis of neurodegenerative diseases. Here, we used zebrafish as a model to investigate how aging and a high-fat diet (HFD) affect brain bioenergetics and behavior. Young (4–6 months) and aged (17–22 months) male zebrafish were fed either a standard diet or an HFD based on boiled chicken egg yolk for 14 days. Brain mitochondria were evaluated using high-resolution respirometry, transmission electron microscopy (TEM), and qRT-PCR. HFD impaired the metabolic health of both young and aged animals, promoting weight gain, increased abdominal length, and elevated fasting glucose levels. Aging intensified the HFD detrimental effects on behavior: aged HFD-fed zebrafish displayed increased anxiety-like behavior in the novel tank test, and impaired cognitive performance in the T-maze test. Notably, HFD had no significant effect on aggressive behavior regardless of age. Mitochondrial responses to HFD differed by age: while cerebral bioenergetic function declined in young fish, aged animals showed an opposite trend. TEM analysis revealed increased accumulation of fragmented mitochondria in HFD group, indicating potential mitochondrial dysfunction. RT-qPCR showed upregulation of genes involved in the electron transport chain, especially in aged zebrafish. In conclusion, our findings demonstrate an age-dependent vulnerability to the effects of HFD on both neurobehavioral and mitochondrial parameters.

Keywords: High fat diet, Aging, Zebrafish, Brain metabolism, Mitochondrial function, Cognition

LISTA DE ABREVIATURAS

ADP	Adenosina difosfato
ATP	Adenosina trifosfato
CNS	Central nervous system
CCCP	Carbonyl cyanide 3-chlorophenylhydrazone
DH	Dieta hiperlipídica
DP	Dieta padrão
HFD	High fat diet
Oligo	Oligomycin
MET	Microscopia eletrônica de transmissão
MIAT	Mirror-induced aggression test
NTT	Novel tank test
PM	Pyruvate and malate
ROT	Rotenone
SD	Standard diet
Succ	Succinate
SV	Synaptic vesicle
TEM	Transmission electron microscopy
TMT	T-maze test

LISTA DE FIGURAS DA REVISÃO BIBLIOGRÁFICA

1. REVISÃO BIBLIOGRÁFICA

1.1. Padrões alimentares e impactos para a saúde humana

Nos últimos 40 anos observamos intensa modificação dos padrões alimentares da população em todo o mundo, com aumento exponencial da produção e consumo de alimentos ultraprocessados. Esses alimentos são altamente palatáveis e caracterizados pela sua pobreza nutricional, contendo baixo teor de fibras, proteínas, vitaminas e minerais, com alta concentração calóricas, e de açúcares e gorduras (Monteiro et al., 2019). Esse novo padrão alimentar está associado a uma piora na qualidade e na expectativa de vida das populações, por conta do aumento da suscetibilidade a diversas doenças (de Miranda et al., 2021; Gomes Gonçalves et al., 2023). O preço acessível e a praticidade de consumo dos alimentos ultraprocessados permitiram a sua incorporação generalizada no dia a dia de indivíduos de todas as classes sociais, inclusive em países pobres e em desenvolvimento (Ferreira et al., 2024; Popkin et al., 2012). Calcula-se que esse tipo alimento colabora para metade do consumo diário de energia em países de alta renda (Baraldi et al., 2018; Moubarac et al., 2017), enquanto em países de média renda, o valor chegue a um terço (Da Costa Louzada et al., 2018; Marrón-Ponce et al., 2018).

O consumo excessivo de açúcares e/ou gorduras causam prejuízo à saúde, impactando a homeostase de diversos órgãos e sistemas do nosso corpo e propiciando o desenvolvimento de doenças como a obesidade, doenças cardiovasculares, síndrome metabólica, dislipidemia, esteatose hepática, diabetes, alguns tipos de cânceres, bem como doenças neurológicas e psiquiátricas (de Miranda et al., 2021; Pagliai et al., 2021). Assim, as alterações nos padrões alimentares têm se tornado um preocupante fator para a saúde pública mundialmente, sendo uma das principais causas da epidemia de obesidade e de suas comorbidades (Popkin et al., 2012; Vandevijvere et al., 2015).

1.1.1. Papel da dieta sobre as alterações metabólicas

O desbalanço entre os macronutrientes, bem como o persistente consumo de calorias em excesso terá impacto sobre a saúde metabólica do indivíduo, sobretudo através do aumento da adipogênese. As disfunções metabólicas, por sua vez, têm sido um tema central de estudo, por seu efeito generalizado na saúde, estando a associada a processos patogênicos (Saklayen, 2018). O

aumento na incidência de diferentes patologias, reduz a qualidade e expetativa de vida, impactando o envelhecimento saudável e promovendo maior gasto com saúde pública, com impacto na capacidade produtiva do país (Lobstein et al., 2023). Por exemplo, indivíduos com síndrome metabólica possuem o dobro de risco de desenvolver doenças cardiovasculares dentro de 5-10 anos, enquanto que os risco para diabetes mellitus quintuplica (Alberti et al., 2009).

A disfunção metabólica pode ser observada a partir de diversas características, como aumentos dos níveis sanguíneos de colesterol, triglicerídeos e glicose, maior circunferência abdominal, resistência sistêmica a hormônios como insulina e leptina, pressão sanguínea alterada e inflamação sistêmica. Assim, diferentes organizações têm se empenhado para desenvolver critérios diagnósticos para a síndrome metabólica, variando pouco entre si. Esse entendimento foi unificado pela associação *American Heart Association/National Heart, Lung and Blood Institute* (AHA/NHLBI) (Alberti et al., 2009) a partir da observação de três dos cinco critérios seguintes: (1) elevação da adiposidade visceral, (2) da glicemia em jejum, (3) da pressão sanguínea, (4) dos triglicerídeos e (5) redução do HDL.

O cálculo da incidência da síndrome metabólica é complicado devido à variação dos critérios diagnósticos, bem como da relação com outras doenças como obesidade e diabetes, dificultando a precisão do diagnóstico. Entretanto, acredita-se que a síndrome metabólica alcance níveis pandêmicos, atingindo mais de um terço da população Estado-Unidense e um quarto da população mundial (NCHS, 2012). Junto com a redução da prática de atividade física, o consumo de dietas desbalanceadas é considerada o principal fator do aumento exorbitante de casos de síndrome metabólica (Saklayen, 2018). Alguns dados epidemiológicos apontam que maus hábitos alimentares é um fator ainda mais relevante que o sedentarismo (Casazza et al., 2009; Hu, 2013). Em um estudo prospectivo envolvendo 120.877 participantes analisados por 12-20 anos, observou-se que os hábitos alimentares tiveram o dobro do efeito no ganho de peso, quando comparado com a prática de atividade física (Mozaffarian et al., 2011). Em um estudo com 6.385 participantes, observou-se uma associação linear entre o consumo de alimentos ultraprocessados e a incidência de síndrome metabólica. Pessoas com ingestão calórica oriunda de ultraprocessados maior do que 71% apresentaram uma prevalência de síndrome metabólica 28% maior que os indivíduos que consumiam menos de 40% de suas calorias em alimentos ultraprocessados (Martínez Steele et al., 2019).

Importante notar que dificilmente a síndrome metabólica se apresentar sozinha, sendo considerada uma comorbidade de doenças como obesidade, diabetes e hipertensão (Monteiro et al., 2019). Por exemplo, a adiposidade visceral, avaliada através da circunferência abdominal, é um dos principais critérios diagnósticos da síndrome metabólica e possui papel central na ativação de mecanismo patogênicos, como inflamação sistêmica e alterações endócrinas. Ao mesmo tempo, essa mesma característica é típica da obesidade (Matsuzawa et al., 2011). Assim, para entender o impacto das alterações alimentares na saúde metabólica é importante ressaltar a tendência de aumento dos casos de obesidade, diabetes mellitus, dentre outras doenças associadas ao consumo de dietas desbalanceadas. No caso da obesidade, calcula-se que a incidência entre 1990 e 2022 tenha duplicado em adultos e quadruplicado em crianças, atingindo 16% da população mundial em 2022 (World Health Organization, 2024), enquanto que a diabetes afetou 415 milhões de pessoas em 2015 (9% da população). No mesmo ano, estima-se que com 5 milhões de mortes e 12% do gasto mundial com saúde estivesse associada com a diabetes (IDF, 2015; Schwingshackl et al., 2017).

1.1.2. Papel da dieta no desenvolvimento de neuropatologias

Hábitos alimentares saudáveis são essenciais para manter a saúde do sistema nervoso central e prevenir neuropatologias no curto prazo, bem como ao longo do envelhecimento. Um estudo recente analisou os componentes da dieta de mais de 180.000 participantes, agrupando-os de acordo com o equilíbrio entre os tipos de alimentos. Observou-se que consumir uma dieta balanceada promoveu melhores escores de bem-estar e saúde cerebral, como demonstrado pela redução de sintomas depressivos, ansiosos, de estresse, de comportamento autodestrutivo e traumas. Houve também redução da suscetibilidade ao desenvolvimento de esquizofrenia, doença de Alzheimer e de Parkinson, acidente vascular cerebral e transtorno de humor bipolar. A cognição foi avaliada por meio de seis testes, dos quais quatro demonstraram o impacto negativo das dietas desbalanceadas. Além disso, uma dieta equilibrada promoveu a manutenção do volume de diversas áreas cerebrais, incluindo áreas relacionadas com a cognição e o processamento de emoções. Tudo isso foi acompanhado por alterações metabólicas que afetam diretamente o SNC, demonstrando como a dieta é capaz de influenciar o funcionamento do SNC de forma generalizada e profunda, geralmente associada com alterações metabólicas sistêmicas (R. Zhang et al., 2024).

No Brasil um estudo longitudinal avaliou os hábitos alimentares de 10.775 participantes por 6-10 anos. Durante esse período a cognição foi avaliada a cada 2-3 anos por meio de seis testes diferentes. Indivíduos com piores hábitos alimentares tiveram um aumento de 28% na taxa de piora na cognição global ao longo do tempo, enquanto o declínio das funções executivas foi 25% maior (Gomes Gonçalves et al., 2023). O consumo de alimentos ultraprocessados foi associado com alterações comportamentais e psiquiátricas em humanos, como o desenvolvimento de sintomas depressivos (Adjibade et al., 2019).

Estudos com modelos animais apontam que as alterações metabólicas induzidas pela dieta tem papel fundamental nas alterações do SNC (de Bem et al., 2021), o que em humanos pode explicar a maior suscetibilidade a doenças psiquiátricas, neurodegenerativas e déficit cognitivo. A predisposição genética a doenças metabólicas é considerada como um fator de risco para o desenvolvimento de doenças psiquiátricas como depressão, transtorno de déficit de atenção, ansiedade e esquizofrenia (Gao et al., 2024). Um estudo de metanálise demonstrou a relação das disfunções metabólicas com o agravamento do quadro de desordem bipolar e o pior desempenho cognitivo (Giménez-Palomo et al., 2022). Em outro estudo envolvendo mais de 2200 indivíduo, observou-se uma correlação entre quadros de depressão atípica e a resistência à leptina, característica comum da obesidade e do consumo crônico de dietas desbalanceada. O efeito foi maior considerando-se a adiposidade corporal, destacando-se o papel da disfunção metabólica na saúde mental (Milaneschi et al., 2017). O consumo de dietas desbalanceadas está associado com o aumento da agressividade e da ansiedade em humanos (Choy, 2023), sendo mais observado em indivíduos obesos e com disfunções metabólicas (Cerniglia et al., 2018; Lindberg et al., 2020). Tais alterações comportamentais e de humor podem ser geradas pelo efeito pró-inflamatório da dieta, que irá impactar localmente o cérebro (Maes et al., 2008). Corroborando esses resultados, um estudo com 336 participantes demonstrou a associação de maior comportamento agressivo com o consumo de dieta desbalanceada e maior circunferência abdominal, bem como menores níveis de triptofano circulante e de serotonina no cérebro (Abiri et al., 2023).

Cabe ressaltar que alguns estudos em humanos apontam para um relação bidirecional entre os hábitos alimentares e sintomas psiquiátricos, comportamentais e/ou cognitivos (Gao et al., 2024; Maksyutynska et al., 2024). Ocorre que os sintomas psiquiátricos e cognitivos podem impelir maus hábitos alimentares, o que por sua vez promove o agravamento dos sintomas neurológicos. Esse

fenômeno é chamado por alguns autores como síndrome do metabolismo-humor, no qual as alterações metabólicas induzidas pela dieta irá ativar mecanismos como desregulação do humor e dos sistemas de reguladores de saciedade aumentando a suscetibilidade ao consumo de dietas desbalanceadas, formando desta forma um ciclo (Mansur et al., 2015). Da mesma forma, observou-se uma relação bidirecional entre a diabetes e a incidência/ gravidade de progressão da depressão, o que foi acompanhado por importantes marcadores de alteração neurológica, i.e. neuroinflamação, alterações estruturais do hipocampo e desregulações autonômicas e neuro-hormonais (Semenkovich et al., 2015).

A aplicação de protocolos com intervenções dietéticas permite elucidar o impacto da dieta sobre o SNC através da modulação de parâmetros metabólicos. Attuquayefio et al. demonstrou que esse efeito pode ocorrer de forma extremamente rápida. 102 indivíduos jovens e saudáveis foram orientados a consumir um café da manhã padrão ou um rico em gordura saturada e açúcar durante 4 dias. Esse curto período de dieta desbalanceada prejudicou a memória dependente do hipocampo de forma proporcional ao aumento dos níveis de glicose circulante (Attuquayefio et al., 2017). Em outro estudo, os hábitos alimentares de 52 crianças com 7-9 anos foram avaliados. O consumo de ácidos graxos não saturados se correlacionaram negativamente com o desempenho cognitivo dependente do hipocampo, demonstrando a vulnerabilidade do SNC à dieta mesmo em tenra idade (Baym et al., 2014). Alterações metabólicas também estão associadas à maior incidência e gravidade da progressão de doenças neurodegenerativas ao longo do envelhecimento (Procaccini et al., 2016), enquanto que a melhora nos parâmetros metabólicos podem reduzir os sintomas da doença de Alzheimer (Watson et al., 2005).

1.2. Modelos animais na investigação das alterações metabólicas e neurológicas induzidas pela dieta

Ainda não se compreende totalmente como a dieta impacta o sistema nervoso central, pois uma série de mecanismos e processos neurofisiológicos estão envolvidos, como a neuroinflamação e, especialmente, a disfunção mitocondrial (McGrattan et al., 2019; Spencer, D'Angelo, et al., 2017a). Modelos animais, sobretudo roedores, são amplamente utilizados para entender como cada um desses mecanismo são ativados e relacionam entre si. Um crescente corpo de evidências sugere que alterações metabólicas sistêmicas induzidas pela dieta, como aumento de lipídios e glicose

circulantes, fatores pró-inflamatórios e distúrbios endócrinos, impactarão localmente o sistema nervoso central, ativando mecanismos moleculares e celulares no cérebro (de Bem et al., 2021; Y. Zhang et al., 2024). Consequentemente, a sobrevivência e o funcionamento neuronal são afetadas, promovendo o aparecimento de sintomas como déficits cognitivos, agressividade e comportamento tipo ansioso e depressivo (Evans et al., 2024; Tan & Norhaizan, 2019).

Nesse contexto, diversos protocolos de dietas desbalanceadas são descritos em modelos animais, com o objetivo de determinar a relação causalista entre os maus hábitos alimentares e parâmetros neurológicos e comportamentais. As dietas hiperlipídica (DH) são bastante aplicadas, evidenciando os efeitos deletérios da ingesta excessiva de lipídeos no SNC. Camundongos alimentados com DH por 12 semanas desenvolveram resistência à insulina e hiperglicemia associado a prejuízos cognitivos avaliado pelo labirinto aquático de Morris. O impacto na cognição foi relacionado com alterações no hipocampo, incluindo inflamação e acúmulo de proteínas Tau-fosforilada (p-Tau) e amiloide-β e estresse oxidativo com efeito na peroxidação lipídica. O tratamento com metformina, um fármaco hipoglicemiante utilizados no tratamento de diabetes, foi eficaz em reduzir as disfunções metabólicas e alterações hipocampais, resultando em uma melhora cognitiva em roedores (FangFang et al., 2017). Resultados similares foram obtidos com ratos alimentados com DH, demonstrando o potencial da metformina em combater as alterações metabólicas, e prevenir as expressão de citocinas e marcadores de ativação glial no hipotálamo, hipocampo, córtex pré-frontal e amídala (Mandwie et al., 2021). Corroborando esses resultados, o tratamento com metformina em humanos com prejuízos cognitivos associados a hiperglicemia também promoveu melhora cognitiva (Lin et al., 2018).

As alterações metabólicas em camundongos alimentados com DH (i.e. aumento de peso, resistência à insulina e hiperglicemia), também foi associada com desenvolvimento de sintomas tipo ansioso e depressivo. Conjuntamente, houve desregulação da sinalização insulínica e do metabolismo de glicose no cérebro, bem como ativação de mecanismos neuroinflamatórios. O controle farmacológico da inflamação foi capaz de remediar o comportamento tipo ansioso induzido pela dieta (Dutheil et al., 2016). Em outro estudo, o consumo de DH por ratas fêmeas promoveu o comportamento tipo ansioso nos testes do campo aberto e no campo claro/escuro. Em associação, houve alteração na expressão de genes relacionados à sinalização de glicocorticoide e a processos inflamatórios no hipocampo, demonstrando que as alterações comportamentais podem

estar relacionadas a mecanismos de sinalização de estresse no hipocampo (Sivanathan et al., 2015). Corroborando esses dados, camundongos alimentados com DH apresentaram maior agressividade e ansiedade, bem como aumento dos níveis séricos de glicocorticoides (Buchenauer et al., 2009).

Mesmo no curto prazo, a dieta hiperlipídica impacta o SNC promovendo déficit cognitivo e alterações comportamentais. Apenas 3 dias de DH já foi capaz de ativar mecanismo de neuroinflamação e apoptose no hipocampo de camundongos, em associação com disfunções metabólicas (Nakandakari et al., 2019), demonstrando como o hipocampo é vulnerável e rapidamente afetado pela dieta. Estudo do nosso grupo de pesquisa mostrou que a cognição e a permeabilidade da barreira hematoencefálica em camundongos foi afetada na primeira semana de ingestão de DH. Sinais de neuroinflamação, disfunção mitocondrial e densidade sináptica reduzida no hipocampo foram verificados após 4 semanas de DH. O tratamento com anti-inflamatório periférico, Infliximab (neutralizador de TNF- α), foi capaz de prevenir o prejuízo cognitivo e o desenvolvimento de comportamento tipo depressivo (de Paula et al., 2021). Em outro estudo, ratos alimentados com DH por 20 dias apresentaram aumento sérico de insulina, leptina e triglicerídeo, além de aumento de massa corporal total e de tecido adiposo branco. Detectou-se marcadores de neuroinflamação e estresse oxidativo no hipocampo, o que foi acompanhado de déficit cognitivo no teste de realocação de objeto após 5 dias de dieta, enquanto não foi observado efeito no teste de reconhecimento do objeto. Tal resultado demonstra a vulnerabilidade do hipocampo aos efeitos da dieta, tendo em vista que o teste de reconhecimento de objeto depende menos do hipocampo e mais de outras áreas cerebrais, quando comparado com o teste de realocação do objeto, que depende majoritariamente do hipocampo (Beilharz et al., 2014).

1.2.1. Peixe-zebra como modelo de obesidade e disfunções metabólicas

O peixe-zebra já é um modelo animal bem consolidado no estudo de diversas doenças humanas como a obesidade, distúrbios metabólicos e doenças neurológicas (Angom & Nakka, 2024; Faillaci et al., 2018; Kalueff et al., 2014). O potencial translacional do peixe-zebra é possibilitado pelas semelhanças com os mamíferos do ponto de vista anatômico, fisiológico e genético (Zang et al., 2018a). O peixe-zebra possui 70% dos genes humanos e cerca de 82% dos genes associados com patologias humanas (Howe et al., 2013). Além disso, o animal possui diversas vantagens, como o pequeno tamanho corporal, ciclo de vida curto, rápida maturação

sexual com grande produção de ovos e facilidade, baixo custo para manutenção em biotério e facilidade de manuseio em laboratório (Bradford et al., 2017; Choi et al., 2021; figura 1). O peixe-zebra também é uma ferramenta poderosa na triagem e desenvolvimento de fármacos, bem como para a avaliação dos efeitos de substâncias presentes no ambiente. Isso é possibilitado pela facilidade de exposição às substancias na água, a possibilidade de se trabalhar com um grande N amostral e pelo desenvolvimento embrionário rápido e visível a olho nu, o que permite avaliar o efeito no desenvolvimento embrionário (Yoganantharjah & Gibert, 2017; figura 2).

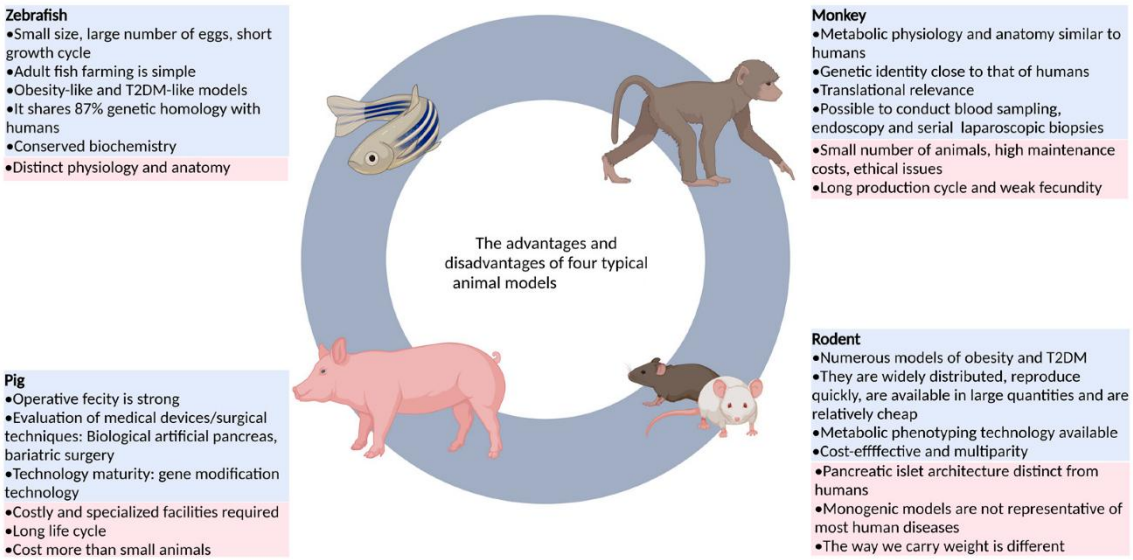

Figura 1. Vantagens e desvantagens dos principais modelos animais no estudo de doenças metabólicas. O uso de diferentes modelos animais permite abordagens experimentais diversas e a avaliação de mecanismos conservados filogeneticamente. O peixe-zebra é considerado um modelo relativamente novo e ainda pouco explorado, apesar de contar com vantagens únicas em relação aos modelos clássicos como transparência corporal, rápido ciclo de vida e facilidade de manipulação genética. Imagem copiada de (Cao et al., 2023) sob licença Creative Commons Attribution 4.0 International.

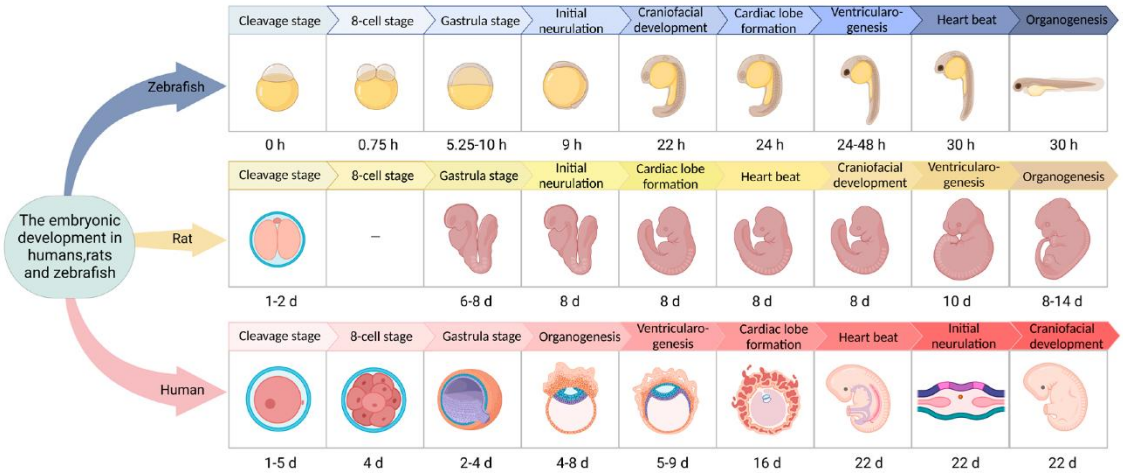

Figura 2. Comparativo do desenvolvimento embrionário entre diferentes espécies. O peixe-zebra possui um desenvolvimento embrionário bastante rápido, com o fim da organogênese ocorrendo 30 horas após a fertilização. Durante esse processo o animal possui transparência corporal e visível por meio de estereomicroscópio facilitando a aplicação de técnicas de microscopia. Imagem copiada de (Cao et al., 2023) sob licença Creative Commons Attribution 4.0 International.

A aplicação do peixe-zebra no estudo da obesidade e alterações metabólicas é possibilitado pela semelhanças fisiológica e celular do tecido adiposo branco e marrom, bem como dos órgãos chaves para o estudo dessas condições (Cao et al., 2023; Seth et al., 2013). Há conservação de mecanismos celulares e bioquímicos associados com o metabolismo de glicose, colesterol e lipídeos (Elo et al., 2007; Oka et al., 2010) e das vias de sinalização da homeostase energética e da sinalização neuroendócrina da saciedade (Gut et al., 2017; Nishio et al., 2012). Além disso, o desenvolvimento embrionário externo e a transparência corporal nas primeiras semanas de vida permitem a fácil aplicação de métodos de microscopia com marcadores de lipídeo para avaliar o desenvolvimento do tecido adiposo e o metabolismo e transporte de lipídeo (Anderson et al., 2011; Flynn et al., 2009).

Os resultados obtidos com o peixe-zebra no estudo da obesidade e alterações metabólicas se assemelham aos dos mamíferos (Cao et al., 2023; Gut et al., 2017; Oka et al., 2010), inclusive em relação ao resultado de abordagens farmacológicas e terapêuticas para tratar ou prevenir tais condições (Asaoka et al., 2013; Misselbeck et al., 2019; Nakayama et al., 2018, 2020). O consumo de DH baseado em gema de ovo por 8 semanas levou a um aumento nos níveis de glicose, triglicerídeos e colesterol circulantes, além do aumento da adiposidade geral e visceral (Landgraf et al., 2017). Uma dieta semelhante foi aplicada por nosso grupo por apenas duas semanas,

promovendo aumento de peso, do index de massa corporal e do comprimento abdominal, um indicativo de adiposidade visceral (Picolo et al., 2021).

1.2.2. Peixe-zebra como modelo de neuropatologias

O sistema nervoso central do peixe-zebra possui conservação em relação aos mamíferos tanto do ponto de vista morfológico, fisiológico e celular, permitindo seu uso como modelo de estudo de diversas neuropatologias. Estão presentes todos os neurotransmissores, neuromoduladores, bem como seus receptores (Soussi-Yanicostas, 2022). Há estruturas homologas às principais áreas cerebrais dos mamíferos relevantes para o estudo da cognição, memória e regulação do humor/ comportamento, como o pálio dorso-lateral (Northcutt, 2006; Portavella et al., 2002), pálio medial (Braford, 1995) e o pálio dorsal (Mueller et al., 2011), que são homólogos ao hipocampo, à amidala e ao isocortex, respectivamente. A disposição dos tipos neuronais em cada região do cérebro se assemelha ao observado em mamíferos, formando conexões e circuitos neuronais semelhantes. (Alsop & Vijayan, 2009; Panula et al., 2006). O sistema serotoninérgico é formado por neurônios em áreas como o hipotálamo, tálamo núcleo da Rafe anterior, cerebelo e glândula pineal (Lillesaar, 2011), e está associado com locomoção (Brustein et al., 2003; Gabriel et al., 2009), comportamento tipo ansioso (Bencan et al., 2009; Sackerman et al., 2010) e comportamento agressivo (Jones & Norton, 2015; Teles et al., 2013). O sistema dopaminérgico também é conservado no peixe e possui papel do controle motor (Anichtchik et al., 2004; Flinn et al., 2008). Neurônios histaminérgicos no hipocampo, amidala e hipotálamo estão associados com regulação da memória, comportamento tipo ansioso e estado de alerta/vigília (Peitsaro et al., 2003).

Há conservação funcional e celular das células da neuroglia, incluindo a micróglia, astrócitos e oligodendrócitos, que estão envolvidas em processos neuropatológicos semelhantes aos humanos. Por exemplo, a resposta da micróglia a moduladores centrais e sistêmicos é semelhante aos mamíferos, resultando em processos neuroinflamatórios considerados centrais na patogênese de doenças neurodegenerativas e psiquiátricas (Zabegalov et al., 2021). Desta forma, o peixe-zebra tem permitido descobertas e avanços no estudo de neuropatologias como, depressão, transtornos de ansiedade e de agressividade (Bally-Cuif & Vernier, 2010; Kyzar et al., 2013; Maximino et al., 2010), distúrbios de memória e aprendizado (Michael Stewart & V. Kalueff,

2012), doenças neurodegenerativas (Kanoh et al., 2024; Panula et al., 2006), além de ser uma excelente ferramenta para a triagem de substancias neuroativas (Kalueff et al., 2014).

O peixe-zebra vem se mostrando um excelente modelo para se avaliar os efeitos das alterações metabólicas sobre o SNC. São descritos tanto modelos genéticos como protocolos de intervenção dietéticas que promovem mudanças metabólicas como aumento de colesterol e triglicerídeos circulantes, aumento de massa gorda, esteatose hepática, alterações endócrinas, incluindo resistência à insulina e leptina e inflamação sistêmica. Em resposta, ocorre a ativação de mecanismos neuropatológicos como disfunção da barreira hematoencefálica, estresse oxidativo, neuroinflamação, resistência hormonal central, e prejuízos à plasticidade neuronal (Ghaddar & Diotel, 2022).

Diversas protocolos de dieta desbalanceadas são descritos e aplicados em peixe-zebra, gerando resultados semelhantes aos observados em roedores (Angom & Nakka, 2024; Seth et al., 2013). No nosso trabalho anterior, demonstramos que apenas 2 semanas de uma DH à base de gema de ovo pode provocar comportamento agressivo e ansioso e cognição prejudicada em peixe-zebra adulto (Picolo et al., 2021). Em outro estudo, após consumir uma DH à base de banha por 8 semanas, os peixes-zebra desenvolveram déficit cognitivo associado à modulação cerebral de genes responsáveis pela defesa antioxidante, integridade da barreira hematoencefálica, função neuronal, apoptose e metabolismo de amiloide- β (Meguro et al., 2019). O consumo de dieta rica em glicose e colesterol por 19 dias promoveu uma robusta resposta pró-inflamatória sistêmica, além de aumento dos níveis séricos de glicose, triglicerídeos e colesterol total. Em associação, houve estimulação do comportamento tipo ansioso com aumento de marcadores de inflamação e apoptose no cérebro (J. Wang et al., 2020). Peixe-zebra obesos superalimentados por 4 semanas apresentaram vazamento da barreira hematoencefálica, neuroinflamação, estresse oxidativo cerebral e neurogênese prejudicada. Uma abordagem terapêutica à base de extrato de planta foi capaz de reverter esses efeitos em associação com uma melhora nos parâmetros metabólicos (Ghaddar et al., 2020).

1.3. Dieta desbalanceada x envelhecimento: Efeito sinergético observado em humanos e modelos animais

Com o aumento considerável da expectativa de vida nos últimos anos, houve um envelhecimento mundial da população sobretudo em países desenvolvidos, acompanhado de

impactos relevantes aos sistemas de saúde. Entre 1990 e 2017, a proporção de pessoas com mais de 65 anos cresceu de 6,1% para 8,8% e estima-se que em 2050 chegue a 16% (Cheng et al., 2020). Com o envelhecimento, uma série de processos fisiológicos, celulares e biomoleculares são prejudicados (López-Otín et al., 2013; figura 3) promovendo um prejuízo generalizado à saúde e aumentando a predisposição a doenças como diabetes, hipercolesterolemia e hipertensão e doenças neurológicas como demência e doenças neurodegenerativas e psiquiátricas (Prince et al., 2015).

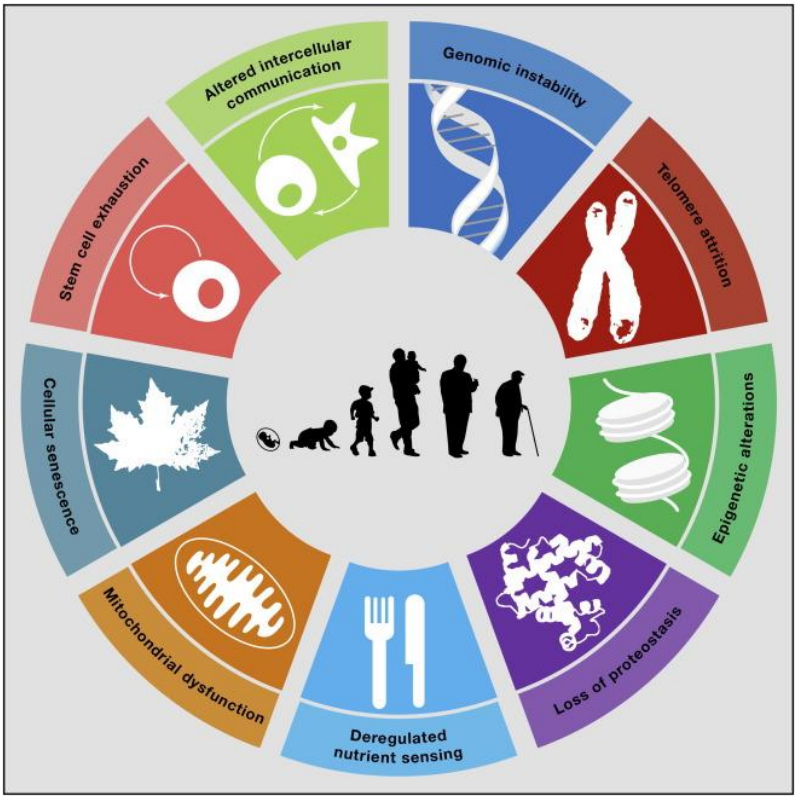

Figura 3. Processos fisiológicos e celulares afetados pelo envelhecimento. A predisposição a doenças pelo envelhecimento está associada com o prejuízo de uma série de processos fisiológicos e celulares, como o acúmulo de mutações genéticas, aumento da morte e quiescência celular, desregulação de processos celulares vitais e disfunções metabólicas e hormonais. Essas mudanças ocorrem de forma generalizada, afetando o funcionamento dos diversos tipos celulares, bem como todos os órgãos e sistemas do nosso corpo. Imagem copiada de (López-Otín et al., 2013) sob licença Creative Commons Attribution 4.0 International.

Os impactos do envelhecimento na saúde são acentuados pelo consumo de dietas desbalanceadas. Ambos são fatores independentes para o desenvolvimento de disfunções metabólicas em humanos, apresentando efeito sinergético quando associados (Stout et al., 2017).

Estudos com modelos animais demonstram que através das alterações metabólicas, tanto o consumo de dietas desequilibradas como o envelhecimento desencadeiam processos deletérios semelhantes no cérebro de humanos, como disfunção da barreira hematoencefálica, estresse oxidativo, neuroinflamação, plasticidade sináptica prejudicada e, especialmente, disfunção mitocondrial. Desta forma, a sobreposição dos fatores irá afetar mais severamente o SNC, aumentando ainda mais a propensão a doenças neurológicas e psiquiátricas. Pesquisa recente com gêmeos demonstrou que os hábitos alimentares podem retardar ou acelerar o processo de envelhecimento biológico geral (Ravi et al., 2025). Fenômeno semelhante é observado localmente no cérebro, já que o consumo de DH pode levar a um envelhecimento prematuro do cérebro, com a instauração precoce de marcadores de neuropatologias como a doença de Alzheimer, i.e. neuroinflamação, acumulação de amiloide- β (Butler et al., 2020; McGrattan et al., 2019; Spencer et al., 2019) e aceleração do processo de poda sináptica através da hiperativação do sistema complemento (Mackey-Alfonso et al., 2024).

Corroborando esse entendimento, um estudo envolvendo 8630 pessoas demonstrou que o consumo de gordura saturada aumentou o risco de desenvolver doença de Alzheimer e demência em 39% e 105%, respectivamente, com desenvolvimento precoce de sintomas associados com as alterações metabólicas induzidas pela dieta (Ruan et al., 2018). Curiosamente, intervenções dietéticas podem aliviar ou até reverter sintomas e alterações neurológicas associadas ao envelhecimento, demonstrando a forte relação entre envelhecimento e dieta (Lobo et al., 2022).

1.3.1. Mecanismos moleculares e fisiológicos dos efeitos da dieta hiperlipídica e do envelhecimento sobre o CNS

O hipocampo e a amígdala cerebral são áreas em destaque na avaliação dos efeitos da dieta e do envelhecimento sobre o CNS, pois desempenham papel fundamental nos processos de memória, aprendizado e regulação do humor, são altamente vulneráveis às alterações induzidas pela dieta e pelo envelhecimento e, desta forma, estão associados aos sintomas observados (Mulati et al., 2021; Zhuang et al., 2022). Curiosamente, Spencer (2017;2019) demostrou que tanto a memória dependente do hipocampo quanto da amígdala foi prejudicada pelo consumo de DH por ratos idosos, mas não em ratos jovens. Além disso, o declínio cognitivo foi acompanhado por neuroinflamação, conforme indicado pelo aumento de IL-1b e marcadores de ativação da micróglia

(Iba1 e cd11b) nos idosos. Já a aplicação de um antagonista do receptor de IL-1b reverteu as alterações cognitivas e mitocondriais, demonstrando a maior vulnerabilidade de indivíduos idosos ao consumo de DH (Spencer et al., 2019; Spencer, D'Angelo, et al., 2017a). Ratos idosos alimentados com DH apresentam prejuízo do mecanismo de potenciação de longo prazo, o que é associado com a incidência de processos neuroinflamatórios especialmente no hipocampo. Quando tratados com um antagonista do receptor de IL-1 também houve uma recuperação da cognição e uma normalização dos parâmetros neurológicos (González Olmo et al., 2023). Em outro estudo, observou-se o efeito sinergético do envelhecimento e do consumo de DH no desenvolvimento de comportamento tipo ansioso e de déficit cognitivo, associado com uma maior ativação microglial. Uma análise proteômica do hipotálamo demonstrou que tanto o envelhecimento como o consumo de DH alteraram vias relacionadas com a sinalização metabólica e com processos neurodegenerativos (Evans et al., 2024). Butler (2020) realizou uma análise lipídica do hipocampo e amídala de ratos jovens e idosos consumindo uma dieta padrão ou um DH por apenas 3 dias. Observou-se que o curto período de consumo de DH promoveu um desbalanço pro-inflamatório do perfil lipídico nas duas áreas cerebrais analisadas. Esse efeito foi potencializado pelo envelhecimento, conjuntamente com a maior ativação microglial e o aumento de citocinas inflamatórias no cérebro dos ratos idosos consumidores de DH (Butler et al., 2020).

Há apenas dois estudos com peixe-zebra onde se avaliou a associação entre o envelhecimento e o consumo de dietas desbalanceadas. Connaughton (2016) demonstrou que peixes-zebra mais velhos possuem maior propensão a desenvolver aumento de glicemia sérica quando exposto a uma dieta hiperglicêmica. Esse resultado se assemelha ao observado em mamíferos, onde indivíduos mais velhos são mais propensos à alterações metabólicas induzidas pela dieta (Connaughton et al., 2016). Outro estudo demonstrou que as alterações metabólicas induzidas por dieta hipercalórica foram responsáveis pelo estabelecimento precoce de mecanismos relacionados ao envelhecimento, i.e. alterações do ciclo circadiano e do tempo de sono, maior comportamento tipo ansioso, escoliose e redução da neurogênese, impactando mais severamente os animais mais velhos (Stankiewicz et al., 2017). Esse modelo tem se mostrado viável para o estudo dos efeitos do envelhecimento sobre o funcionamento do SNC e o desenvolvimento de neuropatologias associados com a idade (Kanoh et al., 2024). Há conservação de processos neuropatológicos observados no envelhecer como a disfunção mitocondrial, quiescência celular, alteração metabólica e endócrina, disfunção da comunicação celular e alterações epigenéticas (Van houcke et al., 2021; X. Wang et al., 2021).

1.3.1.1. Efeito do envelhecimento e da dieta hiperlipídica sobre a disfunção mitocondrial

A disfunção mitocondrial possui papel central em diversas patologias do SNC associadas ao envelhecimento (figura 4). Considerando a alta demanda energética do cérebro, a capacidade mitocondrial é crítica para o funcionamento e sobrevivência das células neuronais (Tan & Norhaizan, 2019) e é considerada um fator limitante para a sinaptogênese e plasticidade neuronal (Benard et al., 2007; Li et al., 2004). Além disso, o estresse oxidativo gerado por mitocôndrias disfuncionais tem um impacto severo na homeostase cerebral, uma vez que esse tecido é especialmente vulnerável a insultos oxidativos (Cobley et al., 2018). Desta forma, a mitocôndria tem sido efetivamente utilizada como alvo terapêutico de diversas neuropatologias como a doença neurodegenerativas (Kurz et al., 2010). Por exemplo, a deficiência de Pink1 em peixe-zebra é considerado um modelo viável da doença de Parkinson, com desenvolvimento de disfunção mitocondrial. Através da triagem de fármacos neuroativos neste modelo, demonstrou-se que a manutenção da homeostase mitocondrial pôde retardar a neurodegeneração (Soman et al., 2017; Y. Zhang et al., 2017).

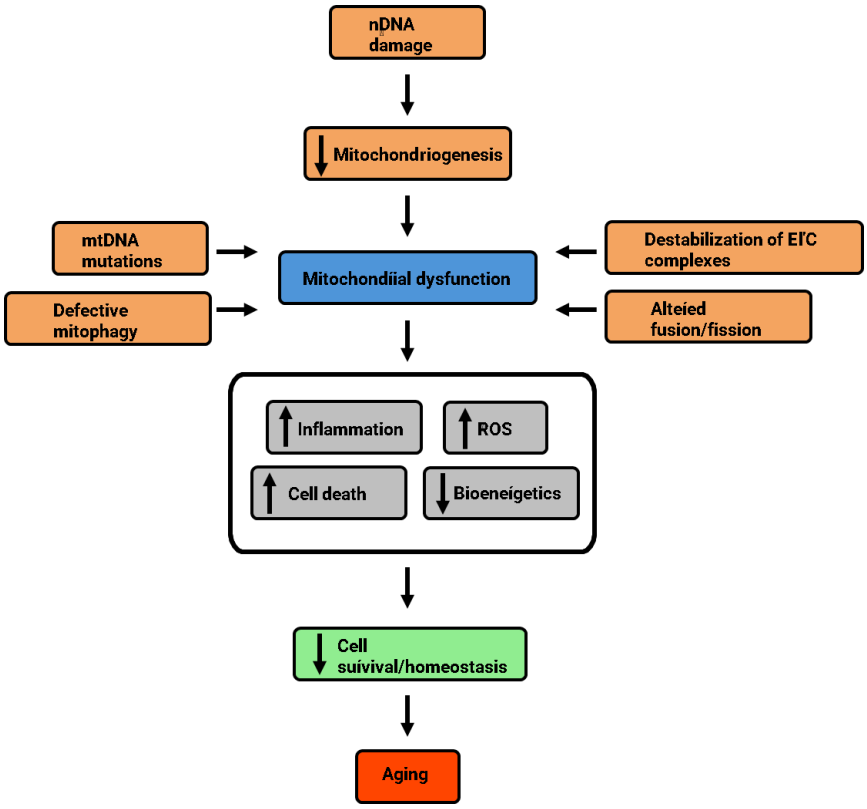

Figura 4. Impacto do envelhecimento sobre a homeostase mitocondrial. A disfunção mitocondrial exerce um papel central nas patologias associadas com a idade. A gravidade e a velocidade em que se desenvolve a disfunção mitocondrial ao longo da vida está associada com fatores genéticos, bem como fatores ambientais, como a dieta. Diversos mecanismo descritos estão por trás da desregulação do funcionamento dessa importante organela. Por sua vez, a homeostase da mitocôndria é essencial para a regulação de processos celulares vitais e garantir o bom funcionamento dos tecidos e órgão. Imagem adaptada de (López-Otín et al., 2013) sob licença Creative Commons Attribution 4.0 International.

O envelhecimento e o consumo de dietas desbalanceadas podem desencadear disfunção mitocondrial por diversos mecanismos em comum ou diversos (Chistiakov et al., 2014). Por exemplo, o acúmulo de mutações no DNA mitocondrial ao longo dos anos pode reduzir a capacidade energética, aumentar a produção de ROS e influenciar processos apoptóticos nos neurônios (Kauppila et al., 2017; Terman et al., 2010). No cérebro de peixe-zebra, há um acúmulo progressivo de mutação no DNA mitocondrial a partir de 12 meses de idade (Shimoda et al., 2014; Zhu & Coffman, 2017). Somando-se a isso, pode ocorrer prejuízos ao processo de mitofagia, levando ao acúmulo de mitocôndrias disfuncionais, redução da capacidade bioenergética, aumento do dano oxidativo e da apoptose (Cavallini et al., 2007; Masiero & Sandri, 2010). Um mecanismo em comum entre o envelhecimento e o consumo de DH é o prejuízo do monitoramento mitocondrial em relação aos substratos disponíveis na célula e sobre as demandas energéticas celulares (López-Otín et al., 2013; Tsilingiris et al., 2021). Desta forma, pode haver uma desregulação da flexibilidade metabólica mitocondrial impactando a homeostase bioenergética celular e estresse oxidativo (Jørgensen et al., 2017; Muoio, 2014).

Camundongos alimentados com DH que desenvolveram alterações metabólicas como resistência central à insulina e hiperglicemia, apresentaram prejuízo da integridade mitocondrial com indução de processos pró-apoptóticos. Camundongos idosos alimentados com DH também apresentaram comprometimento da mitofagia e acúmulo de mitocôndrias danificadas, o que não foi observado nos animais mais jovens alimentados com DH (Galizzi et al., 2021). Em outro estudo, camundongos alimentados com DH apresentaram declínio cognitivo associado à disfunção mitocondrial no hipocampo. Houve uma redução da capacidade mitocondrial e maior produção de espécies oxidativas nos estados C1-LEAK, C1-OXPHOS e C1+C2-OXPHOS. Após a intervenção com exercícios, houve uma normalização de todos esses parâmetros em associação com uma

melhora nos parâmetros metabólicos (H. S. Park et al., 2018). Curiosamente, ratos resistentes ao desenvolvimento de alterações metabólicas induzidas pela dieta e/ou envelhecimento também possuem maior capacidade de manter a homeostase mitocondrial, demonstrando que a perturbação mitocondrial é promovida pelas mudanças metabólicas (Ma et al., 2014).

O peixe-zebra tem sido amplamente aplicado no estudo do papel da disfunção mitocondrial no desenvolvimento de neuropatologias, demonstrando a conservação do funcionamento mitocondrial e da sua relação com mecanismos patogênicos (Otsuka & Matsui, 2023). Entretanto ainda não há estudo com peixe-zebra mostrando o papel da disfunção mitocondrial nas alterações do SNC induzidas pela dieta e/ou pelo envelhecimento. Neste estudo, buscamos aproveitar o potencial inexplorado dos zebrafish para avaliar a interação do consumo de dietas ricas em gorduras e o envelhecimento sobre a cognição, comportamento e parâmetros mitocondriais de zebrafish jovens e idosos. Avaliamos a função mitocondrial no cérebro para entender seu papel nas mudanças neurocomportamentais, usando uma abordagem de respirometria de alta resolução e quantificação da expressão gênica dos complexos da cadeia transportadora de elétrons.

2. JUSTIFICATIVA

A piora dos hábitos alimentares nos últimos anos representa um grave problema de saúde pública mundial pois é fator de risco para obesidade, síndrome metabólica e doenças neurodegenerativas como a demência e a doença de Alzheimer. A situação é agravada pelo atual processo de envelhecimento da população mundial, aumentando a susceptibilidade a essas doenças.

É bem estabelecido na literatura que o consumo de dietas desbalanceadas prejudica o funcionamento do SNC, entretanto pouco se entende como exatamente isso ocorre, tendo em vista que vários mecanismos neuropatológicos estão envolvidos, formando uma complexa rede de interações. Já foi observado também o efeito sinergético da interação entre envelhecimento e maus hábitos alimentares na piora dos parâmetros metabólicos e neurológicos. Modelos animais de intervenção dietética são amplamente aplicados para elucidar esse tema, sendo que diversos protocolos de dieta hiperlipídica (DH) já foram descritos, demonstrando o impacto da ingestão excessiva de lipídeos no SNC.

Dentre esses, o peixe-zebra foi muito pouco explorado e possui grande potencial para ampliar o conhecimento, complementando os dados obtidos com modelos animais clássicos. O peixe-zebra possui alta similaridade com mamíferos sendo bem estabelecido como modelo de estudo de obesidade, disfunções metabólicas, neuropatologias, dentre outras doenças humanas. Porém, poucos estudos avaliaram o efeito da DH sobre parâmetros neurocomportamentais de peixe-zebra, sobretudo quando associado com o envelhecimento. Desta forma, o atual trabalho contribui para estabelecer o peixe-zebra como modelo de estudo e aproveitar as potencialidades e peculiaridades deste animal para avaliar mecanismos por trás do impacto ao SNC induzido pela dieta e pelo envelhecimento, com ênfase no metabolismo mitocondrial como mecanismo neuropatogênico.

3. OBJETIVOS

Objetivo geral

Avaliar o efeito da dieta hiperlipídica à base de gema de ovo em parâmetros morfo-metabólicos, comportamentais e cognitivos em peixe-zebra adulto jovem (4-6 meses de vida) e idoso (17- 22 meses de vida). Investigar o papel da bioenergética mitocondrial como mecanismo associado as alterações comportamentais induzidas pela dieta desbalanceada.

Objetivos específicos

Avaliar o efeito da ingestão de DH em peixe-zebra adulto jovem (4-6 meses de vida) e idoso (17-22 meses de vida) sobre os seguintes parâmetros:

a) Morfo-metabólicos: ganho de massa corporal, comprimento abdominal e glicemia em jejum;

c) Comportamentais: avaliação da locomoção e do comportamento tipo ansioso através do teste do tanque novo, do desempenho cognitivo através do teste do labirinto em "T" e da agressividade através do teste de agressividade induzida por espelho

d) Bioenergéticos do encéfalo: avaliação do consumo de oxigênio no encéfalo dos animais por meio de respirometria de alta resolução em Oroboros em animais adultos jovens e idosos;

e) Análise da expressão de genes da cadeia transportadora de elétron no encéfalo dos animais por qRT-PCR.

f) Análises ultraestruturais do encéfalo por meio de microscopia eletrônica de transmissão.

Como parte integrante desta tese, as seções de Materiais e métodos, de Resultados e de Discussão serão apresentadas abaixo na forma do manuscrito intitulado "**Aging Increases Susceptibility to High-Fat Diet-Induced Neurobehavioral and Mitochondrial Dysfunction in Zebrafish**", que está submetido e em análise na revista "Progress in Neuropsychopharmacology & Biological Psychiatry" (Fator de impacto: 5,3).

Em seguida, a conclusão da tese, perspectivas futuras e a bibliografia utilizada será apresentada em texto à parte nas seções seguintes.

4. MANUSCRITO SUBMETIDO

Manuscrito intitulado "**Aging Increases Susceptibility to High-Fat Diet-Induced Neurobehavioral and Mitochondrial Dysfunction in Zebrafish**", submetido e sobre análise na revista "Progress in Neuropsychopharmacology & Biological Psychiatry" (Fator de impacto: 5,3).

Aging Increases Susceptibility to High-Fat Diet-Induced Neurobehavioral and Mitochondrial Dysfunction in Zebrafish

Victor L. Picolo[a,b,h], Letícia A. Tavares[a,b], Whitney R. Santos[a], Nathasha P. Lopes[a,b,h], Ethiane R. dos Santos[c,h], Wembley R. Vilela[a,h], Paula Q. Bellozi[a,e], Jair T. Goulart[a,f], Cesar K. Grisolia[g], Daniel Ardisson-Araújo[c], Andreza F. de Bem[a,b,g,h*]

[a] Laboratory of Bioenergetic and Metabolism, Institute of Biological Sciences, University of Brasilia, Brasilia, Brazil
[b] Graduate Program in Molecular Pathology, School of Health Sciences, University of Brasilia, Brasilia, Brazil
[c] Laboratory of Insect Virology, Department of Cellular Biology, Institute of Biological Sciences, University of Brasilia, Brasilia, Brazil
[e] Laboratory of Molecular Pharmacology, School of Health Sciences, University of Brasilia, Brasilia, Brazil
[f] Department of Physiological Sciences, Institute of Biological Sciences, University of Brasilia, Brasilia, Brazil
[g] Department of Genetics and Morphology, Institute of Biological Sciences, University of Brasilia, University Campus Darcy Ribeiro, Brasilia, Brazil
[h] National Institute of Science and Technology on Neuroimmunomodulation, Manguinhos, Rio de Janeiro, Brazil

* Correspondence to: de Bem, Andreza
E-mail address: debemandreza@gmail.com; andrezadebem@unb.br

Abstract

Aging and unhealthy eating habits independently and synergistically disrupt central nervous system (CNS) homeostasis, increasing susceptibility to neurological and behavioral disorders. Mitochondria play a critical role in maintaining neuronal survival and activity, representing a central player in the pathogenesis of neurodegenerative diseases. Here, we used zebrafish as a model to investigate how aging and a high-fat diet (HFD) affect brain bioenergetics and behavior. Young (4–6 months) and aged (17–22 months) male zebrafish were fed either a standard diet or an HFD based on boiled chicken egg yolk for 14 days. Brain mitochondria were evaluated using high-resolution respirometry, transmission electron microscopy (TEM), and qRT-PCR. HFD impaired the metabolic health of both young and aged animals, promoting weight gain, increased abdominal length, and elevated fasting glucose levels. Aging intensified the HFD detrimental effects on behavior: aged HFD-fed zebrafish displayed increased anxiety-like behavior in the novel tank test, and impaired cognitive performance in the T-maze test. Notably, HFD had no significant effect on aggressive behavior regardless of age. Mitochondrial responses to HFD differed by age: while cerebral bioenergetic function declined in young fish, aged animals showed an opposite trend. TEM analysis revealed increased accumulation of fragmented mitochondria in HFD group, indicating potential mitochondrial dysfunction. RT-qPCR showed upregulation of genes involved in the electron transport chain, especially in aged zebrafish. In conclusion, our findings demonstrate an age-dependent vulnerability to the effects of HFD on both neurobehavioral and mitochondrial parameters.

Keywords: High fat diet, Aging, Zebrafish, Brain metabolism, Mitochondrial function, Cognitive behavior

HIGHLIGHTS:

• High-fat diet impaired metabolic parameters in both young and aged zebrafish.
• Behavioral deficits induced by HFD—such as anxiety and cognitive decline—were observed only in aged zebrafish.
• Brain mitochondrial responses to HFD were age-specific, with divergent bioenergetic outcomes.
• HFD enhanced transcription of electron transport chain genes, especially in aged zebrafish brains.

ABBREVIATION:

ADP, Adenosine diphosphate; ATP, Adenosine triphosphate; CNS, Central nervous system; CCCP, Carbonyl cyanide 3-chlorophenylhydrazone; HFD, High fat diet; Oligo, Oligomycin; MIAT, Mirror-induced aggression test; NTT, Novel tank test; PM, Pyruvate and malate; ROT, Rotenone; SD, Standard diet; Succ, Succinate; SV, Synaptic vesicle; TMT, T-maze test

1. INTRODUCTION

The worsening of dietary habits observed in the last 40 years is associated with a severe impact on public health system, acting as a major cause of obesity epidemic and its comorbidities (Popkin et al., 2012; Vandevijvere et al., 2015). In parallel, the global population has undergone a process of aging in the last decades. Between 1990 and 2017, the proportion of people over 65 years old grew from 6.1% to 8.8%, and it is estimated that by 2050 this proportion will reach 16% (Cheng et al., 2020). Both aging and unhealthy diets act independently and synergistically to promote the development of metabolic and neurological diseases (López-Otín et al., 2013; Stout et al., 2017). Poor nutritional patterns may accelerate systemic aging processes, including cellular senescence and telomere attrition (Mundstock et al., 2015; Ravi et al., 2025), ultimately contributing to premature brain aging and the early appearance of neuropathological features such as neuroinflammation, beta-amyloid accumulation (Butler et al., 2020; McGrattan et al., 2019; Spencer, Korosi, et al., 2017), and enhanced synaptic pruning (Mackey-Alfonso et al., 2024).

Recent evidence has reinforced the role of poor dietary habits and obesity as major determinants of brain dysfunction, particularly in the context of aging. A large-scale longitudinal study by Zhang et al. (2025) provided compelling evidence that both the degree and duration of obesity exposure significantly impact neurocognitive function in middle-aged and older adults. The authors identified a progressive pattern of brain deterioration associated with increasing obesity severity, marked by widespread alterations in brain morphology and functional connectivity. These structural and functional brain changes were accompanied by varying degrees of cognitive decline, especially in reasoning, visuomotor speed, and working memory (D. Zhang et al., 2025). Supporting this, a Brazilian longitudinal study by Gomes Gonçalves et al. (2023), showed that unhealthy eating habits, including high consumption of ultra-processed foods, were associated with a 28% acceleration in the rate of global cognitive decline and a 25% greater decline in executive functions (Gomes Gonçalves et al., 2023).

Similar results have been consistently reproduced in animal models using various protocols of unbalanced diets, such as high-fat diets (HFD), demonstrating that diet-induced metabolic alterations can trigger pathological events in the brain, including blood-brain barrier dysfunction, oxidative stress, neuroinflammation, impaired synaptic plasticity, and mitochondrial dysfunction (de Bem et al., 2021; Y. Zhang et al., 2024)). Moreover, it has been shown that aged animals are more vulnerable to HFD-induced metabolic changes, resulting in a more pronounced impact on the central nervous system (CNS). Aged rodents fed with HFD are more prone to develop memory impairments dependent on hippocampal and amygdalar function (Spencer, D'Angelo, et al., 2017b), as well as psychobehavioral alterations, such as increased anxiety-like behavior (Evans et al., 2024).

Proper mitochondrial function is essential for maintaining CNS homeostasis, as the brain is both highly energy-demanding and particularly vulnerable to oxidative stress generated by dysfunctional mitochondria (Cobley et al., 2018; Tan & Norhaizan, 2019). Mitochondria play a central role in CNS pathologies, and both aging and HFD consumption can impair mitochondrial homeostasis through overlapping or distinct mechanisms (Chistiakov et al., 2014), including accumulation of mitochondrial DNA mutations (Kauppila et al., 2017; Terman et al., 2010), defective mitophagy (Cavallini et al., 2007; Masiero & Sandri, 2010), and reduced metabolic flexibility and substrate sensing (Jørgensen et al., 2017; Tsilingiris et al., 2021). These alterations ultimately disrupt both metabolic and functional homeostasis in the CNS. The combination of aging and HFD can thus synergistically exacerbate mitochondrial dysfunction, promoting the accumulation of damaged mitochondria and further impairing cellular bioenergetics (Galizzi et al., 2021). Notably, interventions that restore systemic metabolic parameters have been shown to prevent cognitive and behavioral deficits, at least in part, through the enhancement of mitochondrial function (H.-S. Park et al., 2018).

Zebrafish is a well-established model for studying obesity, metabolic disorders (Angom & Nakka, 2024; Gut et al., 2017), and various neuropathologies (Kalueff et al., 2014; Otsuka & Matsui, 2023). A previous study from our group demonstrated that zebrafish fed a HFD develop metabolic alterations alongside behavioral changes, including cognitive decline, anxiety-like behavior, and increased aggressiveness (Picolo et al., 2021). Moreover, HFD-induced cognitive deficits in zebrafish have been linked to redox imbalance, blood-brain barrier disruption, neuronal dysfunction, apoptosis, and alterations in amyloid-β metabolism (Meguro et al., 2019), supporting

the notion of conserved pathogenic mechanisms between fish and mammals. Hallmarks of aging, such as cellular senescence, metabolic alterations, and neuroendocrine changes, are also present in zebrafish (Van houcke et al., 2021; X. Wang et al., 2021), and the impact of aging on CNS function has been extensively characterized in this model (Kanoh et al., 2024), including the contribution of mitochondrial dysfunction to age-related neuropathologies (Otsuka & Matsui, 2023). Interestingly, pharmacological modulation of mitochondrial function has been shown to effectively prevent neurodegeneration in zebrafish models of Parkinson's disease (Soman et al., 2017; Y. Zhang et al., 2017), highlighting the relevance of this model for investigating mitochondria-targeted therapeutic strategies.

However, there is limited evidence in the literature addressing the interaction between aging and unbalanced diets in zebrafish. One study reported that aged zebrafish are more susceptible to diet-induced hyperglycemia (Connaughton et al., 2016), while another demonstrated that metabolic alterations induced by a high-calorie diet led to the early onset of aging-related features, including disruptions in circadian rhythm and sleep duration, increased anxiety-like behavior, scoliosis, and reduced neurogenesis (Stankiewicz et al., 2017). In this study, we investigated the mechanisms underlying the impact of HFD consumption and aging on zebrafish brain metabolism and behavior. We hypothesize that HFD-induced brain alterations are exacerbated by aging, particularly cognitive impairments, through the disruption of cerebral mitochondrial function.

2. MATERIALS AND METHODS

2.1. Animals

Short-fin zebrafish were maintained in a recirculating water system (ZebTec, Tecniplast, Buguggiate, Varese, Italy) at the Department of Genetics and Morphology, University of Brasília, until the start of the experimental protocol. Environmental conditions were kept constant with the water temperature at 27 ± 1°C, conductivity of 650 ± 100 µS/cm, pH 7.0 ± 0.5%, oxygen saturation of 95%, and a 12:12 light-dark cycle. During this period, the fish were fed twice a day with a 1:1 mix of Tetra Colorbits and TetraMin dry food. At the appropriate age for testing, young and aged male fishes (4–6 months old and 17–22 months old) were transferred to 4-liter static water aquariums (10 animals per aquarium). Water quality was monitored using a kit for detecting toxic ammonia (Labcon Test) and changed every 2 days. Only male zebrafish was used on experiments

to avoid hormonal interference and since female fish can direct excess caloric intake to reproductive processes (Landgraf et al., 2017)

2.2. Experimental protocol

The experimental protocol was divided into 2 independent experiments: first, using young adults, and then using aged adults. In each protocol, fishes were divided into 2 experimental groups based on the diet type: Standard Diet (SD), receiving 7.5 mg/fish/day of Tetra ColorBits dry food (6.5% fat, 47.5% protein; 4.9 cal/mg) and high-fat diet (HFD) group, receiving 5 mg/fish/day of ColorBits, supplemented with 15 mg/fish/day of boiled chicken egg yolk. Fishes were fed once a day for two weeks, between 2 and 3 PM. One hour after feeding, any excess food was removed to maintain water quality and ensure that both groups had a similar feeding window (Fig. 1A). Fish were weighed twice a week during the experimental protocol and underwent behavioral tests on days 14 and 15. On day 16, the animals were anesthetized by immersion in room-temperature tricaine 0,2% (MS-222; Sigma-Aldrich), photographed laterally for morphometric measurements and blood was collected from caudal incision for glycemia measurement. Then, euthanasia was performed by immersion in ice-cold water, and the brain was removed for mitochondrial analysis. (Fig. 1A). All procedures were conducted in accordance with the Ethics Committee for Animal Use of the University of Brasília (CEUA- UnB: 040/2020).

2.3. Morphometabolic evaluation

Zebrafish (Young: n= 16-20 per group; aged: n= 31-48 per group) were individually weighed twice a week, using a precision balance. After euthanasia, body length and abdominal length were determined by photographing fish next to a ruler and the dimensions were measured using ImageJ software (NIH, Bethesda, USA). Fasting blood glucose was evaluated immediately after caudal excision using a commercial glucometer (G-Tech, Minas Gerais, Brazil).

2.4. Behavioral tests

Behavioral tests were conducted between 9 AM and 3 PM, alternating between the experimental groups to avoid time-related bias. Fish were tested in a fasting state to avoid experimental divergences related to satiety. The novel tank test (NTT; Young: n= 19-20 per group; aged: n= 30-32 per group) and the mirror-induced aggression test (MIAT; Young: n= 20 per group; aged: n= 31-32 per group) were conducted in 20 cm height x 15 cm width x 18 cm length tanks with 4 liters of the ZebTec system water. The T-maze test (TMT; Young: n= 16-20 per group;

aged: n= 14-23 per group) was conducted in a "T"-shaped tank with three arms (30 cm length x 10 cm x height x 10 cm width, each arm). NTT and MIAT were conducted on the same day, while TMT was conducted on the following day. Behavior was recorded using a webcam positioned frontally (for NTT) or above (for TMT and MIAT) the aquariums. The videos were analyzed using AnyMaze software (Stoelting CO, USA). The analysis of behavioral experiments was conducted by an observer blinded to the experimental groups to minimize bias.

Novel tank test (NTT)

The NTT assesses locomotor capacity and anxiety-like behavior in zebrafish. The test is based on the natural tendency of zebrafish to stay in deeper areas of new environments and gradually explore the upper zones (geotaxis) (Fontana et al., 2019; Kalueff et al., 2013). The fish were transferred to a novel tank and kept isolated while being recorded for six minutes. The apparatus was virtually divided into two horizontal areas of equal size (upper and lower areas). The total distance traveled, absolute turning angle, and maximum speed were evaluated as parameters of locomotor capacity. Anxiety-like behavior was evaluated by recording time spent in the top area, immobility, latency to first enter the top area, and frequency of entrances into the top area (Fig. 2).

T-maze test (TMT)

The TMT assesses zebrafish cognitive capacity by evaluating their spatial memory in response to a neutral episode (Moreira & Luchiari, 2022). The apparatus consists of a "T"-shaped tank with three arms (30 cm length x 10 cm height x 10 cm width, each arm). The maze had a white floor and black walls to minimize external visual interference, and visual cues were placed to help fish distinguish arms. The TMT consists of training and testing phases, each lasting six minutes. In the training, fish can explore the initial arm (the one where they are placed) and one lateral arm (old arm), with the other lateral arm closed with a removable guillotine door (new arm). The testing phase was performed after one hour, when fish were reintroduced to the maze, with all arms accessible. The novel and old arm were alternated between the two lateral arms to minimize any potential preference for one side (Fig. 3A).

The test is based on zebrafish natural preference to explore new environment over familiar ones. Therefore, memory retention was assessed by the time spent in each arm and by calculating a memory index derived from the exploration times of the novel arm (Tn) and the old arm (To), using the formula: $T_n/(T_n+T_o)$. Since memory evaluation requires a proper exploration of all parts

of the T-maze, two exclusion criteria were applied: immobility time higher than 150 seconds (50% test time), and less than 10 seconds in any of the three arms.

Mirror-Induced Aggression Test (MIAT)

This test evaluates aggression by placing a mirror at a 22.5° with the tank. Zebrafish exhibit aggressive behavior when confronted with their own reflection in a mirror, particularly when isolated within an environment. The tank was virtually divided into proximal, central, and distal areas, accordingly with the distance to the mirror, and the fishes were placed into the apparatus for six minutes (Fig. 3C). The test was conducted immediately after the NTT in the same apparatus, which promoted the habituation to the apparatus. Aggressive behavior was defined as high-energy movements towards the reflection in the mirror, like an attack attempt. This behavior was evaluated only in the proximal area of the tank, since it is where the reflection is more visible to the fish. Time spent in each area of the apparatus, the total time of aggressive episodes, and the latency to the first aggressive episode were assessed in the MIAT.

2.5. Brain oxygen consumption

Oxygen consumption was measured using high-resolution respirometry with an Oroboros oxygraph (Oroboros 2k, Innsbruck, Austria) at 28°C. Following euthanasia, brains (Young: n= 11 per group; aged: n= 5-6 per group) per group were promptly transferred to a 5 mL Teflon-glass homogenizer and homogenized in 200 µL of respiration buffer composed of 125 mM sucrose (Sigma-Aldrich, Cat# S9378), 65 mM KCl, 2 mM K_2HPO_4 (Vetec, Cat# 225), 1 mM $MgCl_2$ (Vetec, Cat# 149), 10 mM Hepes (Sigma-Aldrich, Cat# H4034), and 0.2 mM EGTA (Sigma-Aldrich, Cat# E4378). A 100 µL sample was added to the 2 mL Oroboros chamber containing respiration buffer with 1 mg/mL fatty acid-free BSA (Sigma-Aldrich, Cat# A7030). Protein quantification was conducted via the Bradford method (M. Bradford, 1976).

The evaluation of different mitochondrial states was conducted by the sequential addition of substrates and inhibitors of the respiratory chain: State 2 (dependent on complex 1) was assessed after addition of Pyruvate 15 mM (Vetec, Cat# 1054) and Malate 7.5 mM (Sigma-Aldrich, Cat# 240176); State 3 (dependent on complex 1) was assessed after addition of ADP 500 µM (Sigma-Aldrich, Cat# A2754); State 3 (dependent on complexes 1+2) was assessed after addition of Succinate 1mM (Sigma-Aldrich, Cat# V900102); State 4 was assessed after addition of Oligomycin 0.15 µg/mL (Sigma-Aldrich, Cat# O4876); maximum uncoupled respiration was assessed after CCCP 0.3 µM titration (Sigma-Aldrich, Cat# C2759); and maximum uncoupled

respiration (dependent on complex 2) was assessed after addition of Rotenone 0.5 µM (Sigma-Aldrich, Cat# R8875). Residual respiration was assessed with Antimycin A 1 µM, and its value was subtracted from the other states, allowing to evaluate the mitochondrial-derived oxygen consumption. A second protocol was applied using aged zebrafish brain (n= 11 per group), to evaluate complex 2-dependent mitochondrial metabolism. Substrates and inhibitors was added as follow: State 2 was assessed after addition of Rotenone 0.5 µM and Succinate 1mM; State 3 was assessed after addition of ADP 500 µM; State 4 was assessed after addition of Oligomycin 0.15 µg/mL; maximum uncoupled respiration was assessed after CCCP 0.3 µM titration. Residual respiration was assessed with Antimycin A 1 µM, and its value subtracted from the other states, allowing to evaluate the mitochondrial-derived oxygen consumption.

2.6. Evaluation of mRNA expression of electron transportation system complexes

The expression of genes of ETS complexes was evaluated by Quantitative Reverse Transcription PCR (qRT-PCR). The brains (Young: n= 3-4 per group; aged: n= 3-4 per group) were macerated in Tris-EDTA (TE) buffer (10 mM Tris and 1 mM EDTA) at pH 8.0 and centrifuged at 12,000 × g for 5 minutes. RNA was extracted using Trizol™ Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions, with a final volume of 20 µl of RNase-free water. The extracted total RNA was then used for cDNA synthesis with GoScript™ Reverse Transcriptase (Promega) following the manufacturer's instructions.

Following, qRT-PCR was performed to assess the transcription of nuclear-coded genes for ETC complex subunits: *ndufs2* (complex 1), *sdha* (complex 2), *ucrc10* (complex 3), *cox4i1*, *cox6a1*, *cox6c* (complex 4) and *atp5i* (ATP synthase). Brain cDNA samples were diluted 1:10 and further amplified by RT-qPCR performed on a QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific) thermocycler, in a volume of 10 µl containing 2 × mix PowerUp™ SYBR™ Green Master Mix (Applied Biosystems™; Cat# TX78744), 0.5 µM each of forward and reverse primers (Table S1), passive reference Rox (Thermo Fisher Scientific; Cat# 12223012), and nuclease-free water (Sigma-Aldrich, Cat# W4502). The optimized thermal profile was 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 53 °C or 55º C for 10 s, and 72 °C for 15 s. A melting curve (from 55°C to 95°C) was generated at the end of each run to confirm the specificity of the assay. Data acquisition was performed using the Quant dyeStudio^{TM} Design & Analysis Software v1.5.2 software. The reference gene *ef1α* was used to normalize the relative transcriptional changes of target genes, calculated using the $2^{\wedge -\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). The control

expression levels were normalized to 1, and data were then expressed as fold changes relative to the standard control.

2.7. Transmission electron microscopy of brain tissue

Immediately following euthanasia, the zebrafish encephalon was dissected and fixed in Karnovsky's solution (0.1 M sodium cacodylate buffer, pH 7.2, containing 2% glutaraldehyde, 2% paraformaldehyde, 5 mM $CaCl_2$, and 3% sucrose) at 4 °C for 12 hours. Post-fixation was carried out in 0.2 M sodium cacodylate buffer containing 2% osmium tetroxide and 1.6% potassium ferricyanide for 1 hour. Tissues were subsequently stained in bloc with 0.5% aqueous uranyl acetate for 2 hours, dehydrated through a graded acetone series (30–100%, 10 minutes per step), and embedded in Spurr's resin. Ultrathin sections (70 nm) were obtained using an ultramicrotome and examined using a JEOL 1011 transmission electron microscope operated at 80 kV.

2.8. Statistical analysis

Data were analyzed using unpaired Student's t-test or Mann-Whitney test, depending on data's normality, assessed by the Kolmogorov–Smirnov test. Morphometabolic and mitochondrial parameters were presented as mean ± standard error of the mean (SEM), while behavioral data were shown as violin plots with 1-3 quartiles, allowing for visualization of the full data distribution. Statistical analysis was performed using GraphPad Prism 9®. Results were considered statistically significant for $p < 0.05$.

3. RESULTS

3.1. Effect of HFD in young adult zebrafish

HFD induces an obesogenic phenotype in young adult zebrafish

Zebrafish aged 4-6 months exhibited phenotypic changes associated with obesity after consuming a HFD based on egg yolk for two weeks (Fig. 1). There was no difference in the initial body weight between the groups (Fig. 1C; $t_{(38)} = 1.259$, $p = 0.2164$). However, zebrafish in the HFD group showed significantly higher body weight starting from day 8 of the diet until the end of the experimental protocol (Fig. 1C; day 8: $t_{(38)} = 2.237$, $p = 0.0313$; day 10: $t_{(38)} = 3.068$, $p = 0.0040$; day 15: $t_{(38)} = 2.507$, $p = 0.0166$). HFD-fed animals also displayed a significant increase in abdominal length (Fig. 1D; $t_{(37)} = 2.322$, $p = 0.0258$), while body length remained unchanged between groups (Fig. 1E; $t_{(37)} = 1.731$, $p = 0.0918$).

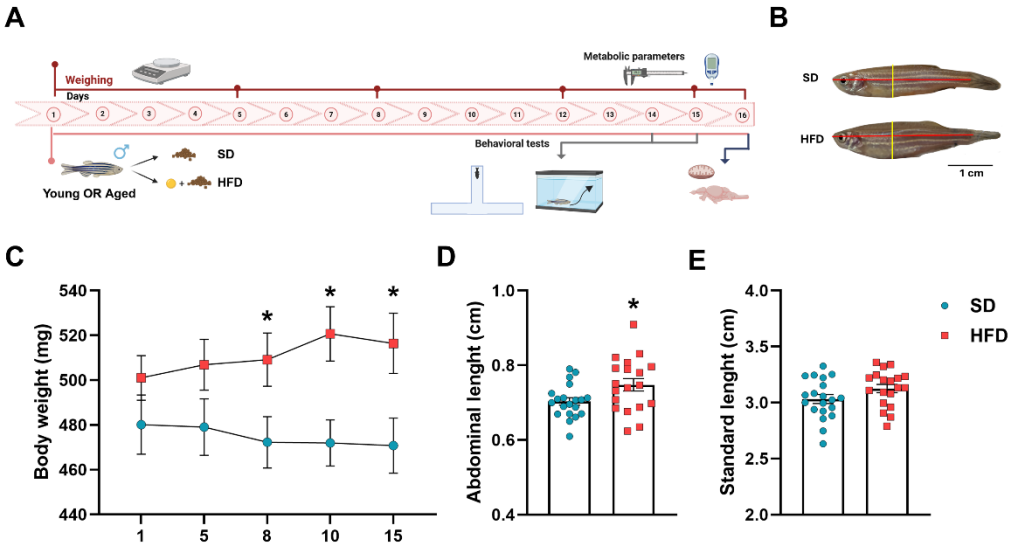

Figure 1. HFD induces an obesogenic phenotype in young adult zebrafish. (A) Experimental design: Two independent experiments were conducted with young (4-6 months) and aged (17-22 months) zebrafish. The animals received either a standard diet (SD) based on commercial dry feed or a high-fat diet (HFD), that includes the SD supplemented with boiled chicken egg yolk as a lipid source. During the two weeks on diet, body weight gain was assessed using a precision scale and the remaining metabolic parameters were evaluated after this period. Behavioral tests were performed on days 14 and 15, and the brain was extracted for molecular and bioenergetic analysis (day 16). (B) Representative image of one individual from each experimental group after two weeks on the diet, showing standard (red line) and abdominal (yellow line) lengths. (C) Body weight registered throughout the experiment. (D-E) Abdominal length and standard length measured using ImageJ software. Data are shown as mean ± standard error of the mean and were analyzed by student t-test. *p < 0.05. n= 19-20 per group.

HFD did not affect locomotion and anxiety-like behavior in young adult zebrafish

Locomotion and anxiety-like behavior were assessed by the novel tank test (Fig. 2). HFD intake did not affect locomotor performance in young adult zebrafish, as indicated by similar values between experimental groups for total distance traveled (Fig. 2A; $t_{(38)}$ = 1.591, p = 0.1199), maximum speed (Fig. S1A; $t_{(38)}$ = 1.651, p = 0.1069), and absolute turning angle (Fig. S1B; $t_{(38)}$ = 1.091, p = 0.2819). No significant differences were observed between groups in the time in the top area (Fig. 2B; U=128.5; p=0.1351), number of entrances into the top area (Fig. 2C; U=173; p=0.4734), or the latency to first entry the top area (Fig. 2D; U=170; p=0.7674), which are the major indicators of anxiety-like behavior. However, total immobility time was significantly higher in the HFD group (Fig. S1C; U= 112; p= 0.0378), suggesting a potential increase in anxiety-like behavior.

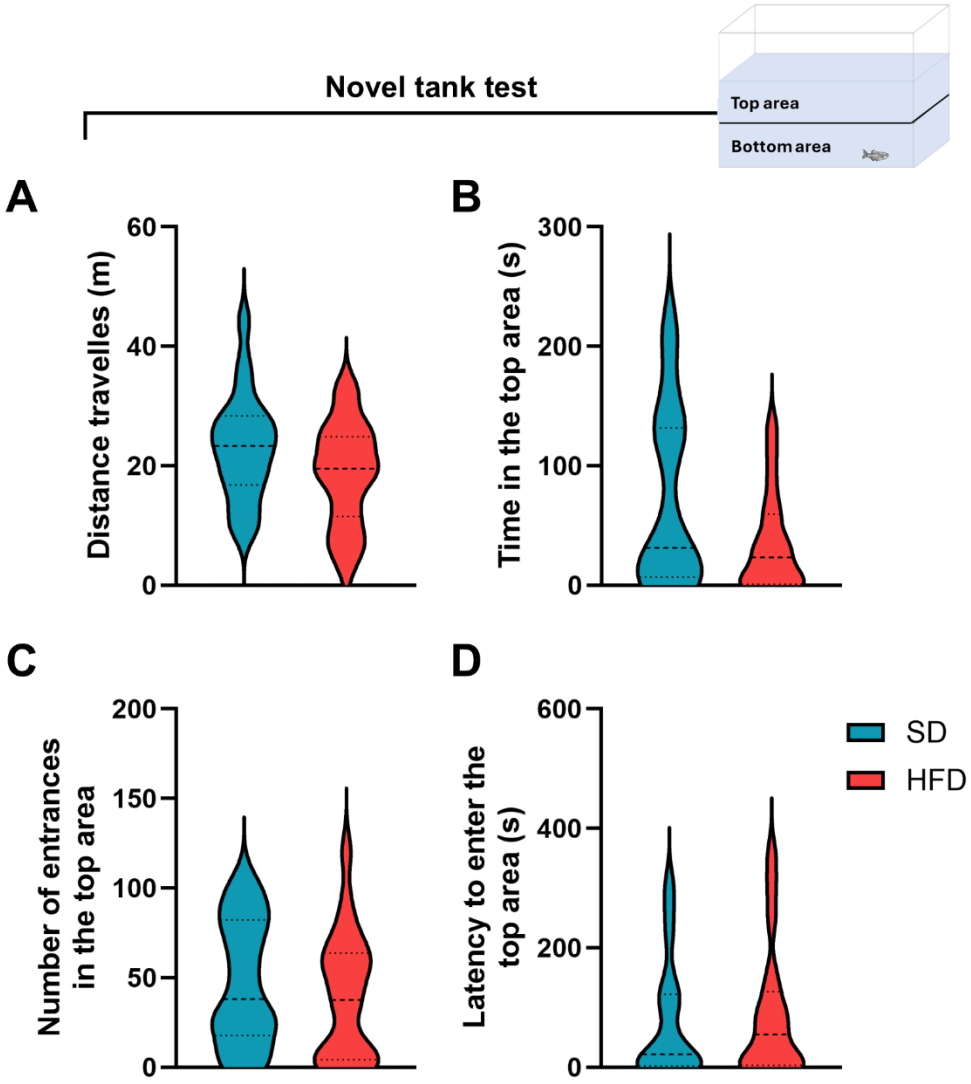

Figure 2. HFD did not affect locomotion and anxiety-like behavior in young adult zebrafish. The novel tank apparatus was virtually divided into two horizontal areas. (A) The total distance traveled evaluated during the 6-minutes behavioral test was used as a locomotion parameter. (B) Time in the top area, (C) number of entries into the top area, and (D) the latency to first entry into the top area were used as anxiety-like behavior parameters. Data full distribution is shown in violin plots with 1-3 quartiles and were analyzed by Student t-test or Mann-Whitney test. *p < 0.05. n= 19-20 per group.

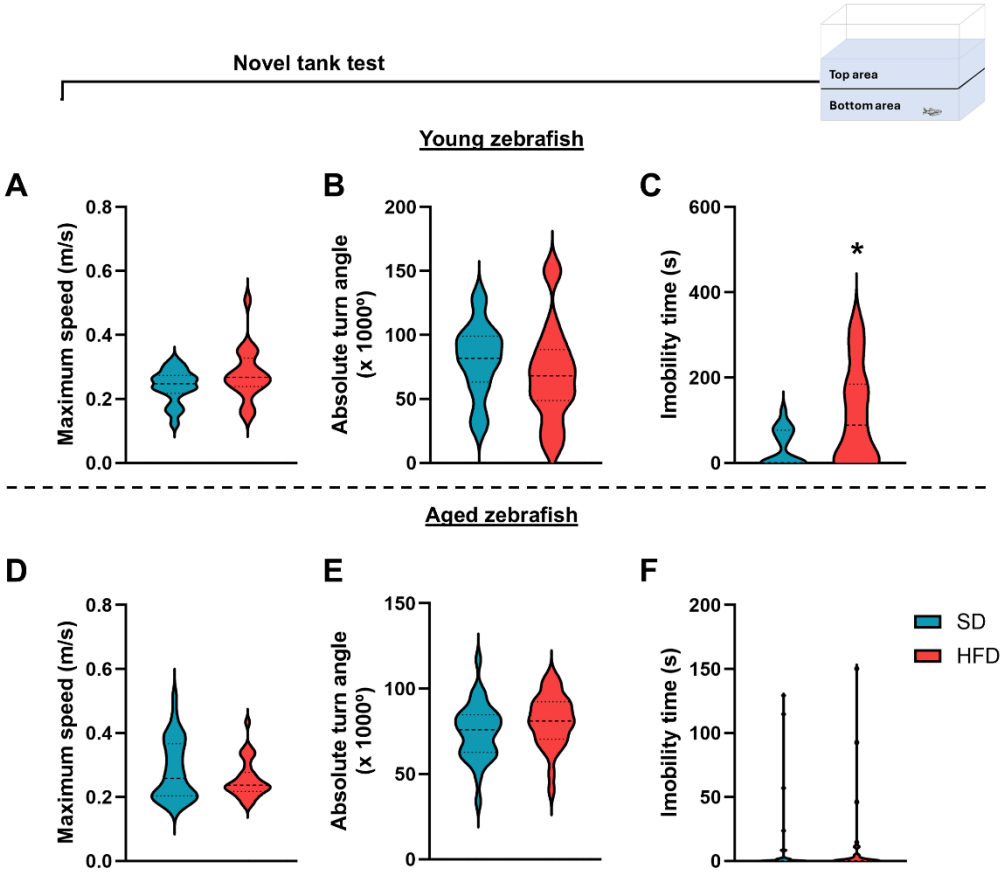

Figure S1. Effect of HFD on locomotion in young and aged adult zebrafish. Maximum speed and absolute turn angle of (A-B) young and (D-E) aged adult zebrafish were evaluated as locomotory parameters in the novel tank test. Immobility time of (C) young and (F) aged adult zebrafish was evaluated as a locomotory pattern parameter. Data full distribution is shown in violin plot with 1-3 quartiles and were analyzed by student t-test or Mann-Whitney test. n= 20-32 per group. *p<0.05.

HFD did not affect cognition and aggressiveness in young adult zebrafish

Short-term spatial memory consolidation was assessed using the T-maze test (Fig. 3A). Time spent in each arm of maze (Fig. 3A; initial: $t_{(33)} = 1.735$, $p = 0.0922$; novel: $t_{(33)} = 1.381$, $p = 0.1764$; old: $t_{(33)} = 0.3520$; $p= 0.7271$) and the memory index value (Fig. 3B; $t_{(33)} = 0.7828$; $p=0.4393$) did not vary between groups, demonstrating that HFD did not affect cognitive performance of young adult zebrafish.

Also, aggressive behavior was not affected by HFD on the mirror-induced aggression test (Fig. 3C). Groups displayed similar values of time spent in each area of the apparatus (Fig. 3C; proximal: $t_{(38)} = 1.178$; $p= 0.2463$; central: $t_{(38)} = 0.983$, $p= 0.3316$; distal: $t_{(38)} = 0.8068$, $p= 0.4248$), of total aggression time during the test (Fig. 3D; $U= 190.5$; $p= 0.8046$), and of the latency to the first aggressive episode (Fig. S2A; $U= 153,5$; $p= 0.4392$).

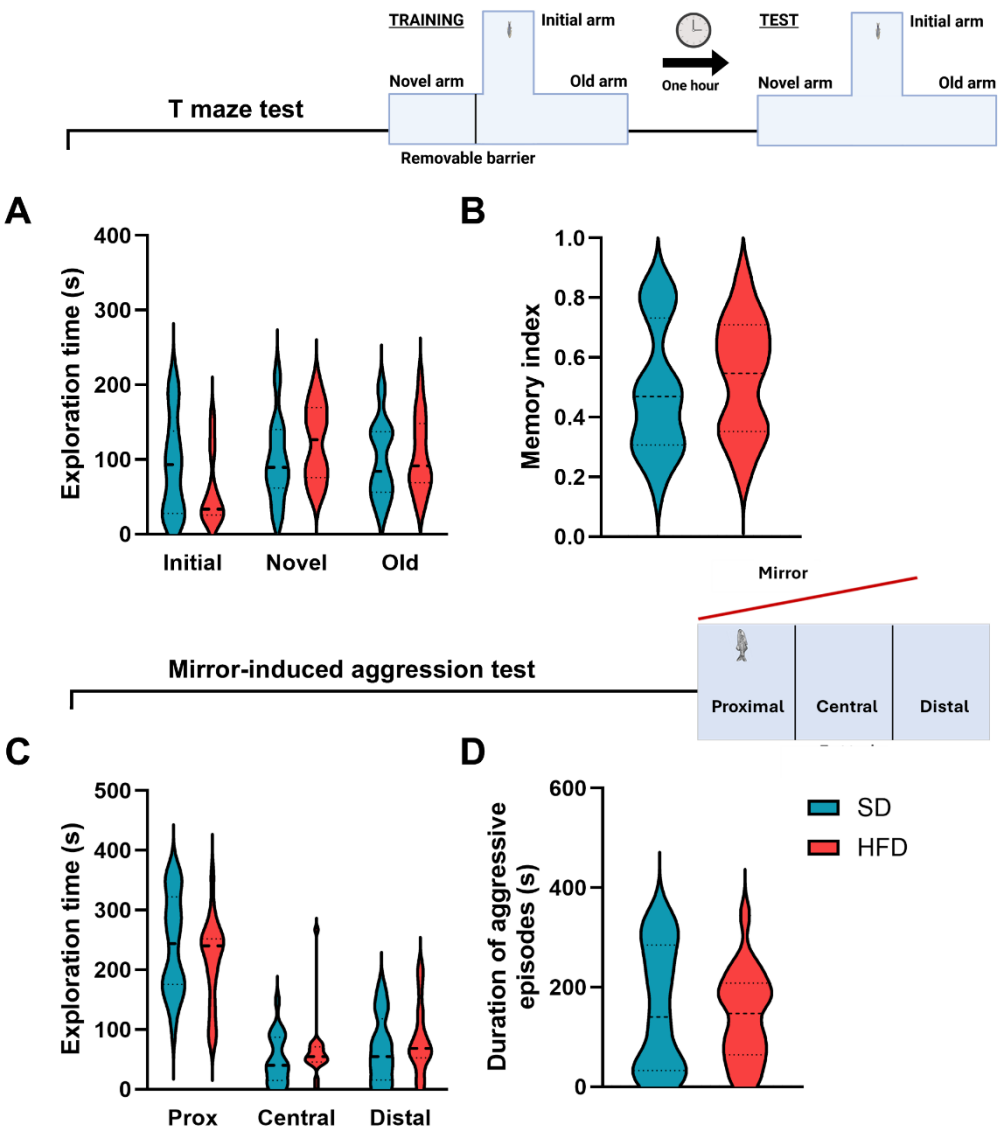

Figure 3. HFD did not affect cognition and aggressiveness in young adult zebrafish. (A) Exploration time in each arm of the T-maze during the testing phase. (B) Memory index calculated as the ratio of time spent in the novel arm to the total time spent on both the novel and old arm. (C) Time in each area, and (D) total time of aggressive episodes on MIAT. Data full distribution is shown in violin plots with 1-3 quartiles and were analyzed by Student t-test or Mann-Whitney test. *$p < 0.05$. n= 16-20 per group (T-maze test) and n= 20 per group (aggressiveness test).

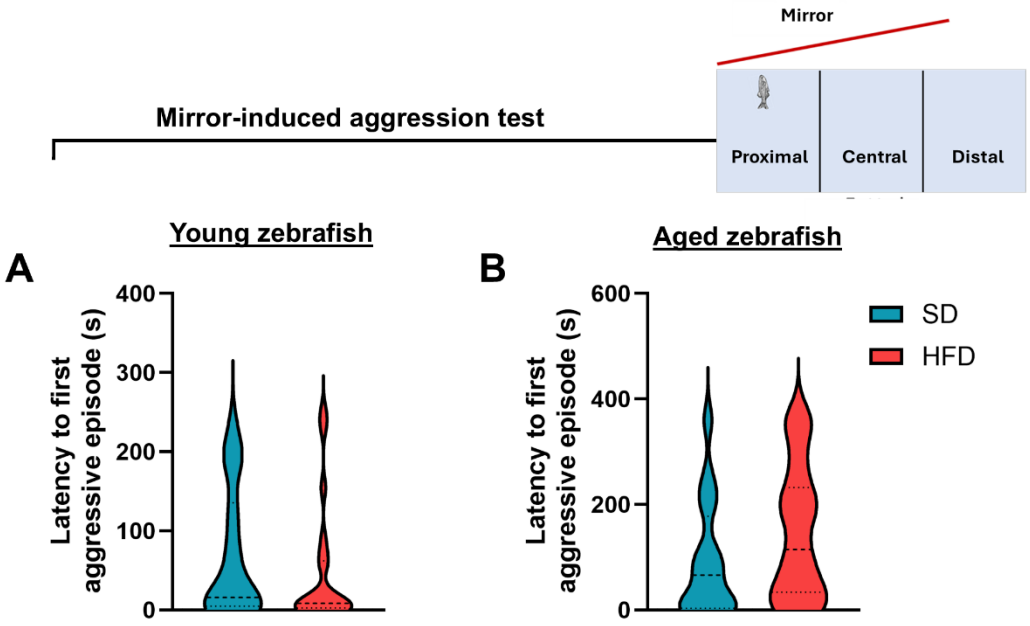

Figure S2. Effect of HFD on aggressive behavior in young and aged adult zebrafish. Latency to first aggressive episode in (A) young and (B) aged adult zebrafish. Data full distribution is shown in violin plot with 1-3 quartiles and were analyzed by student t-test or Mann-Whitney. n= 19-32 per group.

Effect of HFD on brain mitochondrial function in young adult zebrafish

Mitochondrial oxygen consumption in the zebrafish encephalon was assessed using the Oroboros high-resolution respirometry system. Our results revealed a pronounced HFD-induced disruption in mitochondrial function, with a significant reduction in oxygen consumption observed in 4 out of the 6 evaluated respiratory states compared to the standard diet (SD) group (Fig. 4A). Specifically, HFD-fed fish showed decreased respiration in complex I-linked phosphorylation state (C1-state 3; ADP: $t_{(10)} = 2.643$, $p = 0.0246$), ATP synthase-inhibited leak respiration (C1+C2-state 4; oligomycin: $t_{(9)} = 3.075$, $p = 0.0133$), maximal respiratory capacity (CCCP: $t_{(10)} = 2.366$, $p = 0.0396$), and complex II-linked maximal respiratory capacity (after rotenone; $t_{(10)} = 2.686$, $p = 0.0229$).

The mRNA levels of genes coding for the electron transport chain (ETC) subunits were quantified by qRT-PCR using target-specific primers (Fig. 4B, Table S1). HFD significantly increased the expression of *uqcrc10* ($t_{(4)} = 3.731$, p= 0.0203) and *cox6a1* ($t_{(4)} = 3.354$, p= 0.0285) genes by 2.5- and 10.6-fold, respectively. On the other hand, the expression of *ndufs2* ($t_{(6)} = 1.073$,

p= 0.1623), *sdha* ($t_{(6)}$ = 0.1408, p= 0.8926), *cox4i1* ($t_{(6)}$ = 1.532, p= 0.1763), *cox6c* ($t_{(6)}$ = 0.0384, p= 0.9706), *atp5i* ($t_{(6)}$ = 1.568, p= 0.1680) did not differ between the experimental groups.

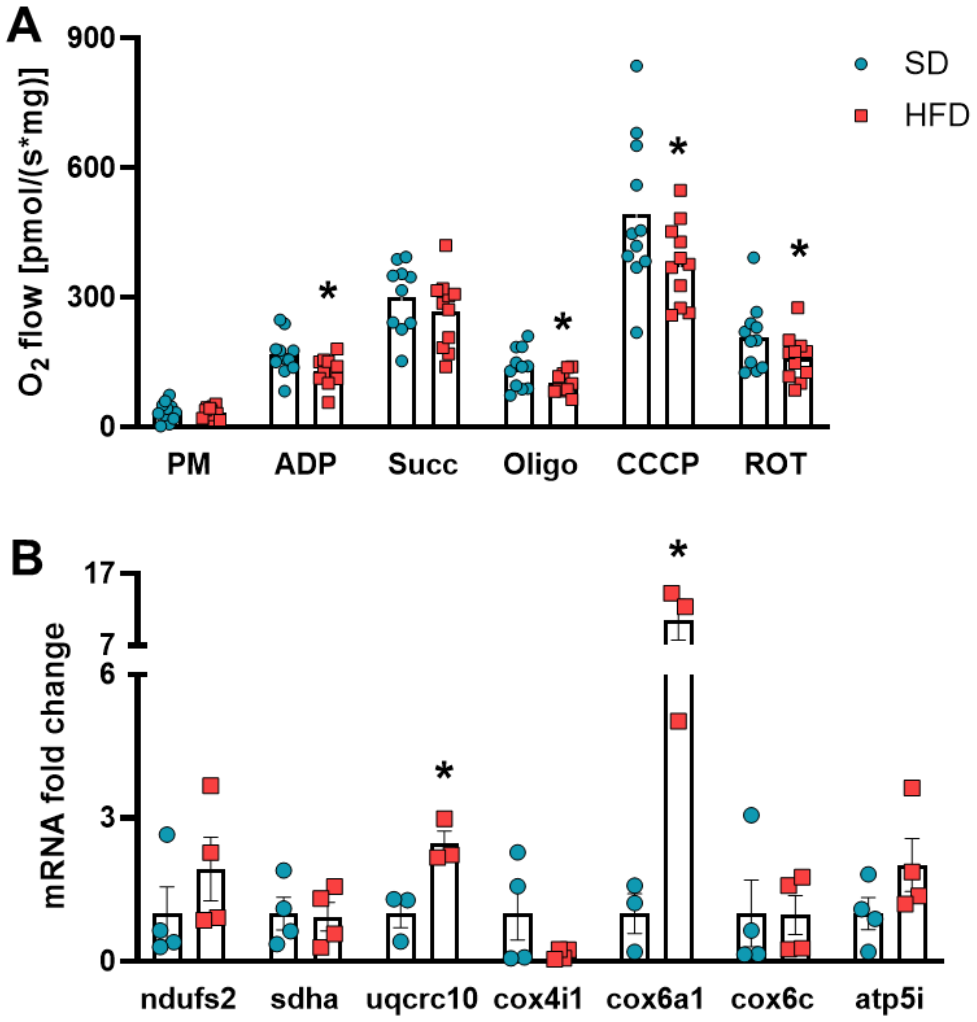

Figure 4. Effect of HFD on brain mitochondria in young adult zebrafish. (A) Oxygen consumption was assessed using the Oroboros oxygraph through the sequential addition of substrates and inhibitors, allowing mitochondrial evaluation in different states: C1- state 2 (PM), C1- state 3 (ADP), C1+ C2- state 3 (Succ), C1+ C2- state 4 (Oligo), C1+ C2- maximun uncoupled capacity (CCCP), and C2- maximum uncoupled capacity (ROT). Data are expressed as mean ± standard error of the mean and were analyzed by Student t-test. (B) The mRNA transcripts were analyzed by qRT-PCR using specific primers for each gene. The data were normalized by EF1a mRNA. *$p < 0.05$. n = 11 per group (high resolution respirometry) and n = 3-4 per group (mRNA expression).

3.2. Effect of HFD in aged adult zebrafish

HFD induces an obesogenic phenotype in aged adult zebrafish

Aged adult zebrafish (17-22 months old) developed phenotypic changes associated with obesity after two weeks on a HFD based on egg yolk (Fig. 1A). We found no difference in the initial body weight between the experimental groups (Fig. 5A; $t_{(93)}$=0.2704; p=0.7875). However, zebrafish fed

the HFD showed significantly higher body weight from day 10 of the diet until the end of the experimental protocol (Fig. 5A; day 10: $t_{(92)}$= 2,106; p= 0.0379; day 15: $t_{(91)}$= 2.054; p= 0.0428). Additionally, the HFD group exhibited increased blood glucose levels (Fig. 5C; $t_{(59)}$=2,325; p= 0.0236) and greater abdominal length (Fig. 5D; U= 607.5; p= 0.0138), whereas standard length did not change between groups (Fig. 5E; U= 800; p= 0.3683).

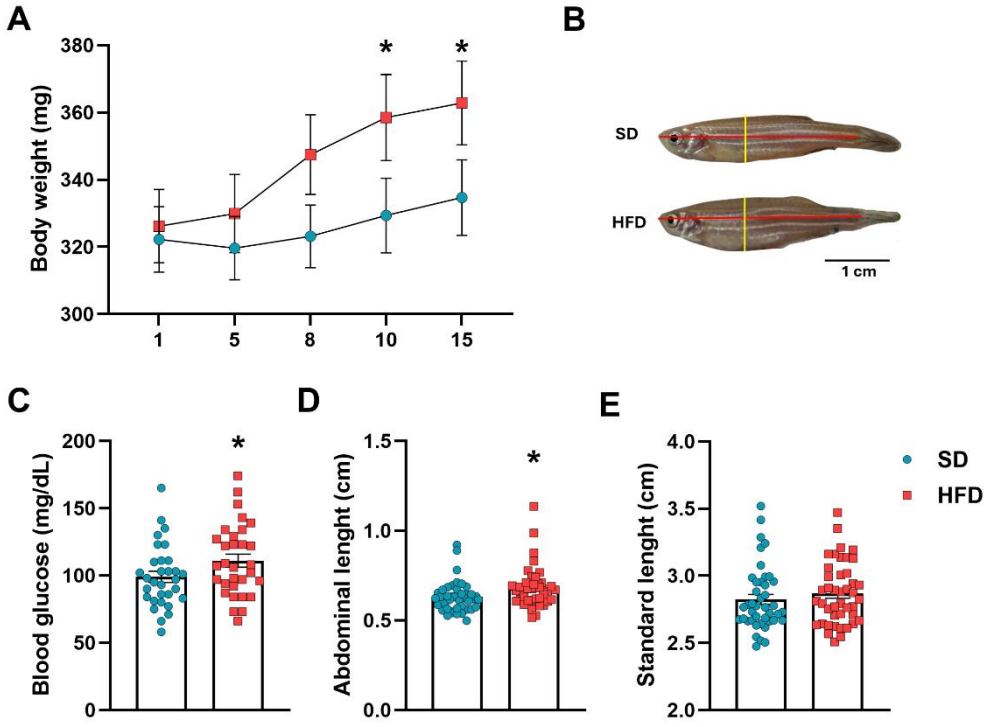

Figure 5. HFD induces an obesogenic phenotype in aged adult zebrafish. (A) Body weight registered throughout the experiment. (B) Representative images of the experimental groups after two weeks on the diet, showing standard (red line) and abdominal (yellow line) lengths. (C) Blood glucose levels, (D) abdominal length and (E) standard length measured using ImageJ software. Data are shown as mean ± standard error of the mean and were analyzed by student t-test. *p< 0.05. n= 31-48 per group.

HFD did not impair locomotion but induced an anxiogenic effect in aged adult zebrafish

Locomotory capacity was not affected by HFD consumption on aged adult zebrafish, as demonstrated by total distance traveled (Fig. 6A; $t_{(61)}$ = 0.7557, p=0.4528), maximum speed (Fig. S1D; U= 452; p=0.5500), and absolute turning angle (Fig. S1E; $t_{(61)}$ = 1,611, p= 0.1124). HFD induced an anxiogenic effect, indicated by reduction of the time spent in the top area (Fig. 6B; U=334.5; p= 0.0393), and of the number of entrances in the top area (Fig. 6C; U=368; p= 0.0379), and increased latency to first entry the top area (Fig. 6D; $t_{(43)}$ = 2.529, p=0.0152). Immobility time did not vary between groups (Fig. S1F; U= 451.5; p= 0.6677).

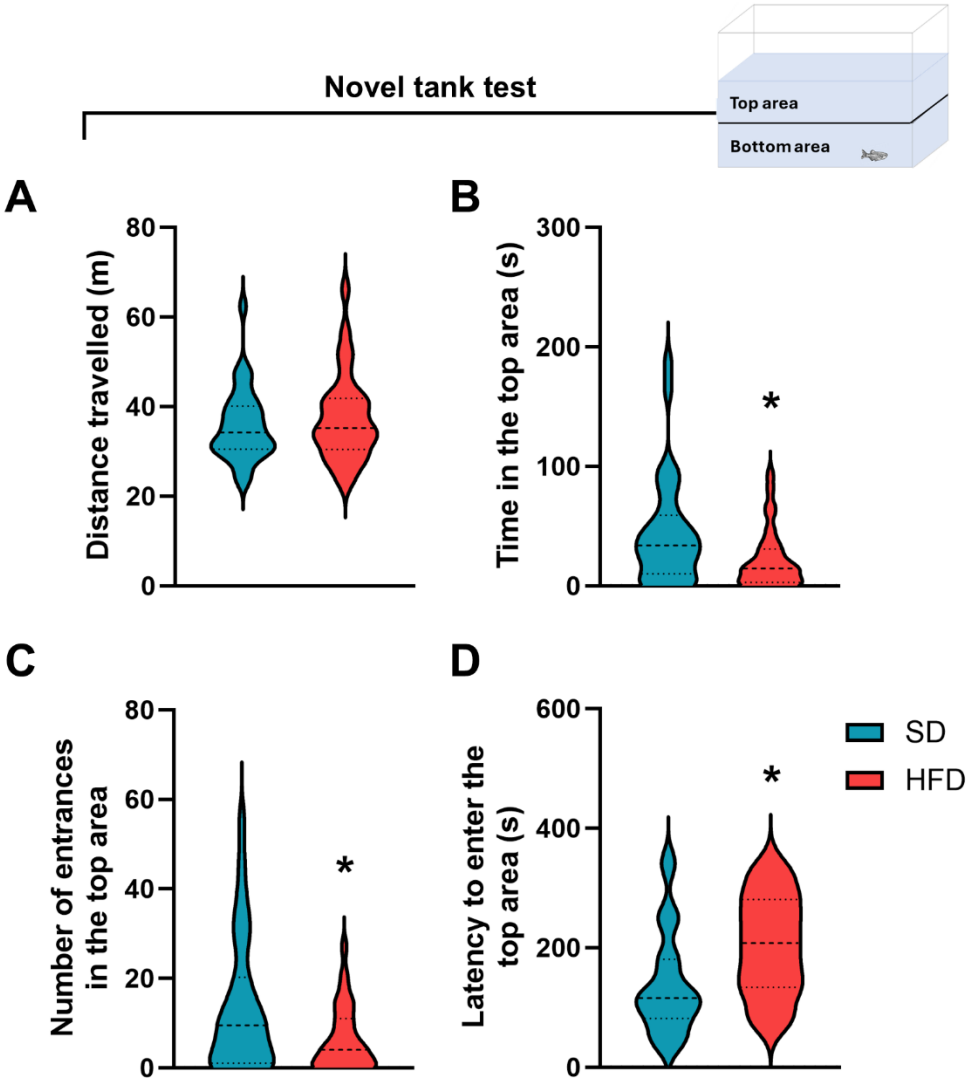

Figure 6. HFD did not impair locomotion but induced an anxiogenic effect in aged zebrafish. The novel tank apparatus was virtually divided into two horizontal areas. (A) The total distance traveled evaluated during the 6-minutes behavioral test was used as a locomotion parameter. (B) Time in the top area, (C) number of entries into the top area, and (D) the latency to first entry into the top area were used as anxiety-like behavior parameters. Data full distribution is shown in violin plots with 1-3 quartiles and were analyzed by student t-test or Mann-Whitney test. *$p < 0.05$. n= 30-32 per group.

HFD induced cognitive decline but did not affect aggressiveness in aged adult zebrafish

T-maze test demonstrated that HFD consumption impairs spatial memory consolidation in aged adult zebrafish (Fig. 7A). During the testing phase, HFD-fed animals spent significantly more time exploring the familiar (old) arm ($t_{(34)} = 2.184$, $p=0.0359$) compared to the novel arm (Fig. 7A; $t_{(34)} = 2.276$, $p=0.0293$). Accordingly, the memory index was significantly reduced in the HFD group (Fig. 7B; $t_{(34)} = 2.613$, $p=0.0133$), indicating a deficit memory performance.

HFD did not affect aggressive behavior in the MIAT. No significant differences were observed between the experimental groups regarding the time spent in each tank zone (Fig. 7C; proximal: $t_{(61)}$ = 0.1900. p= 0.8499; central: $t_{(61)}$ = 0.6631, p= 0.5098; distal: $t_{(61)}$ = 0.7038, p= 0.4842). Similarly, there were no differences in total aggression time across the testing period (Fig. 7D; U= 440; p= 0.4458), nor in the latency to the first aggressive episode (Fig. S2B; U= 368.5; p= 0.0800).

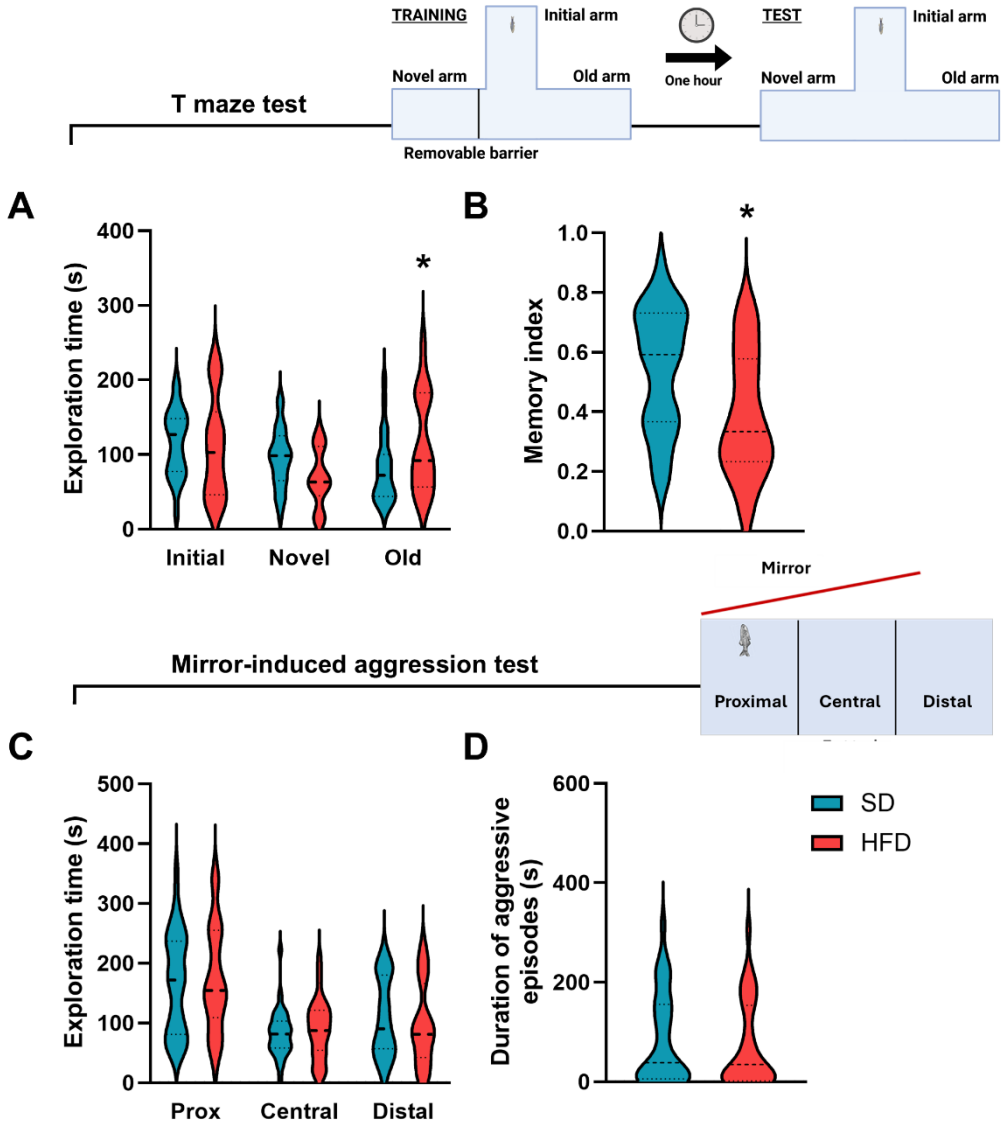

Figure 7. HFD induced cognitive decline but did not affect aggressiveness in aged zebrafish. (A) Exploration time in each arm of the T-maze during the testing phase. (B) Memory index calculated as the ratio of time spent in the novel arm to the total time spent on both the novel and old arm. (C) Time in each area, and (F) total time of aggressive episodes on MIAT. Data full distribution is shown in violin plots with 1-3 quartiles and were analyzed by student t-test or Mann-Whitney test accordingly with normality test result. *p < 0.05. n= 14-23 per group (T-maze test) and n= 31-32 per group (aggressiveness test).

Effect of HFD on brain mitochondria in aged adult zebrafish

Aging differently affects mitochondrial function in the zebrafish brain. High-resolution respirometry revealed that HFD increased mitochondrial oxygen consumption in the brains of aged zebrafish (Fig. 8A), a finding confirmed by an additional assay evaluating complex II-dependent respiration (Fig. S3). In the first oxygraph set (Fig. 8A), which assessed metabolism dependent on both complex I and complex II. We observed an increase in the maximum respiratory capacity (C1+C2) in the HFD upon CCCP titration ($t_{(52)}$ = 2.272; p = 0.0272). No significant differences were observed between groups in the other mitochondrial respiratory states: pyruvate/malate (PM: $t_{(52)}$ = 0.0850, p = 0.9325), ADP-stimulated respiration ($t_{(52)}$ = 0.9653, p = 0.3389), succinate-stimulated respiration ($t_{(52)}$ = 1.080, p = 0.2850), oligomycin-induced LEAK ($t_{(52)}$ = 0.2304, p = 0.8187), and rotenone-inhibited state ($t_{(52)}$ = 0.7363, p = 0.4649). In the second oxygraph set (Fig. S3), where complex I was inhibited with rotenone to isolate complex II-dependent respiration, HFD significantly increased oxygen consumption in state 3 following ADP addition ($t_{(10)}$= 3.225, p = 0.0091) and in the maximum uncoupled state after CCCP titration ($t_{(10)}$ = 2.969, p = 0.0141). No significant differences were observed in the states induced by rotenone + succinate ($t_{(10)}$ = 1.594, p = 0.1420) or by oligomycin ($t_{(10)}$ = 1.464, p = 0.1739).

The mRNA transcriptions of genes related to subunits of ETC were quantified by qRT-PCR using target-specific primers (Fig. 8B). HFD induced an increase in the expression of several ETC-related genes, including *ndufs2* ($t_{(5)}$ = 3.013, p= 0.0297), *cox4i1* ($t_{(6)}$ = 2.044, df=6; p= 0.0435), *cox6a1* ($t_{(5)}$ = 3.937, p= 0.0110), and *cox6c* ($t_{(5)}$ = 0.2836, p= 0.0364) by 31.5-, 180.6-, 148.2-, and 9.4-fold, respectively, when compared with the control group. While genes, *sdha* ($t_{(5)}$ = 1.810, p= 0.1301), *uqcrc10* ($t_{(6)}$ = 1.667, p= 0.1466), and *atp5i* ($t_{(6)}$ = 1.703, p= 0.1395) were not significantly affected by HFD.

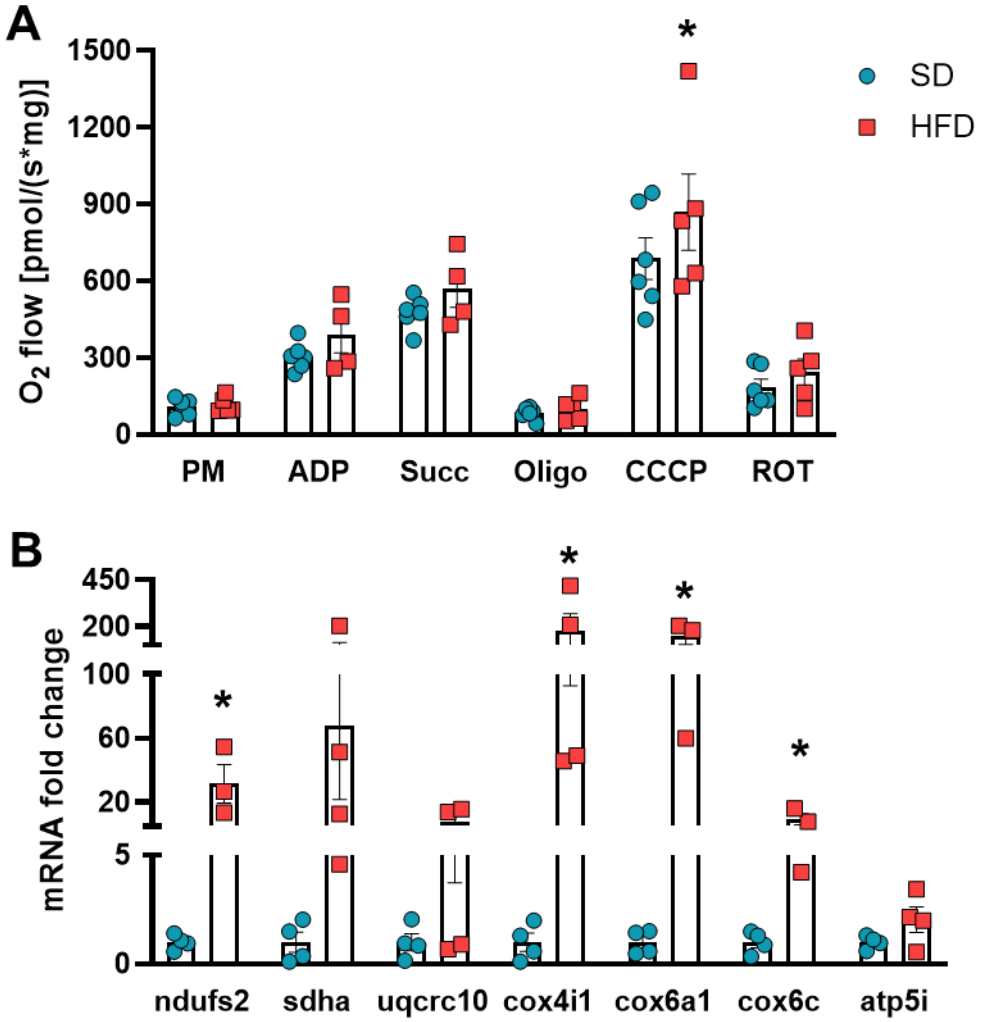

Figure 8. Effect of HFD on brain mitochondria in aged adult zebrafish. (A) Oxygen consumption was assessed using the Oroboros oxygraph through the sequential addition of substrates and inhibitors, allowing mitochondrial evaluation in different states: C1- state 2 (PM), C1- state 3 (ADP), C1+ C2- state 3 (Succ), C1+ C2- state 4 (Oligo), C1+ C2- maximum uncoupled capacity (CCCP), and C2- maximum uncoupled capacity (ROT). Data are expressed as mean ± standard error of the mean and were analyzed by student t-test. (B) The mRNA transcripts were analyzed by qRT-PCR using specific primers for each gene. The data were normalized by EF1a mRNA. Data are expressed as mean ± standard error of the mean and were analyzed by student t-test. *$p < 0.05$. n = 5-6 per group (oximetry) and n = 3-4 per group (mRNA expression).

To further investigate the cellular impact of HFD, we conducted ultrastructural analysis of the brain using transmission electron microscopy (TEM). This analysis was performed exclusively in aged fish, as preliminary observations indicated that young individuals did not exhibit substantial structural alterations under the same dietary conditions. At low magnification (2.5K), mitochondria appeared more abundant and exhibited a fragmented or fissioned morphology in HFD-treated brains compared to controls (Fig. S4). At higher magnification (6K), control fish displayed

mitochondria with well-defined cristae, intact membranes, and a dense matrix (Fig. 9A), while HFD-fed individuals showed notable alterations, including heterogeneity in shape and size, with abnormally arranged cristae (i.e. blurred or disorganized), absence of cristae in distinctive areas and a vesiculated matrix appearance (Fig. 9B). Furthermore, ultrastructural evaluation revealed a marked reduction in synaptic vesicle content in the HFD group (Fig. 9B, S1B) relative to the control group (Fig. 9A, S1A).

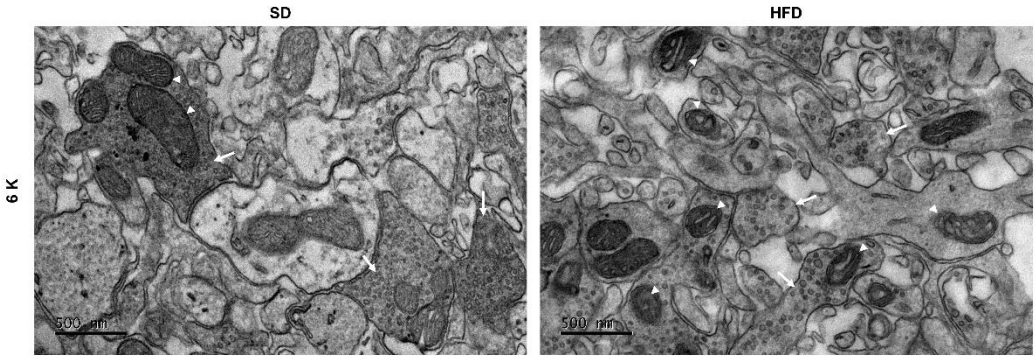

Figure 9. Effect of HFD on brain ultrastructural microscopy in aged zebrafish. Zebrafish brain ultrastructure was analyzed by transmission electron microscopic. Images from brain of zebrafish that received SD or HFD are displayed under magnification of 6K. White arrows indicate mitochondia and white block arrows indicate synaptic vesicles.

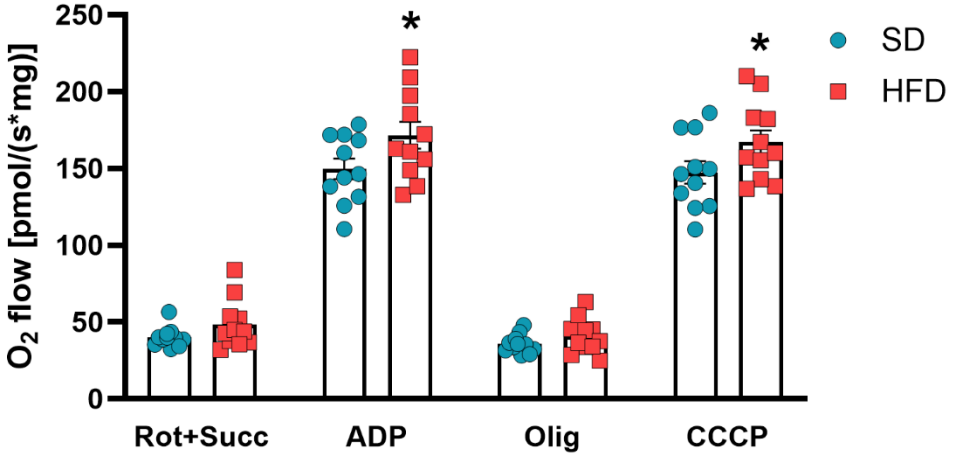

Figure S3. Effect of HFD on C2-dependet mitochondrial metabolism in aged adult zebrafish. (A) Oxygen consumption was assessed using the Oroboros oxygraph through sequential addition of substrates and inhibitors, allowing mitochondrial evaluation in different states: C2- state 2 (Rot+ Succ), C2- state 3 (ADP), C2- state 4 (Oligo), C2- maximum uncoupled capacity (CCCP). Data are expressed as mean ± standard error of the mean and were analyzed by student t-test. *$p < 0.05$. n = 11 per group.

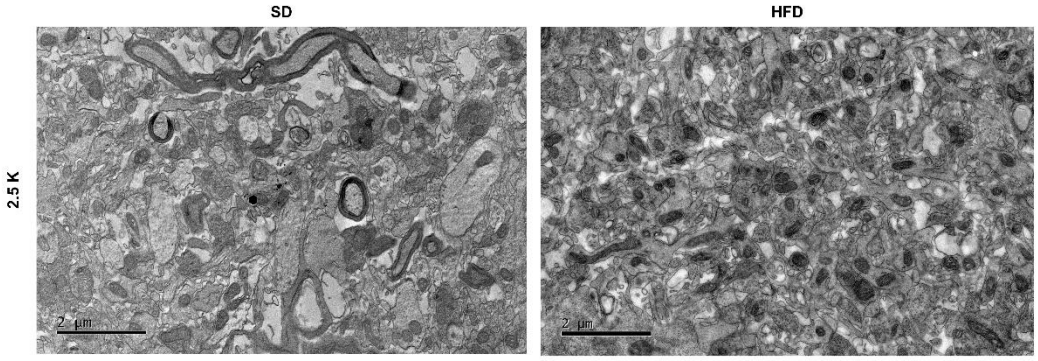

Figure S4. Effect of HFD on brain ultrastructural microscopy in aged zebrafish. Zebrafish brain ultrastructure was analyzed by transmission electron microscopic. Images from brain of zebrafish that received SD or HFD are displayed under magnification of 2.5K.

4. DISCUSSION

The global trend of population aging, combined with the worsening of dietary habits observed in recent decades, represents a new and pressing public health concern that requires deeper understanding. The coexistence of these conditions increases the risk of metabolic and neurological dysregulations, as well as psychobehavioral disorders. In the present study, we investigated the combined effects of age and diet by exposing young and aged zebrafish to either a standard diet or a HFD for 14 days. Consistent with previously reported clinical observations, we found that aging and HFD acted synergistically to promote CNS dysfunction, evidenced by anxiety-like behavior, cognitive impairment, and disrupted brain mitochondrial homeostasis in aged obese zebrafish while young obese zebrafish did not displayed an evident behavioral alteration.

Metabolic dysfunction induced by unbalanced diets, including HFD, is well-documented in zebrafish (Gut et al., 2017; Landgraf et al., 2017; Picolo et al., 2021; Zang et al., 2018b). In this study, we aimed to investigate whether the impact of HFD is more pronounced in aged fish. Regarding metabolic changes, our data expands on previous findings by demonstrating an obesogenic phenotype in zebrafish, regardless of whether they are young or aged. Increased body weight was already evident by the beginning of the second week of treatment, along with a significant increase in abdominal length in both age groups, indicating a greater accumulation of white adipose tissue. This feature is considered the primary diagnostic criterion for metabolic syndrome in humans (Alberti et al., 2009; Bigaard et al., 2005) and plays a key role in the development of metabolic alterations, such as systemic inflammation and endocrine dysfunction (Gomes Gonçalves et al., 2023; Matsuzawa et al., 2011).

In humans, genetic predisposition to metabolic diseases is considered as a risk factor for the development of psychiatric disorders, such as depression, attention deficit disorder, anxiety, and schizophrenia (Gao et al., 2024), underscoring the role of metabolic dysfunction in the development of neuropathologies. Our findings align with previous evidence indicating that aging exacerbates the brain's vulnerability to metabolic challenges induced by HFD, as evidenced by cognitive impairment and anxiety-like behavior only in aged obese zebrafish but not in their younger counterparts. Similarly, Spencer and D'Angelo et al. (2017) demonstrated that short-term HFD served as a neuroinflammatory trigger in aged, but not young adult rats, resulting in marked cognitive impairment. These impairments were paralleled by increased microglial activation, as indicated by elevated Iba1 and CD11b expression exclusively in aged animals, along with an enhanced expression of MHCII and elevated IL-1β protein levels in hippocampus (Spencer, D'Angelo, et al., 2017b). In another study, the inhibition of the IL-1β receptor reversed long-term potentiation impairment induced by HFD only in older mice. The synergistic effect between aging and HFD consumption was also observed in the development of anxiety-like behavior. Additionally, both aging and HFD consumption altered pathways related to metabolic signaling and neurodegenerative processes(Evans et al., 2024).

The consumption of unbalanced diets in humans is associated with accelerated overall aging processes, affecting the CNS locally (Ravi et al., 2025). In a study involving 8,630 individuals, consumption of saturated fats increased the risk of Alzheimer's disease and dementia by 39% and 105%, respectively, with diet-induced metabolic alterations associated with the early development of symptoms (Ruan et al., 2018). Furthermore, elevated BMI demonstrates a positive association with brain atrophy (Gustafson et al., 2004), constituting a significant risk factor for dementia (Whitmer et al., 2008) and doubling the likelihood of developing Alzheimer's disease (Anstey et al., 2011). On the other hand, improvements in dietary habits have neuroprotective effects in humans, particularly in older individuals (Hadem et al., 2019), being effective in managing dementia (Wahl et al., 2016) and improving mood alterations (Redman & Ravussin, 2011), in association with enhanced metabolic health.

The synergistic impact of aging and poor dietary habits on the CNS stems from the fact that both factors promote the activation of pathological mechanisms in the brain. Among these, mitochondrial dysfunction stands out due to its critical importance for the basic functioning and survival of neuronal cells (Tan & Norhaizan, 2019), being a limiting factor for synaptogenesis and

neuronal plasticity (Benard et al., 2007; Li et al., 2004). Moreover, mitochondrial dysfunction plays a central role in the pathogenesis of various neurological and psychiatric disorders, activating processes such as oxidative stress, neurodegeneration, and neuroinflammation (Cobley et al., 2018; Penna et al., 2020). Consequently, mitochondria have been effectively used as therapeutic targets for various neuropathologies, including neurodegenerative diseases (Kurz et al., 2010). For example, the effect of mitochondrial dysfunction induced by Pink1 deficiency in zebrafish was evidenced as a viable Parkinson's disease model. Through the screening of neuroactive drugs, it was demonstrated that the maintenance of mitochondrial homeostasis could delay neurodegeneration (Soman et al., 2017; Y. Zhang et al., 2017).

Neurobehavioral alterations, such as cognitive deficits, anxiety-like behavior, and depressive symptoms, are often accompanied by mitochondrial changes (de Paula et al., 2021). Herein we performed a detailed evaluation of brain mitochondria in zebrafish using three complementary approaches: high-resolution respirometry to assess mitochondrial metabolism, gene expression of respiratory chain components, and ultrastructural mitochondrial microscopy. Our data reveals an age-dependent effect of HFD on brain mitochondrial metabolism. In young animals, HFD led to a reduction in cerebral bioenergetic capacity, including decreased oxidative phosphorylation and maximum respiration. In contrast, aging zebrafish exhibited an upregulation of brain mitochondrial oxygen consumption in response to HFD. The HFD-induced increase in mitochondrial metabolism in aged fish was further confirmed using a protocol dependent exclusively on complex II, by inhibiting complex I with rotenone.

Alterations in brain mitochondrial metabolism have been reported in rodent models of obesity and are frequently associated with neuronal dysfunction, reduced synaptic density, and cognitive decline. Several mechanisms may underlie these effects, including impaired bioenergetics, mitochondrial calcium imbalance, activation of apoptotic signaling pathways (via caspases and cytochrome c), and increased oxidative stress. The oxidative damage to mitochondrial components, including electron transport chain complexes, may further compromise mitochondrial function and integrity, leading to lower ATP production and exacerbated oxidative stress (Freeman et al., 2014). Central resistance to metabolic hormones like insulin may also contribute to the dysregulation of pathways responsible for mitochondrial activation and maintenance. Interestingly, physical exercise has been shown to reverse mitochondrial impairments, ameliorate cognitive deficits, and suppress apoptosis in HFD-fed mice (H.-S. Park et al., 2018).

There are several explanations for the increased mitochondrial metabolism observed in aged zebrafish fed with HFD. Mitochondria are highly dynamic organelles capable of monitoring available energy sources to prioritize one metabolic pathway over others. For instance, the β-oxidation or pyruvate oxidation pathways derived from glycolysis may be activated while others are inhibited (Tsilingiris et al., 2021). Unbalanced diets may interfere with the mitochondrial substrate-sensing process, reducing the metabolic flexibility. Consequently, metabolic pathways could become overstimulated, increasing substrate flux into mitochondria and promoting hyperactivation of the ETC with higher production of reactive oxygen species (ROS), without an improvement in ATP production (Jørgensen et al., 2017; Muoio, 2014).

Another explanation involves the process of mitophagy, which is impaired by aging and is closely associated with the development of age-related neuropathologies, including neurodegenerative diseases (Nixon, 2013). Unbalanced diets also impact the mitophagy process, more severely in older individuals, leading to a higher accumulation of dysfunctional mitochondria. These mitochondria appear in a more fragmented morphology due to disrupted mitochondrial dynamics, which are also synergistically impacted by aging and HFD consumption (Galizzi et al., 2021). Consequently, mitochondrial accumulation may promote an apparent increase in mitochondrial metabolism under experimental conditions, without reflecting a genuinely improved bioenergetic status within the cellular environment. Supporting this hypothesis, ultrastructural microscopy revealed a higher number of mitochondria in HFD brains, with a more fragmented morphology. TEM analysis also demonstrated that the HFD reduced the number of synaptic vesicles (SV). This phenomenon may be related to mitochondrial dysfunction, given that this organelle plays a central role in neural plasticity and neurotransmission (Todorova & Blokland, 2017). Proper mitochondrial functioning is essential for maintaining SV homeostasis through calcium regulation (Walters & Usachev, 2023) and bioenergetic support (Palikaras & Tavernarakis, 2020). Thus, inhibition of the ETC in the brain is expected to impact the regeneration of SV and their release into the synaptic cleft (Ivannikov et al., 2013).

Interestingly, we observed a HFD-induced increase in the expression of ETC genes, more pronounced in older animals. All genes evaluated are localized and expressed in the cell nucleus, and its expression is finely regulated to meet cellular bioenergetic demands (Cogliati et al., 2018). It is worth noting that there are few studies evaluating the expression of such genes in response to unbalanced diets in zebrafish. Controversially, those studies report reductions in ETC genes

expression in the brain (Selfridge et al., 2015), skeletal muscle (Sparks et al., 2005), and white adipose tissue (M.-S. Choi et al., 2015). Selfridge et al. evaluated mitochondrial regulation and function in the brain in response to two HFD: one ketogenic, which was metabolically beneficial, and the other obesogenic. They reported increase in transcripts of CREB, PGC1α, and NRFS2 in response to both HFD, highlighting the activation of mitochondrial biogenesis pathways responsible for mRNA transcription for the ETC. However, they also observed a reduction in COX4I1 mRNA with the obesogenic diet and a reduction in mtDNA with both HFDs, indicating lower mitochondrial content. The authors suggest that increased mitophagy, which occurs in response to HFD-induced mitochondrial dysfunction, might explain these findings (Selfridge et al., 2015).

Our ultrastructural findings align with previous reports showing that HFD induces prominent alterations in aged animals. In HFD mice, glial cells related to blood-liquor crosstalk, exhibited remarkable ultrastructural anomalies, including altered alignment, reduced junctions, degenerating organelles, and higher content of lipid droplets, lysosomes, and autophagosomes (Severi et al., 2021). Authors found that the mitochondria were very heterogeneous in shape and size, and presented swellings and internal alterations, including abnormally arranged cristae, absence of cristae in distinctive areas and, sometimes, the presence of myelin-like structures. This cellular event is characterized by progressive changes in mitochondrial morphology: they first enlarge, the cristae disappear, and lastly, the organelle appears empty. Moreover, a high-fat diet (HFD) could likely disrupt neurotransmitter vesicle dynamics, potentially reducing the availability of vesicles for release and impair synaptic transmission, which could be related to the change in fish behavior. Supporting these findings, Yoon et al. (2019) reported that HFD leads to downregulation of multiple mRNAs associated with neurogenesis and synaptic function, as well as changes in non-coding RNA profiles, suggesting that diet exerts multifaceted control over neural gene expression and plasticity (Yoon et al., 2019).

Therefore, the increase in mRNA transcripts observed in our study may be related to the activation of biogenesis pathways as a compensatory mechanism for increased mitophagy and mitochondrial bioenergetic impairment. However, future experiments are needed to confirm these hypotheses and elucidate possible underlying mechanisms. Our data suggests an age-dependent HFD effect on mitochondrial parameters, which has been scarcely explored in the literature and could pave the way for new insights and the development of therapeutic approaches.

5. CONCLUSION

Here we described for the first time the synergetic impact of aging and HFD consumption in neurobehavioral parameters of zebrafish, as evidenced by cognitive impairment and anxiety-like behavior of aged animals fed with HFD. We describe an age-specific effect of HFD on mitochondrial homeostasis on zebrafish encephalon, with a reduction of its function in young animals and an increase in aged animals. We hypothesize that this increase is associated with an accumulation of dysfunctional mitochondria, since mitophagy process is synergistically impaired by aging and HFD consumption. Another possibility is that both factors work together to disrupt mitochondrial substrate screening, leading to ETC overload and hyperactivation. We also observed a robust increase in mRNA transcription for proteins integrating the ETC complexes, which may represent a compensatory mechanism to reestablish cellular bioenergetic, mainly in aged individuals, which were more affected by HFD consumption. This study underscores the translational value of zebrafish as a model for investigating the intersection between diet, aging, and brain health, which can be further explored to evaluate the role of mitochondrial dysfunction on neurological alteration, and to help develop future therapeutic approaches to avoid neurological complications.

Contributions

VLP: Conceptualization, Methodology, Bibliographic revision and foundation, Experiment design, Investigation, Data Curation, Writing; LAT: Methodology, Investigation; WRS: Methodology, Investigation; NMC: Investigation; ERS: Methodology, Investigation; WRV: Writing review, Investigation; PMQ: Investigation, Writing - Review & Editing; JTG: Data Curation, Supervision; CKG: Resources, Writing - Review & Editing; DMP: Methodology, Resources, Supervision; AFB: Conceptualization, Bibliographic revision and foundation, Methodology, Data Curation, Writing - Review & Editing, Project administration, Funding acquisition, Resources, Supervision.

Ethical Statement

I declare that all procedures were performed in accordance with the current laws of the country in which they were performed. This study was approved by the Ethics Committee for Animal Use of the University of Brasília (Protocol CEUA- UnB: 040/2020).

Declaration of Competing Interest

The authors declare that no competing interests exist.

Acknowledgements

Funding Information: This work was founded by the Fundação de Apoio à Pesquisa do Distrito Federal -FAPDF (00193-00002348/2022-07 and 00193-00000884/2021-89); Conselho Nacional de Desenvolvimento Científico e Tecnológico- CNPq (404466/2023-0 and 201648/2024-5); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - (88881.465507/2019-01); and Instituto Nacional de Ciência e Tecnologia e Neuro-ImunoModulação - INCT-NIM (485489/2014-1).

References

Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., Fruchart, J. C., James, W. P. T., Loria, C. M., & Smith, S. C. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International. In Circulation. https://doi.org/10.1161/CIRCULATIONAHA.109.192644

Angom, R. S., & Nakka, N. M. R. (2024). Zebrafish as a Model for Cardiovascular and Metabolic Disease: The Future of Precision Medicine. Biomedicines, 12(3), 693. https://doi.org/10.3390/biomedicines12030693

Anstey, K. J., Cherbuin, N., Budge, M., & Young, J. (2011). Body mass index in midlife and late-life as a risk factor for dementia: A meta-analysis of prospective studies. Obesity Reviews. https://doi.org/10.1111/j.1467-789X.2010.00825.x

Benard, G., Bellance, N., James, D., Parrone, P., Fernandez, H., Letellier, T., & Rossignol, R. (2007). Mitochondrial bioenergetics and structural network organization. Journal of Cell Science, 120(5), 838–848. https://doi.org/10.1242/jcs.03381

Bigaard, J., Frederiksen, K., Tjønneland, A., Thomsen, B. L., Overvad, K., Heitmann, B. L., & Sørensen, T. (2005). Waist circumference and body composition in relation to all-cause mortality in middle-aged men and women. International Journal of Obesity. https://doi.org/10.1038/sj.ijo.0802976

Bradford, M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry, 72(1–2), 248–254. https://doi.org/10.1006/abio.1976.9999

Butler, M. J., Cole, R. M., Deems, N. P., Belury, M. A., & Barrientos, R. M. (2020). Fatty food, fatty acids, and microglial priming in the adult and aged hippocampus and amygdala. Brain, Behavior, and Immunity, 89, 145–158. https://doi.org/10.1016/j.bbi.2020.06.010

Cavallini, G., Donati, A., Taddei, M., & Bergamini, E. (2007). Evidence for Selective Mitochondrial Autophagy and Failure in Aging. Autophagy, 3(1), 26–27. https://doi.org/10.4161/auto.3268

Cheng, X., Yang, Y., Schwebel, D. C., Liu, Z., Li, L., Cheng, P., Ning, P., & Hu, G. (2020). Population ageing and mortality during 1990–2017: A global decomposition analysis. PLOS Medicine, 17(6), e1003138. https://doi.org/10.1371/journal.pmed.1003138

Chistiakov, D. A., Sobenin, I. A., Revin, V. V., Orekhov, A. N., & Bobryshev, Y. V. (2014). Mitochondrial Aging and Age-Related Dysfunction of Mitochondria. BioMed Research International, 2014, 1–7. https://doi.org/10.1155/2014/238463

Choi, M.-S., Kim, Y.-J., Kwon, E.-Y., Ryoo, J. Y., Kim, S. R., & Jung, U. J. (2015). High-fat diet decreases energy expenditure and expression of genes controlling lipid metabolism, mitochondrial function and skeletal system development in the adipose tissue, along with increased expression of extracellular matrix remodelling- and inflamm. British Journal of Nutrition, 113(6), 867–877. https://doi.org/10.1017/S0007114515000100

Cobley, J. N., Fiorello, M. L., & Bailey, D. M. (2018). 13 Reasons Why the Brain Is Susceptible To Oxidative Stress. Redox Biology, 15(January), 490–503. https://doi.org/10.1016/j.redox.2018.01.008

Cogliati, S., Lorenzi, I., Rigoni, G., Caicci, F., & Soriano, M. E. (2018). Regulation of Mitochondrial Electron Transport Chain Assembly. Journal of Molecular Biology, 430(24), 4849–4873. https://doi.org/10.1016/j.jmb.2018.09.016

Connaughton, V. P., Baker, C., Fonde, L., Gerardi, E., & Slack, C. (2016). Alternate Immersion in an External Glucose Solution Differentially Affects Blood Sugar Values in Older Versus Younger Zebrafish Adults. Zebrafish, 13(2), 87–94. https://doi.org/10.1089/zeb.2015.1155

de Bem, A. F., Krolow, R., Farias, H. R., de Rezende, V. L., Gelain, D. P., Moreira, J. C. F., Duarte, J. M. das N., & de Oliveira, J. (2021). Animal Models of Metabolic Disorders in the Study of Neurodegenerative Diseases: An Overview. Frontiers in Neuroscience, 14(January), 1–22. https://doi.org/10.3389/fnins.2020.604150

de Paula, G. C., Brunetta, H. S., Engel, D. F., Gaspar, J. M., Velloso, L. A., Engblom, D., de Oliveira, J., & de Bem, A. F. (2021). Hippocampal Function Is Impaired by a Short-Term High-Fat Diet in Mice: Increased Blood–Brain Barrier Permeability and Neuroinflammation as Triggering Events. Frontiers in Neuroscience, 15(November), 1–12. https://doi.org/10.3389/fnins.2021.734158

Evans, A. K., Saw, N. L., Woods, C. E., Vidano, L. M., Blumenfeld, S. E., Lam, R. K., Chu, E. K., Reading, C., & Shamloo, M. (2024). Impact of high-fat diet on cognitive behavior and central

and systemic inflammation with aging and sex differences in mice. Brain, Behavior, and Immunity, 118, 334–354. https://doi.org/10.1016/j.bbi.2024.02.025

Fontana, B. D., Cleal, M., Clay, J. M., & Parker, M. O. (2019). Zebrafish (Danio rerio) behavioral laterality predicts increased short-term avoidance memory but not stress-reactivity responses. Animal Cognition, 22(6), 1051–1061. https://doi.org/10.1007/s10071-019-01296-9

Freeman, L. R., Haley-Zitlin, V., Rosenberger, D. S., & Granholm, A.-C. (2014). Damaging effects of a high-fat diet to the brain and cognition: A review of proposed mechanisms NIH Public Access. Nutr Neurosci, 17(6), 241–251. https://doi.org/10.1179/1476830513Y.0000000092

Galizzi, G., Palumbo, L., Amato, A., Conigliaro, A., Nuzzo, D., Terzo, S., Caruana, L., Picone, P., Alessandro, R., Mulè, F., & Di Carlo, M. (2021). Altered insulin pathway compromises mitochondrial function and quality control both in in vitro and in vivo model systems. Mitochondrion, 60, 178–188. https://doi.org/10.1016/j.mito.2021.08.014

Gao, X., Qin, Y., Jiao, S., Hao, J., Zhao, J., Wang, J., Wen, Y., & Wang, T. (2024). Genetic evidence for the causal relations between metabolic syndrome and psychiatric disorders: a Mendelian randomization study. Translational Psychiatry, 14(1), 46. https://doi.org/10.1038/s41398-024-02759-5

Gomes Gonçalves, N., Vidal Ferreira, N., Khandpur, N., Martinez Steele, E., Bertazzi Levy, R., Andrade Lotufo, P., Bensenor, I. M., Caramelli, P., Alvim De Matos, S. M., Marchioni, D. M., & Suemoto, C. K. (2023). Association between Consumption of Ultraprocessed Foods and Cognitive Decline. JAMA Neurology, 80(2), 142–150. https://doi.org/10.1001/jamaneurol.2022.4397

Gustafson, D., Lissner, L., Bengtsson, C., Björkelund, C., & Skoog, I. (2004). A 24-year follow-up of body mass index and cerebral atrophy. In Neurology. https://doi.org/10.1212/01.WNL.0000141850.47773.5F

Gut, P., Reischauer, S., Stainier, D. Y. R., & Arnaout, R. (2017). Little fish, big data: Zebrafish as a model for cardiovascular and metabolic disease. Physiological Reviews, 97(3), 889–938. https://doi.org/10.1152/physrev.00038.2016

Hadem, I. K. H., Majaw, T., Kharbuli, B., & Sharma, R. (2019). Beneficial effects of dietary restriction in aging brain. Journal of Chemical Neuroanatomy, 95, 123–133. https://doi.org/10.1016/j.jchemneu.2017.10.001

Ivannikov, M. V., Sugimori, M., & Llinás, R. R. (2013). Synaptic Vesicle Exocytosis in Hippocampal Synaptosomes Correlates Directly with Total Mitochondrial Volume. Journal of Molecular Neuroscience, 49(1), 223–230. https://doi.org/10.1007/s12031-012-9848-8

Jørgensen, W., Rud, K. A., Mortensen, O. H., Frandsen, L., Grunnet, N., & Quistorff, B. (2017). Your mitochondria are what you eat: a high-fat or a high-sucrose diet eliminates metabolic flexibility in isolated mitochondria from rat skeletal muscle. Physiological Reports, 5(6), 1–10. https://doi.org/10.14814/phy2.13207

Kalueff, A. V., Gebhardt, M., Stewart, A. M., Cachat, J. M., Brimmer, M., Chawla, J. S., Craddock, C., Kyzar, E. J., Roth, A., Landsman, S., Gaikwad, S., Robinson, K., Baatrup, E., Tierney, K., Shamchuk, A., Norton, W., Miller, N., Nicolson, T., Braubach, O., … Schneider, H. (2013).

Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. In Zebrafish (Vol. 10, Issue 1, pp. 70–86). Mary Ann Liebert, Inc. https://doi.org/10.1089/zeb.2012.0861

Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. In Trends in Pharmacological Sciences (Vol. 35, Issue 2, pp. 63–75). NIH Public Access. https://doi.org/10.1016/j.tips.2013.12.002

Kanoh, T., Mizoguchi, T., Tonoki, A., & Itoh, M. (2024). Modeling of age-related neurological disease: utility of zebrafish. Frontiers in Aging Neuroscience, 16. https://doi.org/10.3389/fnagi.2024.1399098

Kauppila, T. E. S., Kauppila, J. H. K., & Larsson, N.-G. (2017). Mammalian Mitochondria and Aging: An Update. Cell Metabolism, 25(1), 57–71. https://doi.org/10.1016/j.cmet.2016.09.017

Kurz, C., Ungerer, I., Lipka, U., Kirr, S., Schütt, T., Eckert, A., Leuner, K., & Müller, W. (2010). The metabolic enhancer piracetam ameliorates the impairment of mitochondrial function and neurite outgrowth induced by ß-amyloid peptide. British Journal of Pharmacology, 160(2), 246–257. https://doi.org/10.1111/j.1476-5381.2010.00656.x

Landgraf, K., Schuster, S., Meusel, A., Garten, A., Riemer, T., Schleinitz, D., Kiess, W., & Körner, A. (2017). Short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development of metabolically healthy versus unhealt. BMC Physiology, 17(1), 4. https://doi.org/10.1186/s12899-017-0031-x

Li, Z., Okamoto, K.-I., Hayashi, Y., & Sheng, M. (2004). The Importance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses. Cell, 119(6), 873–887. https://doi.org/10.1016/j.cell.2004.11.003

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods, 25(4), 402–408. https://doi.org/10.1006/meth.2001.1262

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The Hallmarks of Aging. Cell, 153(6), 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Mackey-Alfonso, S. E., Butler, M. J., Taylor, A. M., Williams-Medina, A. R., Muscat, S. M., Fu, H., & Barrientos, R. M. (2024). Short-term high fat diet impairs memory, exacerbates the neuroimmune response, and evokes synaptic degradation via a complement-dependent mechanism in a mouse model of Alzheimer's disease. Brain, Behavior, and Immunity, 121, 56–69. https://doi.org/10.1016/j.bbi.2024.07.021

Masiero, E., & Sandri, M. (2010). Autophagy inhibition induces atrophy and myopathy in adult skeletal muscles. Autophagy, 6(2), 307–309. https://doi.org/10.4161/auto.6.2.11137

Matsuzawa, Y., Funahashi, T., & Nakamura, T. (2011). The Concept of Metabolic Syndrome: Contribution of Visceral Fat Accumulation and Its Molecular Mechanism. Journal of Atherosclerosis and Thrombosis, 18(8), 629–639. https://doi.org/10.5551/jat.7922

McGrattan, A. M., McGuinness, B., McKinley, M. C., Kee, F., Passmore, P., Woodside, J. V., & McEvoy, C. T. (2019). Diet and Inflammation in Cognitive Ageing and Alzheimer's Disease. Current Nutrition Reports, 8(2), 53–65. https://doi.org/10.1007/s13668-019-0271-4

Meguro, S., Hosoi, S., & Hasumura, T. (2019). High-fat diet impairs cognitive function of zebrafish. Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-53634-z

Moreira, A. L. P., & Luchiari, A. C. (2022). Effects of oxybenzone on zebrafish behavior and cognition. Science of The Total Environment, 808, 152101. https://doi.org/10.1016/J.SCITOTENV.2021.152101

Mundstock, E., Sarria, E. E., Zatti, H., Mattos Louzada, F., Kich Grun, L., Herbert Jones, M., Guma, F. T. C. R., Mazzola in Memoriam, J., Epifanio, M., Stein, R. T., Barbé-Tuana, F. M., & Mattiello, R. (2015). Effect of obesity on telomere length: Systematic review and meta-analysis. Obesity, 23(11), 2165–2174. https://doi.org/10.1002/oby.21183

Muoio, D. M. (2014). Metabolic inflexibility: When mitochondrial indecision leads to metabolic gridlock. Cell, 159(6), 1253–1262. https://doi.org/10.1016/j.cell.2014.11.034

Nixon, R. A. (2013). The role of autophagy in neurodegenerative disease. Nature Medicine, 19(8), 983–997. https://doi.org/10.1038/nm.3232

Otsuka, T., & Matsui, H. (2023). Fish Models for Exploring Mitochondrial Dysfunction Affecting Neurodegenerative Disorders. International Journal of Molecular Sciences, 24(8). https://doi.org/10.3390/ijms24087079

Palikaras, K., & Tavernarakis, N. (2020). Regulation and roles of mitophagy at synapses. Mechanisms of Ageing and Development, 187, 111216. https://doi.org/10.1016/j.mad.2020.111216

Park, H.-S., Cho, H.-S., & Kim, T.-W. (2018). Physical exercise promotes memory capability by enhancing hippocampal mitochondrial functions and inhibiting apoptosis in obesity-induced insulin resistance by high fat diet. Metabolic Brain Disease, 33(1), 283–292. https://doi.org/10.1007/s11011-017-0160-8

Penna, E., Pizzella, A., Cimmino, F., Trinchese, G., Cavaliere, G., Catapano, A., Allocca, I., Chun, J. T., Campanozzi, A., Messina, G., Precenzano, F., Lanzara, V., Messina, A., Monda, V., Monda, M., Perrone-Capano, C., Mollica, M. P., & Crispino, M. (2020). Neurodevelopmental disorders: Effect of high-fat diet on synaptic plasticity and mitochondrial functions. Brain Sciences, 10(11), 1–15. https://doi.org/10.3390/brainsci10110805

Picolo, V. L., Quadros, V. A., Canzian, J., Grisolia, C. K., Goulart, J. T., Pantoja, C., de Bem, A. F., & Rosemberg, D. B. (2021). Short-term high-fat diet induces cognitive decline, aggression, and anxiety-like behavior in adult zebrafish. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 110, 110288. https://doi.org/10.1016/j.pnpbp.2021.110288

Popkin, B. M., Adair, L. S., & Ng, S. W. (2012). Global nutrition transition and the pandemic of obesity in developing countries. Nutrition Reviews, 70(1), 3–21. https://doi.org/10.1111/j.1753-4887.2011.00456.x

Ravi, S., Kankaanpää, A., Bogl, L. H., Heikkinen, A., Pietiläinen, K. H., Kaprio, J., Ollikainen, M., & Sillanpää, E. (2025). Suboptimal dietary patterns are associated with accelerated biological aging in young adulthood: A study with twins. Clinical Nutrition, 45, 10–21. https://doi.org/10.1016/j.clnu.2024.12.018

Redman, L. M., & Ravussin, E. (2011). Caloric Restriction in Humans: Impact on Physiological,

Psychological, and Behavioral Outcomes. Antioxidants & Redox Signaling, 14(2), 275–287. https://doi.org/10.1089/ars.2010.3253

Ruan, Y., Tang, J., Guo, X., Li, K., & Li, D. (2018). Dietary Fat Intake and Risk of Alzheimer's Disease and Dementia: A Meta-Analysis of Cohort Studies. Current Alzheimer Research, 15(9), 869–876. https://doi.org/10.2174/1567205015666180427142350

Selfridge, J. E., Wilkins, H. M., E, L., Carl, S. M., Koppel, S., Funk, E., Fields, T., Lu, J., Tang, E. P., Slawson, C., Wang, W., Zhu, H., & Swerdlow, R. H. (2015). Effect of one month duration ketogenic and non-ketogenic high fat diets on mouse brain bioenergetic infrastructure. Journal of Bioenergetics and Biomembranes, 47(1–2), 1–11. https://doi.org/10.1007/s10863-014-9570-z

Severi, I., Fosca, M., Colleluori, G., Marini, F., Imperatori, L., Senzacqua, M., Di Vincenzo, A., Barbatelli, G., Fiori, F., Rau, J. V., & Giordano, A. (2021). High-Fat Diet Impairs Mouse Median Eminence: A Study by Transmission and Scanning Electron Microscopy Coupled with Raman Spectroscopy. International Journal of Molecular Sciences, 22(15), 8049. https://doi.org/10.3390/ijms22158049

Soman, S., Keatinge, M., Moein, M., Da Costa, M., Mortiboys, H., Skupin, A., Sugunan, S., Bazala, M., Kuznicki, J., & Bandmann, O. (2017). Inhibition of the mitochondrial calcium uniporter rescues dopaminergic neurons in pink1 − / − zebrafish. European Journal of Neuroscience, 45(4), 528–535. https://doi.org/10.1111/ejn.13473

Sparks, L. M., Xie, H., Koza, R. A., Mynatt, R., Hulver, M. W., Bray, G. A., & Smith, S. R. (2005). A High-Fat Diet Coordinately Downregulates Genes Required for Mitochondrial Oxidative Phosphorylation in Skeletal Muscle. Diabetes, 54(7), 1926–1933. https://doi.org/10.2337/diabetes.54.7.1926

Spencer, S. J., D'Angelo, H., Soch, A., Watkins, L. R., Maier, S. F., & Barrientos, R. M. (2017). High-fat diet and aging interact to produce neuroinflammation and impair hippocampal- and amygdalar-dependent memory. Neurobiology of Aging, 58. https://doi.org/10.1016/j.neurobiolaging.2017.06.014

Spencer, S. J., Korosi, A., Layé, S., Shukitt-Hale, B., & Barrientos, R. M. (2017). Food for thought: how nutrition impacts cognition and emotion. Npj Science of Food, 1(1), 7. https://doi.org/10.1038/s41538-017-0008-y

Stankiewicz, A., McGowan, E., Yu, L., & Zhdanova, I. (2017). Impaired Sleep, Circadian Rhythms and Neurogenesis in Diet-Induced Premature Aging. International Journal of Molecular Sciences, 18(11), 2243. https://doi.org/10.3390/ijms18112243

Stout, M. B., Justice, J. N., Nicklas, B. J., & Kirkland, J. L. (2017). Physiological Aging: Links Among Adipose Tissue Dysfunction, Diabetes, and Frailty. Physiology, 32(1), 9–19. https://doi.org/10.1152/physiol.00012.2016

Tan, B. L., & Norhaizan, M. E. (2019). Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function. Nutrients, 11(11), 1–22. https://doi.org/10.3390/nu11112579

Terman, A., Kurz, T., Navratil, M., Arriaga, E. A., & Brunk, U. T. (2010). Mitochondrial Turnover

and Aging of Long-Lived Postmitotic Cells: The Mitochondrial–Lysosomal Axis Theory of Aging. Antioxidants & Redox Signaling, 12(4), 503–535. https://doi.org/10.1089/ars.2009.2598

Todorova, V., & Blokland, A. (2017). Mitochondria and Synaptic Plasticity in the Mature and Aging Nervous System. Current Neuropharmacology, 15(1), 166–173. https://doi.org/10.2174/1570159X14666160414111821

Tsilingiris, D., Tzeravini, E., Koliaki, C., Dalamaga, M., & Kokkinos, A. (2021). The Role of Mitochondrial Adaptation and Metabolic Flexibility in the Pathophysiology of Obesity and Insulin Resistance: an Updated Overview. Current Obesity Reports, 10(3), 191–213. https://doi.org/10.1007/s13679-021-00434-0

Van houcke, J., Mariën, V., Zandecki, C., Seuntjens, E., Ayana, R., & Arckens, L. (2021). Modeling Neuroregeneration and Neurorepair in an Aging Context: The Power of a Teleost Model. Frontiers in Cell and Developmental Biology, 9. https://doi.org/10.3389/fcell.2021.619197

Vandevijvere, S., Chow, C. C., Hall, K. D., Umali, E., & Swinburn, B. A. (2015). Increased food energy supply as a major driver of the obesity epidemic: a global analysis. Bulletin of the World Health Organization. https://doi.org/10.2471/blt.14.150565

Wahl, D., Cogger, V. C., Solon-Biet, S. M., Waern, R. V. R., Gokarn, R., Pulpitel, T., Cabo, R. de, Mattson, M. P., Raubenheimer, D., Simpson, S. J., & Le Couteur, D. G. (2016). Nutritional strategies to optimise cognitive function in the aging brain. Ageing Research Reviews, 31, 80–92. https://doi.org/10.1016/j.arr.2016.06.006

Walters, G. C., & Usachev, Y. M. (2023). Mitochondrial calcium cycling in neuronal function and neurodegeneration. Frontiers in Cell and Developmental Biology, 11. https://doi.org/10.3389/fcell.2023.1094356

Wang, X., Zhang, J.-B., He, K.-J., Wang, F., & Liu, C.-F. (2021). Advances of Zebrafish in Neurodegenerative Disease: From Models to Drug Discovery. Frontiers in Pharmacology, 12. https://doi.org/10.3389/fphar.2021.713963

Whitmer, R. A., Gustafson, D. R., Barrett-Connor, E., Haan, M. N., Gunderson, E. P., & Yaffe, K. (2008). Central obesity and increased risk of dementia more than three decades later. Neurology, 71(14), 1057–1064. https://doi.org/10.1212/01.wnl.0000306313.89165.ef

Yoon, G., Cho, K. A., Song, J., & Kim, Y.-K. (2019). Transcriptomic Analysis of High Fat Diet Fed Mouse Brain Cortex. Frontiers in Genetics, 10. https://doi.org/10.3389/fgene.2019.00083

Zang, L., Maddison, L. A., & Chen, W. (2018). Zebrafish as a model for obesity and diabetes. Frontiers in Cell and Developmental Biology, 6(AUG), 1–13. https://doi.org/10.3389/fcell.2018.00091

Zhang, D., Shen, C., Chen, N., Liu, C., Hu, J., Lau, K. K., Wen, Z., & Qiu, A. (2025). Long-term obesity impacts brain morphology, functional connectivity and cognition in adults. Nature Mental Health, 3(4), 466–478. https://doi.org/10.1038/s44220-025-00396-5

Zhang, Y., Luo, C., Huang, P., Cheng, Y., Ma, Y., Gao, J., & Ding, H. (2024). Diosmetin Ameliorates HFD-induced Cognitive Impairments via Inhibiting Metabolic Disorders,

Mitochondrial Dysfunction and Neuroinflammation in Male SD Rats. Molecular Neurobiology, 61(10), 8069–8085. https://doi.org/10.1007/s12035-024-04083-x

Zhang, Y., Nguyen, D. T., Olzomer, E. M., Poon, G. P., Cole, N. J., Puvanendran, A., Phillips, B. R., & Hesselson, D. (2017). Rescue of Pink1 Deficiency by Stress-Dependent Activation of Autophagy. Cell Chemical Biology, 24(4), 471-480.e4. https://doi.org/10.1016/j.chembiol.2017.03.005

5. CONCLUSÕES E PERSPECTIVAS FUTURAS

O agravamento dos hábitos alimentares observados nos últimos 40 anos está associado a um impacto severo no sistema público de saúde, atuando como uma das principais causas da epidemia de obesidade e de suas comorbidades (Popkin et al., 2012; Vandevijvere et al., 2015), além de aumentar a incidência de doenças neurológicas e psiquiátricas (de Miranda et al., 2021; Pagliai et al., 2021). Esse problema é ainda mais acentuado pela recente tendência de envelhecimento da população global, e a combinação desses fatores acelera o processo de envelhecimento (Ravi et al., 2025), resultando em um agravamento precoce dos parâmetros neurológicos e maior suscetibilidade à doenças do SNC (Ruan et al., 2018).

O impacto no SNC promovido pela associação entre envelhecimento e consumo de DH foi amplamente estudado em modelos de roedores (Evans et al., 2024; Spencer et al., 2019). Em contraste, este é o primeiro estudo a descrever esse fenômeno no modelo de peixe-zebra, o que representa um avanço na área com novas oportunidade de experimentações e descobertas.

Demonstramos pela primeira vez o impacto da DH no desenvolvimento da obesidade e das alterações metabólicas em peixe-zebra envelhecidos, o que também ocorreu nos animais jovens. Além disso, a DH induziu comportamentos tipo ansioso e comprometimento cognitivo apenas nos animais mais velhos, demonstrando que o envelhecimento aumenta a vulnerabilidade do SNC ao consumo de dietas desbalanceadas também em peixe-zebra.

As análises mitocondriais evidenciaram um efeito relacionado à idade: redução na bioenergética mitocondrial nos animais mais jovens, enquanto nos mais velhos ocorreu um aumento. Ademais, o aumento na transcrição de mRNA das subunidades formadoras da CTE foi detectado em ambas as faixas etárias, embora de forma muito mais robusta nos animais envelhecidos. Esses achados reforçam o papel central da disfunção mitocondrial no desenvolvimento de neuropatologias, evidenciando o efeito diferencial da associação entre envelhecimento e o consumo de dietas desbalanceadas.

Por fim, estabelecemos na Universidade de Brasília um novo modelo experimental para investigar as alterações metabólicas, neurocomportamentais e mitocondriais induzidas pela associação entre envelhecimento e consumo de DH em peixe-zebra. Junto com isso, desenvolvemos a infraestrutura necessárias para realizar diversas análises comportamentais na nossa Universidade.

Concluindo, este estudo reforça o potencial translacional do peixe-zebra reafirmando a conservação de mecanismos patológicos, e abre novas possibilidades para avaliar como o SNC é impactado e para o desenvolvimento de futuras abordagens que visem mitigar os impactos neurológicos.

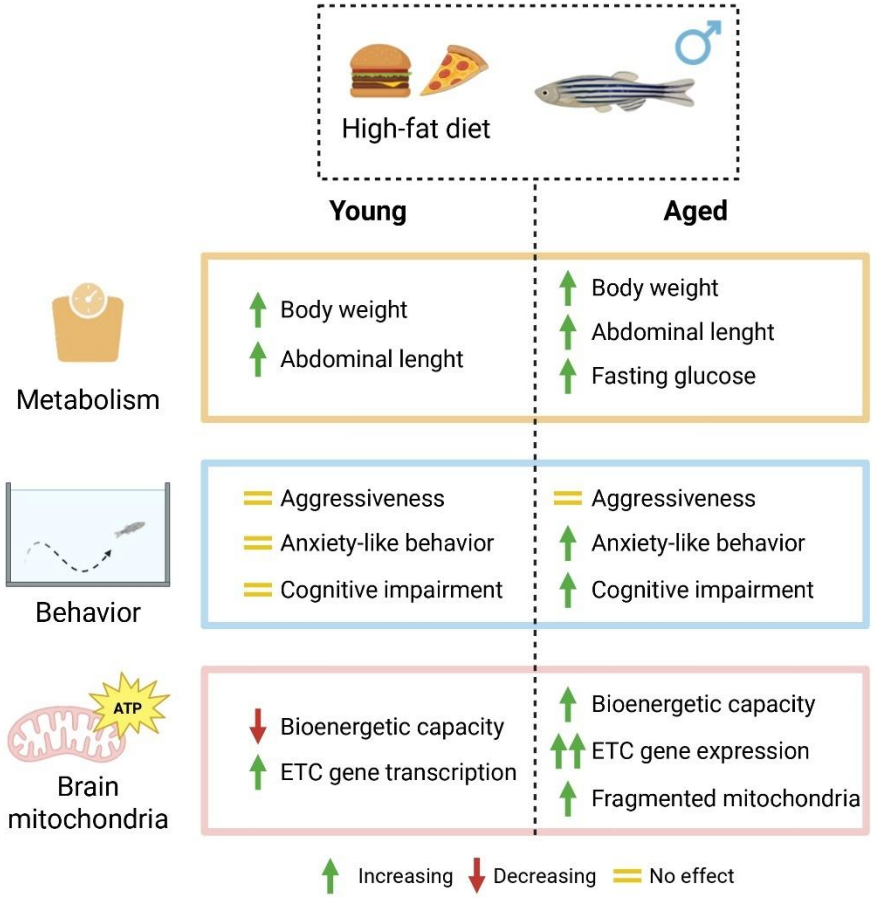

Figura 4. Resumo gráfico dos resultados obtidos. O efeito sinergético entre o envelhecimento e o consumo de dietas desbalanceadas foi investigado através da exposição de peixe-zebra adulto jovem (4-6 meses de idade) e adulto envelhecido (17-22 meses de idade) à uma dieta hiperlipídica (DH) por duas semanas. A DH promoveu um fenótipo obesogênico semelhante em ambas as faixas de idade, porém os animais envelhecidos foram mais suscetíveis às alterações comportamentais e cognitivas induzidas pela dieta, como demonstrado por maior comportamento tipo ansioso e por comprometimento cognitivo. A DH também promoveu alterações mitocondriais no cérebro diferente em cada intervalo de idade, demonstrando uma interação entre o envelhecimento e o consumo de dietas desbalanceadas na ativação de mecanismo neuropatológicos e a maior vulnerabilidade de indivíduos mais velhos às alterações do sistema nervoso central induzida pela dieta.

6. REFERÊNCIAS BIBLIOGRÁFICAS

Abiri, B., Amini, S., Ehsani, H., Ehsani, M., Adineh, P., Mohammadzadeh, H., & Hashemi, S. (2023). Evaluation of dietary food intakes and anthropometric measures in middle-aged men with aggressive symptoms. *BMC Nutrition*, *9*(1), 75. https://doi.org/10.1186/s40795-023-00730-z

Adjibade, M., Julia, C., Allès, B., Touvier, M., Lemogne, C., Srour, B., Hercberg, S., Galan, P., Assmann, K. E., & Kesse-Guyot, E. (2019). Prospective association between ultra-processed food consumption and incident depressive symptoms in the French NutriNet-Santé cohort. *BMC Medicine*, *17*(1), 78. https://doi.org/10.1186/s12916-019-1312-y

Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., Fruchart, J. C., James, W. P. T., Loria, C. M., & Smith, S. C. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International. In *Circulation*. https://doi.org/10.1161/CIRCULATIONAHA.109.192644

Alsop, D., & Vijayan, M. (2009). The zebrafish stress axis: Molecular fallout from the teleost-specific genome duplication event. *General and Comparative Endocrinology*, *161*(1), 62–66. https://doi.org/10.1016/j.ygcen.2008.09.011

Anderson, J. L., Carten, J. D., & Farber, S. A. (2011). Zebrafish Lipid Metabolism: From Mediating Early Patterning to the Metabolism of Dietary Fat and Cholesterol. *Methods in Cell Biology*, *101*, 111–141. https://doi.org/10.1016/B978-0-12-387036-0.00005-0

Angom, R. S., & Nakka, N. M. R. (2024). Zebrafish as a Model for Cardiovascular and Metabolic Disease: The Future of Precision Medicine. *Biomedicines*, *12*(3), 693. https://doi.org/10.3390/biomedicines12030693

Anichtchik, O. V., Kaslin, J., Peitsaro, N., Scheinin, M., & Panula, P. (2004). Neurochemical and behavioural changes in zebrafish Danio rerio after systemic administration of 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Journal of Neurochemistry*, *88*(2), 443–53. https://doi.org/10.1111/j.1471-4159.2004.02190.x

Anstey, K. J., Cherbuin, N., Budge, M., & Young, J. (2011). Body mass index in midlife and late-life as a risk factor for dementia: A meta-analysis of prospective studies. *Obesity Reviews*. https://doi.org/10.1111/j.1467-789X.2010.00825.x

Asaoka, Y., Terai, S., Sakaida, I., & Nishina, H. (2013). The expanding role of fish models in understanding non-alcoholic fatty liver disease. *Disease Models & Mechanisms*. https://doi.org/10.1242/dmm.011981

Attuquayefio, T., Stevenson, R. J., Oaten, M. J., & Francis, H. M. (2017). A four-day Western-style dietary intervention causes reductions in hippocampal-dependent learning and memory and interoceptive sensitivity. *PLoS ONE*, *12*(2). https://doi.org/10.1371/journal.pone.0172645

Bally-Cuif, L., & Vernier, P. (2010). Organization and physiology of the zebrafish nervous

system. In *Fish Physiology*. https://doi.org/10.1016/S1546-5098(10)02902-X

Baraldi, L. G., Martinez Steele, E., Canella, D. S., & Monteiro, C. A. (2018). Consumption of ultra-processed foods and associated sociodemographic factors in the USA between 2007 and 2012: evidence from a nationally representative cross-sectional study. *BMJ Open*, *8*(3), e020574. https://doi.org/10.1136/bmjopen-2017-020574

Baym, C. L., Khan, N. A., Monti, J. M., Raine, L. B., Drollette, E. S., Moore, R. D., Scudder, M. R., Kramer, A. F., Hillman, C. H., & Cohen, N. J. (2014). Dietary lipids are differentially associated with hippocampal-dependent relational memory in prepubescent children. *American Journal of Clinical Nutrition*, *99*(5), 1026–1033. https://doi.org/10.3945/ajcn.113.079624

Beilharz, J. E., Maniam, J., & Morris, M. J. (2014). Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. *Brain, Behavior, and Immunity*, *37*, 134–141. https://doi.org/10.1016/j.bbi.2013.11.016

Benard, G., Bellance, N., James, D., Parrone, P., Fernandez, H., Letellier, T., & Rossignol, R. (2007). Mitochondrial bioenergetics and structural network organization. *Journal of Cell Science*, *120*(5), 838–848. https://doi.org/10.1242/jcs.03381

Bencan, Z., Sledge, D., & Levin, E. D. (2009). Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. *Pharmacology Biochemistry and Behavior*, *94*(1), 75–80. https://doi.org/10.1016/j.pbb.2009.07.009

Bigaard, J., Frederiksen, K., Tjønneland, A., Thomsen, B. L., Overvad, K., Heitmann, B. L., & Sørensen, T. (2005). Waist circumference and body composition in relation to all-cause mortality in middle-aged men and women. *International Journal of Obesity*. https://doi.org/10.1038/sj.ijo.0802976

Bradford, M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1006/abio.1976.9999

Bradford, Y. M., Toro, S., Ramachandran, S., Ruzicka, L., Howe, D. G., Eagle, A., Kalita, P., Martin, R., Moxon, S. A. T., Schaper, K., & Westerfield, M. (2017). Zebrafish models of human disease: Gaining insight into human disease at ZFIN. *ILAR Journal*, *58*(1), 4–16. https://doi.org/10.1093/ilar/ilw040

Braford, M. R. (1995). Comparative aspects of forebrain organization in the ray-finned fishes: touchstones or not? *Brain, Behavior and Evolution*, *46*, 259–274. https://doi.org/10.1159/000113278

Brustein, E., Saint-Amant, L., Buss, R. R., Chong, M., McDearmid, J. R., & Drapeau, P. (2003). Steps during the development of the zebrafish locomotor network. *Journal of Physiology Paris*, *97*(1), 77–86. https://doi.org/10.1016/j.jphysparis.2003.10.009

Buchenauer, T., Behrendt, P., Bode, F. J., Horn, R., Brabant, G., Stephan, M., & Nave, H. (2009). Diet-induced obesity alters behavior as well as serum levels of corticosterone in F344 rats. *Physiology and Behavior*, *98*(5), 563–569. https://doi.org/10.1016/j.physbeh.2009.09.003

Butler, M. J., Cole, R. M., Deems, N. P., Belury, M. A., & Barrientos, R. M. (2020). Fatty food,

fatty acids, and microglial priming in the adult and aged hippocampus and amygdala. *Brain, Behavior, and Immunity*, *89*, 145–158. https://doi.org/10.1016/j.bbi.2020.06.010

Cao, Y., Chen, Q., Liu, Y., Jin, L., & Peng, R. (2023). Research Progress on the Construction and Application of a Diabetic Zebrafish Model. *International Journal of Molecular Sciences*, *24*(6), 5195. https://doi.org/10.3390/ijms24065195

Casazza, K., Dulin-Keita, A., Gower, B. A., & Fernandez, J. R. (2009). Differential Influence of Diet and Physical Activity on Components of Metabolic Syndrome in a Multiethnic Sample of Children. *Journal of the American Dietetic Association*, *109*(2), 236–244. https://doi.org/10.1016/j.jada.2008.10.054

Cavallini, G., Donati, A., Taddei, M., & Bergamini, E. (2007). Evidence for Selective Mitochondrial Autophagy and Failure in Aging. *Autophagy*, *3*(1), 26–27. https://doi.org/10.4161/auto.3268

Cerniglia, L., Cimino, S., Erriu, M., Jezek, S., Almenara, C. A., & Tambelli, R. (2018). Trajectories of aggressive and depressive symptoms in male and female overweight children: Do they share a common path or do they follow different routes? *PLOS ONE*, *13*(1), e0190731. https://doi.org/10.1371/journal.pone.0190731

Cheng, X., Yang, Y., Schwebel, D. C., Liu, Z., Li, L., Cheng, P., Ning, P., & Hu, G. (2020). Population ageing and mortality during 1990–2017: A global decomposition analysis. *PLOS Medicine*, *17*(6), e1003138. https://doi.org/10.1371/journal.pmed.1003138

Chistiakov, D. A., Sobenin, I. A., Revin, V. V., Orekhov, A. N., & Bobryshev, Y. V. (2014). Mitochondrial Aging and Age-Related Dysfunction of Mitochondria. *BioMed Research International*, *2014*, 1–7. https://doi.org/10.1155/2014/238463

Choi, M.-S., Kim, Y.-J., Kwon, E.-Y., Ryoo, J. Y., Kim, S. R., & Jung, U. J. (2015). High-fat diet decreases energy expenditure and expression of genes controlling lipid metabolism, mitochondrial function and skeletal system development in the adipose tissue, along with increased expression of extracellular matrix remodelling- and inflamm. *British Journal of Nutrition*, *113*(6), 867–877. https://doi.org/10.1017/S0007114515000100

Choi, T.-Y., Choi, T.-I., Lee, Y.-R., Choe, S.-K., & Kim, C.-H. (2021). Zebrafish as an animal model for biomedical research. *Experimental & Molecular Medicine*, *53*(3), 310–317. https://doi.org/10.1038/s12276-021-00571-5

Choy, O. (2023). Nutritional factors associated with aggression. *Frontiers in Psychiatry*, *14*, 1176061. https://doi.org/10.3389/fpsyt.2023.1176061

Cobley, J. N., Fiorello, M. L., & Bailey, D. M. (2018). 13 Reasons Why the Brain Is Susceptible To Oxidative Stress. *Redox Biology*, *15*(January), 490–503. https://doi.org/10.1016/j.redox.2018.01.008

Cogliati, S., Lorenzi, I., Rigoni, G., Caicci, F., & Soriano, M. E. (2018). Regulation of Mitochondrial Electron Transport Chain Assembly. *Journal of Molecular Biology*, *430*(24), 4849–4873. https://doi.org/10.1016/j.jmb.2018.09.016

Connaughton, V. P., Baker, C., Fonde, L., Gerardi, E., & Slack, C. (2016). Alternate Immersion in an External Glucose Solution Differentially Affects Blood Sugar Values in Older Versus

Younger Zebrafish Adults. *Zebrafish*, *13*(2), 87–94. https://doi.org/10.1089/zeb.2015.1155

Da Costa Louzada, M. L., Ricardo, C. Z., Steele, E. M., Levy, R. B., Cannon, G., & Monteiro, C. A. (2018). The share of ultra-processed foods determines the overall nutritional quality of diets in Brazil. *Public Health Nutrition*, *21*(1), 94–102. https://doi.org/10.1017/S1368980017001434

de Bem, A. F., Krolow, R., Farias, H. R., de Rezende, V. L., Gelain, D. P., Moreira, J. C. F., Duarte, J. M. das N., & de Oliveira, J. (2021). Animal Models of Metabolic Disorders in the Study of Neurodegenerative Diseases: An Overview. *Frontiers in Neuroscience*, *14*(January), 1–22. https://doi.org/10.3389/fnins.2020.604150

de Miranda, R. C., Rauber, F., & Levy, R. B. (2021). Impact of ultra-processed food consumption on metabolic health. *Current Opinion in Lipidology*, *32*(1), 24–37. https://doi.org/10.1097/MOL.0000000000000728

de Paula, G. C., Brunetta, H. S., Engel, D. F., Gaspar, J. M., Velloso, L. A., Engblom, D., de Oliveira, J., & de Bem, A. F. (2021). Hippocampal Function Is Impaired by a Short-Term High-Fat Diet in Mice: Increased Blood–Brain Barrier Permeability and Neuroinflammation as Triggering Events. *Frontiers in Neuroscience*, *15*(November), 1–12. https://doi.org/10.3389/fnins.2021.734158

Dutheil, S., Ota, K. T., Wohleb, E. S., Rasmussen, K., & Duman, R. S. (2016). High-Fat Diet Induced Anxiety and Anhedonia: Impact on Brain Homeostasis and Inflammation. *Neuropsychopharmacology*, *41*(7), 1874–1887. https://doi.org/10.1038/npp.2015.357

Elo, B., Villano, C. M., Govorko, D., & White, L. A. (2007). Larval zebrafish as a model for glucose metabolism: Expression of phosphoenolpyruvate carboxykinase as a marker for exposure to anti-diabetic compounds. *Journal of Molecular Endocrinology*, *38*, 433–440. https://doi.org/10.1677/JME-06-0037

Evans, A. K., Saw, N. L., Woods, C. E., Vidano, L. M., Blumenfeld, S. E., Lam, R. K., Chu, E. K., Reading, C., & Shamloo, M. (2024). Impact of high-fat diet on cognitive behavior and central and systemic inflammation with aging and sex differences in mice. *Brain, Behavior, and Immunity*, *118*, 334–354. https://doi.org/10.1016/j.bbi.2024.02.025

Faillaci, F., Milosa, F., Critelli, R. M., Turola, E., Schepis, F., & Villa, E. (2018). Obese zebrafish: A small fish for a major human health condition. *Animal Models and Experimental Medicine*, *1*(4), 255–265. https://doi.org/10.1002/ame2.12042

FangFang, Li, H., Qin, T., Li, M., & Ma, S. (2017). Thymol improves high-fat diet-induced cognitive deficits in mice via ameliorating brain insulin resistance and upregulating NRF2/HO-1 pathway. *Metabolic Brain Disease*, *32*(2), 385–393. https://doi.org/10.1007/s11011-016-9921-z

Ferreira, S. R. G., Macotela, Y., Velloso, L. A., & Mori, M. A. (2024). Determinants of obesity in Latin America. *Nature Metabolism*, 32. https://doi.org/10.1038/s42255-024-00977-1

Flinn, L., Bretaud, S., Lo, C., Ingham, P. W., & Bandmann, O. (2008). Zebrafish as a new animal model for movement disorders. *Journal of Neurochemistry*, *106*, 7. https://doi.org/10.1111/j.1471-4159.2008.05463.x

Flynn, E. J., Trent, C. M., Rawls, J. F., & Rawls, J. F. (2009). Ontogeny and nutritional control of adipogenesis in zebrafish (Danio rerio). *Journal of Lipid Research*, *50*(8), 1641–1652. https://doi.org/10.1194/jlr.M800590-JLR200

Fontana, B. D., Cleal, M., Clay, J. M., & Parker, M. O. (2019). Zebrafish (Danio rerio) behavioral laterality predicts increased short-term avoidance memory but not stress-reactivity responses. *Animal Cognition*, *22*(6), 1051–1061. https://doi.org/10.1007/s10071-019-01296-9

Freeman, L. R., Haley-Zitlin, V., Rosenberger, D. S., & Granholm, A.-C. (2014). Damaging effects of a high-fat diet to the brain and cognition: A review of proposed mechanisms NIH Public Access. *Nutr Neurosci*, *17*(6), 241–251. https://doi.org/10.1179/1476830513Y.0000000092

Gabriel, J. P., Mahmood, R., Kyriakatos, A., Söll, I., Hauptmann, G., Calabrese, R. L., & El Manira, A. (2009). Serotonergic modulation of locomotion in zebrafish - Endogenous release and synaptic mechanisms. *Journal of Neuroscience*, *29*(33), 10387–10395. https://doi.org/10.1523/JNEUROSCI.1978-09.2009

Galizzi, G., Palumbo, L., Amato, A., Conigliaro, A., Nuzzo, D., Terzo, S., Caruana, L., Picone, P., Alessandro, R., Mulè, F., & Di Carlo, M. (2021). Altered insulin pathway compromises mitochondrial function and quality control both in in vitro and in vivo model systems. *Mitochondrion*, *60*, 178–188. https://doi.org/10.1016/j.mito.2021.08.014

Gao, X., Qin, Y., Jiao, S., Hao, J., Zhao, J., Wang, J., Wen, Y., & Wang, T. (2024). Genetic evidence for the causal relations between metabolic syndrome and psychiatric disorders: a Mendelian randomization study. *Translational Psychiatry*, *14*(1), 46. https://doi.org/10.1038/s41398-024-02759-5

Ghaddar, B., & Diotel, N. (2022). Zebrafish: A New Promise to Study the Impact of Metabolic Disorders on the Brain. *International Journal of Molecular Sciences*, *23*(10), 5372. https://doi.org/10.3390/ijms23105372

Ghaddar, B., Veeren, B., Rondeau, P., Bringart, M., Lefebvre d'Hellencourt, C., Meilhac, O., Bascands, J.-L., & Diotel, N. (2020). Impaired brain homeostasis and neurogenesis in diet-induced overweight zebrafish: a preventive role from A. borbonica extract. *Scientific Reports*, *10*(1), 14496. https://doi.org/10.1038/s41598-020-71402-2

Giménez-Palomo, A., Gomes-da-Costa, S., Dodd, S., Pachiarotti, I., Verdolini, N., Vieta, E., & Berk, M. (2022). Does metabolic syndrome or its component factors alter the course of bipolar disorder? A systematic review. *Neuroscience & Biobehavioral Reviews*, *132*, 142–153. https://doi.org/10.1016/j.neubiorev.2021.11.026

Gomes Gonçalves, N., Vidal Ferreira, N., Khandpur, N., Martinez Steele, E., Bertazzi Levy, R., Andrade Lotufo, P., Bensenor, I. M., Caramelli, P., Alvim De Matos, S. M., Marchioni, D. M., & Suemoto, C. K. (2023). Association between Consumption of Ultraprocessed Foods and Cognitive Decline. *JAMA Neurology*, *80*(2), 142–150. https://doi.org/10.1001/jamaneurol.2022.4397

González Olmo, B. M., Bettes, M. N., DeMarsh, J. W., Zhao, F., Askwith, C., & Barrientos, R. M. (2023). Short-term high-fat diet consumption impairs synaptic plasticity in the aged

hippocampus via IL-1 signaling. *Npj Science of Food*, *7*(1), 35. https://doi.org/10.1038/s41538-023-00211-4

Gustafson, D., Lissner, L., Bengtsson, C., Björkelund, C., & Skoog, I. (2004). A 24-year follow-up of body mass index and cerebral atrophy. In *Neurology*. https://doi.org/10.1212/01.WNL.0000141850.47773.5F

Gut, P., Reischauer, S., Stainier, D. Y. R., & Arnaout, R. (2017). Little fish, big data: Zebrafish as a model for cardiovascular and metabolic disease. *Physiological Reviews*, *97*(3), 889–938. https://doi.org/10.1152/physrev.00038.2016

Hadem, I. K. H., Majaw, T., Kharbuli, B., & Sharma, R. (2019). Beneficial effects of dietary restriction in aging brain. *Journal of Chemical Neuroanatomy*, *95*, 123–133. https://doi.org/10.1016/j.jchemneu.2017.10.001

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., Collins, J. E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J. C., Koch, R., Rauch, G. J., White, S., … Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, *496*, 498–503. https://doi.org/10.1038/nature12111

Hu, F. B. (2013). Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. *Obesity Reviews*, *14*(8), 606–619. https://doi.org/10.1111/obr.12040

IDF. (2015). *(International Diabetes Federation): Diabetes Atlas*. https://diabetesatlas.org/idfawp/resource-files/2012/07/IDF_diabetes_atlas_seventh_edition_en.pdf

Ivannikov, M. V., Sugimori, M., & Llinás, R. R. (2013). Synaptic Vesicle Exocytosis in Hippocampal Synaptosomes Correlates Directly with Total Mitochondrial Volume. *Journal of Molecular Neuroscience*, *49*(1), 223–230. https://doi.org/10.1007/s12031-012-9848-8

Jones, L. J., & Norton, W. H. J. (2015). Using zebrafish to uncover the genetic and neural basis of aggression, a frequent comorbid symptom of psychiatric disorders. *Behavioural Brain Research*, *276*(;HY:^**I98;171-180), 171–180. https://doi.org/10.1016/j.bbr.2014.05.055

Jørgensen, W., Rud, K. A., Mortensen, O. H., Frandsen, L., Grunnet, N., & Quistorff, B. (2017). Your mitochondria are what you eat: a high-fat or a high-sucrose diet eliminates metabolic flexibility in isolated mitochondria from rat skeletal muscle. *Physiological Reports*, *5*(6), 1–10. https://doi.org/10.14814/phy2.13207

Kalueff, A. V., Gebhardt, M., Stewart, A. M., Cachat, J. M., Brimmer, M., Chawla, J. S., Craddock, C., Kyzar, E. J., Roth, A., Landsman, S., Gaikwad, S., Robinson, K., Baatrup, E., Tierney, K., Shamchuk, A., Norton, W., Miller, N., Nicolson, T., Braubach, O., … Schneider, H. (2013). Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. In *Zebrafish* (Vol. 10, Issue 1, pp. 70–86). Mary Ann Liebert, Inc. https://doi.org/10.1089/zeb.2012.0861

Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. In *Trends in Pharmacological Sciences* (Vol. 35, Issue 2, pp. 63–

75). NIH Public Access. https://doi.org/10.1016/j.tips.2013.12.002

Kanoh, T., Mizoguchi, T., Tonoki, A., & Itoh, M. (2024). Modeling of age-related neurological disease: utility of zebrafish. *Frontiers in Aging Neuroscience*, *16*. https://doi.org/10.3389/fnagi.2024.1399098

Kauppila, T. E. S., Kauppila, J. H. K., & Larsson, N.-G. (2017). Mammalian Mitochondria and Aging: An Update. *Cell Metabolism*, *25*(1), 57–71. https://doi.org/10.1016/j.cmet.2016.09.017

Kurz, C., Ungerer, I., Lipka, U., Kirr, S., Schütt, T., Eckert, A., Leuner, K., & Müller, W. (2010). The metabolic enhancer piracetam ameliorates the impairment of mitochondrial function and neurite outgrowth induced by ß-amyloid peptide. *British Journal of Pharmacology*, *160*(2), 246–257. https://doi.org/10.1111/j.1476-5381.2010.00656.x

Kyzar, E., Stewart, A. M., Landsman, S., Collins, C., Gebhardt, M., Robinson, K., & Kalueff, A. V. (2013). Behavioral effects of bidirectional modulators of brain monoamines reserpine and d-amphetamine in zebrafish. *Brain Research*, *1527*, 108–116. https://doi.org/10.1016/j.brainres.2013.06.033

Landgraf, K., Schuster, S., Meusel, A., Garten, A., Riemer, T., Schleinitz, D., Kiess, W., & Körner, A. (2017). Short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development of metabolically healthy versus unhealt. *BMC Physiology*, *17*(1), 4. https://doi.org/10.1186/s12899-017-0031-x

Li, Z., Okamoto, K.-I., Hayashi, Y., & Sheng, M. (2004). The Importance of Dendritic Mitochondria in the Morphogenesis and Plasticity of Spines and Synapses. *Cell*, *119*(6), 873–887. https://doi.org/10.1016/j.cell.2004.11.003

Lillesaar, C. (2011). The serotonergic system in fish. *Journal of Chemical Neuroanatomy*, *41*(4), 294–308. https://doi.org/10.1016/j.jchemneu.2011.05.009

Lin, Y., Wang, K., Ma, C., Wang, X., Gong, Z., Zhang, R., Zang, D., & Cheng, Y. (2018). Evaluation of Metformin on Cognitive Improvement in Patients With Non-dementia Vascular Cognitive Impairment and Abnormal Glucose Metabolism. *Frontiers in Aging Neuroscience*, *10*. https://doi.org/10.3389/fnagi.2018.00227

Lindberg, L., Hagman, E., Danielsson, P., Marcus, C., & Persson, M. (2020). Anxiety and depression in children and adolescents with obesity: A nationwide study in Sweden. *BMC Medicine*, *18*(1). https://doi.org/10.1186/s12916-020-1498-z

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Lobo, F., Haase, J., & Brandhorst, S. (2022). The Effects of Dietary Interventions on Brain Aging and Neurological Diseases. *Nutrients*, *14*(23), 5086. https://doi.org/10.3390/nu14235086

Lobstein, T., Jackson-Leach, R., Powis, J., Brinsden, H., & Gray, M. (2023). World Obesity Atlas 2023. *World Obesity Federation*, *March*, 5–25. www.johnclarksondesign.co.uk

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The Hallmarks

of Aging. *Cell*, *153*(6), 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Ma, W., Yuan, L., Yu, H., Xi, Y., & Xiao, R. (2014). Mitochondrial dysfunction and oxidative damage in the brain of diet-induced obese rats but not in diet-resistant rats. *Life Sciences*, *110*(2), 53–60. https://doi.org/10.1016/j.lfs.2014.07.018

Mackey-Alfonso, S. E., Butler, M. J., Taylor, A. M., Williams-Medina, A. R., Muscat, S. M., Fu, H., & Barrientos, R. M. (2024). Short-term high fat diet impairs memory, exacerbates the neuroimmune response, and evokes synaptic degradation via a complement-dependent mechanism in a mouse model of Alzheimer's disease. *Brain, Behavior, and Immunity*, *121*, 56–69. https://doi.org/10.1016/j.bbi.2024.07.021

Maes, M., Kubera, M., & Leunis, J.-C. (2008). The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuro Endocrinology Letters*, *29*(1), 117–124. http://www.ncbi.nlm.nih.gov/pubmed/18283240

Maksyutynska, K., Stogios, N., Prasad, F., Gill, J., Hamza, Z., De, R., Smith, E., Horta, A., Goldstein, B. I., Korczak, D., Graff-Guerrero, A., Hahn, M. K., & Agarwal, S. M. (2024). Neurocognitive correlates of metabolic dysregulation in individuals with mood disorders: A systematic review and meta-analysis. *Psychological Medicine*. https://doi.org/10.1017/S0033291724000345

Mandwie, M., Karunia, J., Niaz, A., Keay, K. A., Musumeci, G., Rennie, C., McGrath, K., Al-Badri, G., & Castorina, A. (2021). Metformin Treatment Attenuates Brain Inflammation and Rescues PACAP/VIP Neuropeptide Alterations in Mice Fed a High-Fat Diet. *International Journal of Molecular Sciences*, *22*(24), 13660. https://doi.org/10.3390/ijms222413660

Mansur, R. B., Brietzke, E., & McIntyre, R. S. (2015). Is there a "metabolic-mood syndrome"? A review of the relationship between obesity and mood disorders. *Neuroscience & Biobehavioral Reviews*, *52*, 89–104. https://doi.org/10.1016/j.neubiorev.2014.12.017

Marrón-Ponce, J. A., Sánchez-Pimienta, T. G., Louzada, M. L. da C., & Batis, C. (2018). Energy contribution of NOVA food groups and sociodemographic determinants of ultra-processed food consumption in the Mexican population. *Public Health Nutrition*, *21*(1), 87–93. https://doi.org/10.1017/S1368980017002129

Martínez Steele, E., Juul, F., Neri, D., Rauber, F., & Monteiro, C. A. (2019). Dietary share of ultra-processed foods and metabolic syndrome in the US adult population. *Preventive Medicine*, *125*, 40–48. https://doi.org/10.1016/j.ypmed.2019.05.004

Masiero, E., & Sandri, M. (2010). Autophagy inhibition induces atrophy and myopathy in adult skeletal muscles. *Autophagy*, *6*(2), 307–309. https://doi.org/10.4161/auto.6.2.11137

Matsuzawa, Y., Funahashi, T., & Nakamura, T. (2011). The Concept of Metabolic Syndrome: Contribution of Visceral Fat Accumulation and Its Molecular Mechanism. *Journal of Atherosclerosis and Thrombosis*, *18*(8), 629–639. https://doi.org/10.5551/jat.7922

Maximino, C., de Brito, T. M., da Silva Batista, A. W., Herculano, A. M., Morato, S., & Gouveia, A. (2010). Measuring anxiety in zebrafish: A critical review. In *Behavioural Brain*

Research. https://doi.org/10.1016/j.bbr.2010.05.031

McGrattan, A. M., McGuinness, B., McKinley, M. C., Kee, F., Passmore, P., Woodside, J. V., & McEvoy, C. T. (2019). Diet and Inflammation in Cognitive Ageing and Alzheimer's Disease. *Current Nutrition Reports*, *8*(2), 53–65. https://doi.org/10.1007/s13668-019-0271-4

Meguro, S., Hosoi, S., & Hasumura, T. (2019). High-fat diet impairs cognitive function of zebrafish. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-53634-z

Michael Stewart, A., & V. Kalueff, A. (2012). The Developing Utility of Zebrafish Models for Cognitive Enhancers Research. *Current Neuropharmacology*, *10*, 263–271. https://doi.org/10.2174/157015912803217323

Milaneschi, Y., Lamers, F., Bot, M., Drent, M. L., & Penninx, B. W. J. H. (2017). Leptin Dysregulation Is Specifically Associated With Major Depression With Atypical Features: Evidence for a Mechanism Connecting Obesity and Depression. *Biological Psychiatry*, *81*(9), 807–814. https://doi.org/10.1016/j.biopsych.2015.10.023

Misselbeck, K., Parolo, S., Lorenzini, F., Savoca, V., Leonardelli, L., Bora, P., Morine, M. J., Mione, M. C., Domenici, E., & Priami, C. (2019). A network-based approach to identify deregulated pathways and drug effects in metabolic syndrome. *Nature Communications*, *10*(1), 5215. https://doi.org/10.1038/s41467-019-13208-z

Monteiro, C. A., Cannon, G., Levy, R. B., Moubarac, J.-C., Louzada, M. L., Rauber, F., Khandpur, N., Cediel, G., Neri, D., Martinez-Steele, E., Baraldi, L. G., & Jaime, P. C. (2019). Ultra-processed foods: what they are and how to identify them. *Public Health Nutrition*, *22*(5), 936–941. https://doi.org/10.1017/S1368980018003762

Moreira, A. L. P., & Luchiari, A. C. (2022). Effects of oxybenzone on zebrafish behavior and cognition. *Science of The Total Environment*, *808*, 152101. https://doi.org/10.1016/J.SCITOTENV.2021.152101

Moubarac, J.-C., Batal, M., Louzada, M. L., Martinez Steele, E., & Monteiro, C. A. (2017). Consumption of ultra-processed foods predicts diet quality in Canada. *Appetite*, *108*, 512–520. https://doi.org/10.1016/j.appet.2016.11.006

Mozaffarian, D., Hao, T., Rimm, E. B., Willett, W. C., & Hu, F. B. (2011). Changes in Diet and Lifestyle and Long-Term Weight Gain in Women and Men. *New England Journal of Medicine*, *364*(25), 2392–2404. https://doi.org/10.1056/NEJMoa1014296

Mueller, T., Dong, Z., Berberoglu, M. A., & Guo, S. (2011). The dorsal pallium in zebrafish, Danio rerio (Cyprinidae, Teleostei). *Brain Research*, *1381*, 95–105. https://doi.org/10.1016/j.brainres.2010.12.089

Mulati, A., Zhang, X., Zhao, T., Ren, B., Wang, L., Liu, X., Lan, Y., & Liu, X. (2021). Isorhamnetin attenuates high-fat and high-fructose diet induced cognitive impairments and neuroinflammation by mediating MAPK and NFκB signaling pathways. *Food & Function*, *12*(19), 9261–9272. https://doi.org/10.1039/D0FO03165H

Mundstock, E., Sarria, E. E., Zatti, H., Mattos Louzada, F., Kich Grun, L., Herbert Jones, M., Guma, F. T. C. R., Mazzola in Memoriam, J., Epifanio, M., Stein, R. T., Barbé-Tuana, F. M., & Mattiello, R. (2015). Effect of obesity on telomere length: Systematic review and

meta-analysis. *Obesity*, *23*(11), 2165–2174. https://doi.org/10.1002/oby.21183

Muoio, D. M. (2014). Metabolic inflexibility: When mitochondrial indecision leads to metabolic gridlock. *Cell*, *159*(6), 1253–1262. https://doi.org/10.1016/j.cell.2014.11.034

Nakandakari, S. C. B. R., Muñoz, V. R., Kuga, G. K., Gaspar, R. C., Sant'Ana, M. R., Pavan, I. C. B., da Silva, L. G. S., Morelli, A. P., Simabuco, F. M., da Silva, A. S. R., de Moura, L. P., Ropelle, E. R., Cintra, D. E., & Pauli, J. R. (2019). Short-term high-fat diet modulates several inflammatory, ER stress, and apoptosis markers in the hippocampus of young mice. *Brain, Behavior, and Immunity*, *79*(May 2018), 284–293. https://doi.org/10.1016/j.bbi.2019.02.016

Nakayama, H., Hata, K., Matsuoka, I., Zang, L., Kim, Y., Chu, D., Juneja, L. R., Nishimura, N., & Shimada, Y. (2020). Anti-Obesity Natural Products Tested in Juvenile Zebrafish Obesogenic Tests and Mouse 3T3-L1 Adipogenesis Assays. *Molecules*, *25*(24), 5840. https://doi.org/10.3390/molecules25245840

Nakayama, H., Shimada, Y., Zang, L., Terasawa, M., Nishiura, K., Matsuda, K., Toombs, C., Langdon, C., & Nishimura, N. (2018). Novel Anti-Obesity Properties of Palmaria mollis in Zebrafish and Mouse Models. *Nutrients*, *10*(10), 1401. https://doi.org/10.3390/nu10101401

NCHS. (2012). *No Crude and age-adjusted percentage of civilian, noninsti tutionalized adults with diagnosed diabetes, United States, 1980- 2010.* National Center for Health Statistics, Division of Health Interview Statistics.

Nishio, S. I., Gibert, Y., Berekelya, L., Bernard, L., Brunet, F., Guillot, E., Le Bail, J. C., Sánchez, J. A., Galzin, A. M., Triqueneaux, G., & Laudet, V. (2012). Fasting induces CART down-regulation in the zebrafish nervous system in a cannabinoid receptor 1-dependent manner. *Molecular Endocrinology*, *26*, 1316–1326. https://doi.org/10.1210/me.2011-1180

Nixon, R. A. (2013). The role of autophagy in neurodegenerative disease. *Nature Medicine*, *19*(8), 983–997. https://doi.org/10.1038/nm.3232

Northcutt, R. G. (2006). Connections of the lateral and medial divisions of the goldfish telencephalic pallium. *Journal of Comparative Neurology*, *494*, 903–943. https://doi.org/10.1002/cne.20853

Oka, T., Nishimura, Y., Zang, L., Hirano, M., Shimada, Y., Wang, Z., Umemoto, N., Kuroyanagi, J., Nishimura, N., & Tanaka, T. (2010). Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity. *BMC Physiology*, *10*(1), 21. https://doi.org/10.1186/1472-6793-10-21

Otsuka, T., & Matsui, H. (2023). Fish Models for Exploring Mitochondrial Dysfunction Affecting Neurodegenerative Disorders. *International Journal of Molecular Sciences*, *24*(8). https://doi.org/10.3390/ijms24087079

Pagliai, G., Dinu, M., Madarena, M. P., Bonaccio, M., Iacoviello, L., & Sofi, F. (2021). Consumption of ultra-processed foods and health status: a systematic review and meta-analysis. *British Journal of Nutrition*, *125*(3), 308–318. https://doi.org/10.1017/S0007114520002688

Palikaras, K., & Tavernarakis, N. (2020). Regulation and roles of mitophagy at synapses.

Mechanisms of Ageing and Development, *187*, 111216. https://doi.org/10.1016/j.mad.2020.111216

Panula, P., Sallinen, V., Sundvik, M., Kolehmainen, J., Torkko, V., Tiittula, A., Moshnyakov, M., & Podlasz, P. (2006). Modulatory neurotransmitter systems and behavior: Towards zebrafish models of neurodegenerative diseases. *Zebrafish*, *3*(2), 235–247. https://doi.org/10.1089/zeb.2006.3.235

Park, H.-S., Cho, H.-S., & Kim, T.-W. (2018). Physical exercise promotes memory capability by enhancing hippocampal mitochondrial functions and inhibiting apoptosis in obesity-induced insulin resistance by high fat diet. *Metabolic Brain Disease*, *33*(1), 283–292. https://doi.org/10.1007/s11011-017-0160-8

Park, H. S., Cho, H. S., & Kim, T. W. (2018). Physical exercise promotes memory capability by enhancing hippocampal mitochondrial functions and inhibiting apoptosis in obesity-induced insulin resistance by high fat diet. *Metabolic Brain Disease*, *33*(1), 283–292. https://doi.org/10.1007/s11011-017-0160-8

Peitsaro, N., Kaslin, J., Anichtchik, O. V., & Panula, P. (2003). Modulation of the histaminergic system and behaviour by α-fluoromethylhistidine in zebrafish. *Journal of Neurochemistry*, *86*, 432–41. https://doi.org/10.1046/j.1471-4159.2003.01850.x

Penna, E., Pizzella, A., Cimmino, F., Trinchese, G., Cavaliere, G., Catapano, A., Allocca, I., Chun, J. T., Campanozzi, A., Messina, G., Precenzano, F., Lanzara, V., Messina, A., Monda, V., Monda, M., Perrone-Capano, C., Mollica, M. P., & Crispino, M. (2020). Neurodevelopmental disorders: Effect of high-fat diet on synaptic plasticity and mitochondrial functions. *Brain Sciences*, *10*(11), 1–15. https://doi.org/10.3390/brainsci10110805

Picolo, V. L., Quadros, V. A., Canzian, J., Grisolia, C. K., Goulart, J. T., Pantoja, C., de Bem, A. F., & Rosemberg, D. B. (2021). Short-term high-fat diet induces cognitive decline, aggression, and anxiety-like behavior in adult zebrafish. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *110*, 110288. https://doi.org/10.1016/j.pnpbp.2021.110288

Popkin, B. M., Adair, L. S., & Ng, S. W. (2012). Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition Reviews*, *70*(1), 3–21. https://doi.org/10.1111/j.1753-4887.2011.00456.x

Portavella, M., Vargas, J. P., Torres, B., & Salas, C. (2002). The effects of telencephalic pallial lesions on spatial, temporal, and emotional learning in goldfish. *Brain Research Bulletin*, *57*, 397–399. https://doi.org/10.1016/S0361-9230(01)00699-2

Prince, M. J., Wu, F., Guo, Y., Gutierrez Robledo, L. M., O'Donnell, M., Sullivan, R., & Yusuf, S. (2015). The burden of disease in older people and implications for health policy and practice. *The Lancet*, *385*(9967), 549–562. https://doi.org/10.1016/S0140-6736(14)61347-7

Procaccini, C., Santopaolo, M., Faicchia, D., Colamatteo, A., Formisano, L., De Candia, P., Galgani, M., De Rosa, V., & Matarese, G. (2016). Role of metabolism in neurodegenerative disorders. In *Metabolism: Clinical and Experimental*. https://doi.org/10.1016/j.metabol.2016.05.018

Ravi, S., Kankaanpää, A., Bogl, L. H., Heikkinen, A., Pietiläinen, K. H., Kaprio, J., Ollikainen, M., & Sillanpää, E. (2025). Suboptimal dietary patterns are associated with accelerated biological aging in young adulthood: A study with twins. *Clinical Nutrition*, *45*, 10–21. https://doi.org/10.1016/j.clnu.2024.12.018

Redman, L. M., & Ravussin, E. (2011). Caloric Restriction in Humans: Impact on Physiological, Psychological, and Behavioral Outcomes. *Antioxidants & Redox Signaling*, *14*(2), 275–287. https://doi.org/10.1089/ars.2010.3253

Ruan, Y., Tang, J., Guo, X., Li, K., & Li, D. (2018). Dietary Fat Intake and Risk of Alzheimer's Disease and Dementia: A Meta-Analysis of Cohort Studies. *Current Alzheimer Research*, *15*(9), 869–876. https://doi.org/10.2174/1567205015666180427142350

Sackerman, J., Donegan, J. J., Cunningham, C. S., Nguyen, N. N., Lawless, K., Long, A., Benno, R. H., & Gould, G. G. (2010). Zebrafish Behavior in Novel Environments: Effects of Acute Exposure to Anxiolytic Compounds and Choice of Danio rerio Line. *International Journal of Comparative Psychology*, *23*(1), 43–61.

Saklayen, M. G. (2018). The Global Epidemic of the Metabolic Syndrome. *Current Hypertension Reports*, *20*(2), 12. https://doi.org/10.1007/s11906-018-0812-z

Schwingshackl, L., Hoffmann, G., Lampousi, A.-M., Knüppel, S., Iqbal, K., Schwedhelm, C., Bechthold, A., Schlesinger, S., & Boeing, H. (2017). Food groups and risk of type 2 diabetes mellitus: a systematic review and meta-analysis of prospective studies. *European Journal of Epidemiology*, *32*(5), 363–375. https://doi.org/10.1007/s10654-017-0246-y

Selfridge, J. E., Wilkins, H. M., E, L., Carl, S. M., Koppel, S., Funk, E., Fields, T., Lu, J., Tang, E. P., Slawson, C., Wang, W., Zhu, H., & Swerdlow, R. H. (2015). Effect of one month duration ketogenic and non-ketogenic high fat diets on mouse brain bioenergetic infrastructure. *Journal of Bioenergetics and Biomembranes*, *47*(1–2), 1–11. https://doi.org/10.1007/s10863-014-9570-z

Semenkovich, K., Brown, M. E., Svrakic, D. M., & Lustman, P. J. (2015). Depression in Type 2 Diabetes Mellitus: Prevalence, Impact, and Treatment. *Drugs*, *75*(6), 577–587. https://doi.org/10.1007/s40265-015-0347-4

Seth, A., Stemple, D. L., & Barroso, I. (2013). The emerging use of zebrafish to model metabolic disease. *Disease Models & Mechanisms*, *6*(5), 1080–1088. https://doi.org/10.1242/dmm.011346

Severi, I., Fosca, M., Colleluori, G., Marini, F., Imperatori, L., Senzacqua, M., Di Vincenzo, A., Barbatelli, G., Fiori, F., Rau, J. V., & Giordano, A. (2021). High-Fat Diet Impairs Mouse Median Eminence: A Study by Transmission and Scanning Electron Microscopy Coupled with Raman Spectroscopy. *International Journal of Molecular Sciences*, *22*(15), 8049. https://doi.org/10.3390/ijms22158049

Shimoda, N., Izawa, T., Yoshizawa, A., Yokoi, H., Kikuchi, Y., & Hashimoto, N. (2014). Decrease in cytosine methylation at CpG island shores and increase in DNA fragmentation during zebrafish aging. *AGE*, *36*(1), 103–115. https://doi.org/10.1007/s11357-013-9548-5

Sivanathan, S., Thavartnam, K., Arif, S., Elegino, T., & McGowan, P. O. (2015). Chronic high

fat feeding increases anxiety-like behaviour and reduces transcript abundance of glucocorticoid signalling genes in the hippocampus of female rats. *Behavioural Brain Research*. https://doi.org/10.1016/j.bbr.2015.02.036

Soman, S., Keatinge, M., Moein, M., Da Costa, M., Mortiboys, H., Skupin, A., Sugunan, S., Bazala, M., Kuznicki, J., & Bandmann, O. (2017). Inhibition of the mitochondrial calcium uniporter rescues dopaminergic neurons in pink1 − / − zebrafish. *European Journal of Neuroscience*, *45*(4), 528–535. https://doi.org/10.1111/ejn.13473

Soussi-Yanicostas, N. (2022). Zebrafish as a Model for Neurological Disorders. *International Journal of Molecular Sciences*, *23*(8), 4321. https://doi.org/10.3390/ijms23084321

Sparks, L. M., Xie, H., Koza, R. A., Mynatt, R., Hulver, M. W., Bray, G. A., & Smith, S. R. (2005). A High-Fat Diet Coordinately Downregulates Genes Required for Mitochondrial Oxidative Phosphorylation in Skeletal Muscle. *Diabetes*, *54*(7), 1926–1933. https://doi.org/10.2337/diabetes.54.7.1926

Spencer, S. J., Basri, B., Sominsky, L., Soch, A., Ayala, M. T., Reineck, P., Gibson, B. C., & Barrientos, R. M. (2019). High-fat diet worsens the impact of aging on microglial function and morphology in a region-specific manner. *Neurobiology of Aging*, *74*, 121–134. https://doi.org/10.1016/j.neurobiolaging.2018.10.018

Spencer, S. J., D'Angelo, H., Soch, A., Watkins, L. R., Maier, S. F., & Barrientos, R. M. (2017a). High-fat diet and aging interact to produce neuroinflammation and impair hippocampal- and amygdalar-dependent memory. *Neurobiology of Aging*, *58*, 88–101. https://doi.org/10.1016/j.neurobiolaging.2017.06.014

Spencer, S. J., D'Angelo, H., Soch, A., Watkins, L. R., Maier, S. F., & Barrientos, R. M. (2017b). High-fat diet and aging interact to produce neuroinflammation and impair hippocampal- and amygdalar-dependent memory. *Neurobiology of Aging*, *58*. https://doi.org/10.1016/j.neurobiolaging.2017.06.014

Spencer, S. J., Korosi, A., Layé, S., Shukitt-Hale, B., & Barrientos, R. M. (2017). Food for thought: how nutrition impacts cognition and emotion. *Npj Science of Food*, *1*(1), 7. https://doi.org/10.1038/s41538-017-0008-y

Stankiewicz, A., McGowan, E., Yu, L., & Zhdanova, I. (2017). Impaired Sleep, Circadian Rhythms and Neurogenesis in Diet-Induced Premature Aging. *International Journal of Molecular Sciences*, *18*(11), 2243. https://doi.org/10.3390/ijms18112243

Stout, M. B., Justice, J. N., Nicklas, B. J., & Kirkland, J. L. (2017). Physiological Aging: Links Among Adipose Tissue Dysfunction, Diabetes, and Frailty. *Physiology*, *32*(1), 9–19. https://doi.org/10.1152/physiol.00012.2016

Tan, B. L., & Norhaizan, M. E. (2019). Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function. *Nutrients*, *11*(11), 1–22. https://doi.org/10.3390/nu11112579

Teles, M. C., Dahlbom, S. J., Winberg, S., & Oliveira, R. F. (2013). Social modulation of brain monoamine levels in zebrafish. *Behavioural Brain Research*, *253*, 17–24. https://doi.org/10.1016/j.bbr.2013.07.012

Terman, A., Kurz, T., Navratil, M., Arriaga, E. A., & Brunk, U. T. (2010). Mitochondrial Turnover and Aging of Long-Lived Postmitotic Cells: The Mitochondrial–Lysosomal Axis Theory of Aging. *Antioxidants & Redox Signaling*, *12*(4), 503–535. https://doi.org/10.1089/ars.2009.2598

Todorova, V., & Blokland, A. (2017). Mitochondria and Synaptic Plasticity in the Mature and Aging Nervous System. *Current Neuropharmacology*, *15*(1), 166–173. https://doi.org/10.2174/1570159X14666160414111821

Tsilingiris, D., Tzeravini, E., Koliaki, C., Dalamaga, M., & Kokkinos, A. (2021). The Role of Mitochondrial Adaptation and Metabolic Flexibility in the Pathophysiology of Obesity and Insulin Resistance: an Updated Overview. *Current Obesity Reports*, *10*(3), 191–213. https://doi.org/10.1007/s13679-021-00434-0

Van houcke, J., Mariën, V., Zandecki, C., Seuntjens, E., Ayana, R., & Arckens, L. (2021). Modeling Neuroregeneration and Neurorepair in an Aging Context: The Power of a Teleost Model. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.619197

Vandevijvere, S., Chow, C. C., Hall, K. D., Umali, E., & Swinburn, B. A. (2015). Increased food energy supply as a major driver of the obesity epidemic: a global analysis. *Bulletin of the World Health Organization*. https://doi.org/10.2471/blt.14.150565

Wahl, D., Cogger, V. C., Solon-Biet, S. M., Waern, R. V. R., Gokarn, R., Pulpitel, T., Cabo, R. de, Mattson, M. P., Raubenheimer, D., Simpson, S. J., & Le Couteur, D. G. (2016). Nutritional strategies to optimise cognitive function in the aging brain. *Ageing Research Reviews*, *31*, 80–92. https://doi.org/10.1016/j.arr.2016.06.006

Walters, G. C., & Usachev, Y. M. (2023). Mitochondrial calcium cycling in neuronal function and neurodegeneration. *Frontiers in Cell and Developmental Biology*, *11*. https://doi.org/10.3389/fcell.2023.1094356

Wang, J., Li, Y., Lai, K., Zhong, Q., Demin, K. A., Kalueff, A. V., & Song, C. (2020). High-glucose/high-cholesterol diet in zebrafish evokes diabetic and affective pathogenesis: The role of peripheral and central inflammation, microglia and apoptosis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *96*, 109752. https://doi.org/10.1016/j.pnpbp.2019.109752

Wang, X., Zhang, J.-B., He, K.-J., Wang, F., & Liu, C.-F. (2021). Advances of Zebrafish in Neurodegenerative Disease: From Models to Drug Discovery. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.713963

Watson, G. S., Cholerton, B. A., Reger, M. A., Baker, L. D., Plymate, S. R., Asthana, S., Fishel, M. A., Kulstad, J. J., Green, P. S., Cook, D. G., Kahn, S. E., Keeling, M. L., & Craft, S. (2005). Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study. *The American Journal of Geriatric Psychiatry : Official Journal of the American Association for Geriatric Psychiatry*, *13*, 950–958. https://doi.org/10.1176/appi.ajgp.13.11.950

Whitmer, R. A., Gustafson, D. R., Barrett-Connor, E., Haan, M. N., Gunderson, E. P., & Yaffe, K. (2008). Central obesity and increased risk of dementia more than three decades later.

Neurology, *71*(14), 1057–1064. https://doi.org/10.1212/01.wnl.0000306313.89165.ef

World Health Organization. (2024). *Obesity and overweight: Key Facts*. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Yoganantharjah, P., & Gibert, Y. (2017). The Use of the Zebrafish Model to Aid in Drug Discovery and Target Validation. *Current Topics in Medicinal Chemistry*, *17*(18). https://doi.org/10.2174/1568026617666170130112109

Yoon, G., Cho, K. A., Song, J., & Kim, Y.-K. (2019). Transcriptomic Analysis of High Fat Diet Fed Mouse Brain Cortex. *Frontiers in Genetics*, *10*. https://doi.org/10.3389/fgene.2019.00083

Zabegalov, K. N., Wang, D., Yang, L., Wang, J., Hu, G., Serikuly, N., Alpyshov, E. T., Khatsko, S. L., Zhdanov, A., Demin, K. A., Galstyan, D. S., Volgin, A. D., de Abreu, M. S., Strekalova, T., Song, C., Amstislavskaya, T. G., Sysoev, Y., Musienko, P. E., & Kalueff, A. V. (2021). Decoding the role of zebrafish neuroglia in CNS disease modeling. *Brain Research Bulletin*, *166*, 44–53. https://doi.org/10.1016/j.brainresbull.2020.09.020

Zang, L., Maddison, L. A., & Chen, W. (2018a). Zebrafish as a model for obesity and diabetes. In *Frontiers in Cell and Developmental Biology* (Vol. 6, Issue AUG). Frontiers Media S.A. https://doi.org/10.3389/fcell.2018.00091

Zang, L., Maddison, L. A., & Chen, W. (2018b). Zebrafish as a model for obesity and diabetes. *Frontiers in Cell and Developmental Biology*, *6*(AUG), 1–13. https://doi.org/10.3389/fcell.2018.00091

Zhang, D., Shen, C., Chen, N., Liu, C., Hu, J., Lau, K. K., Wen, Z., & Qiu, A. (2025). Long-term obesity impacts brain morphology, functional connectivity and cognition in adults. *Nature Mental Health*, *3*(4), 466–478. https://doi.org/10.1038/s44220-025-00396-5

Zhang, R., Zhang, B., Shen, C., Sahakian, B. J., Li, Z., Zhang, W., Zhao, Y., Li, Y., Feng, J., & Cheng, W. (2024). Associations of dietary patterns with brain health from behavioral, neuroimaging, biochemical and genetic analyses. *Nature Mental Health*, *2*(5), 535–552. https://doi.org/10.1038/s44220-024-00226-0

Zhang, Y., Luo, C., Huang, P., Cheng, Y., Ma, Y., Gao, J., & Ding, H. (2024). Diosmetin Ameliorates HFD-induced Cognitive Impairments via Inhibiting Metabolic Disorders, Mitochondrial Dysfunction and Neuroinflammation in Male SD Rats. *Molecular Neurobiology*, *61*(10), 8069–8085. https://doi.org/10.1007/s12035-024-04083-x

Zhang, Y., Nguyen, D. T., Olzomer, E. M., Poon, G. P., Cole, N. J., Puvanendran, A., Phillips, B. R., & Hesselson, D. (2017). Rescue of Pink1 Deficiency by Stress-Dependent Activation of Autophagy. *Cell Chemical Biology*, *24*(4), 471-480.e4. https://doi.org/10.1016/j.chembiol.2017.03.005

Zhu, S., & Coffman, J. A. (2017). Simple and fast quantification of DNA damage by real-time PCR, and its application to nuclear and mitochondrial DNA from multiple tissues of aging zebrafish. *BMC Research Notes*, *10*(1), 269. https://doi.org/10.1186/s13104-017-2593-x

Zhuang, H., Yao, X., Li, H., Li, Q., Yang, C., Wang, C., Xu, D., Xiao, Y., Gao, Y., Gao, J., Bi, M., Liu, R., Teng, G., & Liu, L. (2022). Long-term high-fat diet consumption by mice

throughout adulthood induces neurobehavioral alterations and hippocampal neuronal remodeling accompanied by augmented microglial lipid accumulation. *Brain, Behavior, and Immunity*, *100*, 155–171. https://doi.org/10.1016/j.bbi.2021.11.018

7. ANEXOS

7.1.Artigo publicado como primeiro autor

Progress in Neuropsychopharmacology & Biological Psychiatry 110 (2021) 110288

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Short-term high-fat diet induces cognitive decline, aggression, and anxiety-like behavior in adult zebrafish

Victor L. Picolo [a,b], Vanessa A. Quadros [c,d], Julia Canzian [c,d], Cesar K. Grisolia [e], Jair T. Goulart [a], Carlos Pantoja [f], Andreza F. de Bem [a,b,**], Denis B. Rosemberg [c,d,g,*]

7.2.Apresentação do trabalho em eventos científicos

CERTIFICADO

15 e 16 DEZEMBRO
XI SIMPÓSIO
Biologia Molecular

Certifico que o trabalho dos autores
Victor L. Picolo, Paula Maria Q. Bellozi, Daniel L. Fagundes, Natasha P. Lopes, Whitney R. Santos, Letícia A. Tavares, Ana Clara Dutra, Jair T. Goulart, Cesar K. Grisólia, Andreza F. de Bem
intitulado
Effect of high fat diet on neurochemical and behavioral parameters in zebrafish
foi apresentado em formato de pôster no XI Simpósio do Programa de Pós-graduação em Biologia Molecular da Universidade de Brasília.

Brasília, 16 de dezembro de 2022

Prof.ª Dra. Andréa Queiroz Maranhão
Coordenadora PPGBioMol - IB - UnB

UnB
Pós-Graduação Biologia Molecular
CAPES

ALZHEIMER'S ASSOCIATION
AAIC 23

POSTER PRESENTER

Victor Picolo

presented "**Short-term high-fat diet feeding induces cognitive decline, aggressiveness and anxiety-like behavior in adult zebrafish (*Danio rerio*)**"

at the Alzheimer's Association International Conference® (AAIC®).

Sergio T. Ferreira, PhD
Co-Chair, AAIC Scientific Program Committee

Wiesje van der Flier, PhD
Co-Chair, AAIC Scientific Program Committee

RAI Amsterdam
Amsterdam, Netherlands
and/or virtually

XII SIMPÓSIO

Programa de Pós-Graduação em Ciências Biológicas (Biologia Molecular) - 7 e 8/DEZ

CERTIFICADO

Certifico que o trabalho dos autores
Victor Luna Picolo, Vanessa Quadros, Júlia Canzian, Cesar Koppe Grisólia, Jair Trapé Goulart, Denis Broock Rosemberg, Daniel Mendes Pereira Ardisson de Araujo, Andreza Fabro de Bem
intitulado **Short-term high-fat diet feeding induces cognitive decline, aggressiveness and anxiety-like behavior in adult zebrafish (Danio rerio)**
foi apresentado no formato pôster no XII Simpósio do Programa de Pós-graduação em Ciências Biológicas (Biologia Molecular) da Universidade de Brasília.

Brasília, 10 de janeiro de 2024

UnB Pós-Graduação Biologia Molecular CAPES

João Barbosa

Prof. Dr. João Alexandre R. G. Barbosa
Coordenador PPGBioMol - IB - UnB

ALZHEIMER'S ASSOCIATION | Neuroscience Next | POA Hub

CERTIFICATE OF POSTER PRESENTATION

Victor Picolo

has presented the work "*High-fat diet induce obesity and cognitive impairment in zebrafish.*" at AAIC Neuroscience Next 2024 - Porto Alegre Hub.

Eduardo R. Zimmer

Eduardo R. Zimmer, PhD
AAIC NeuroNext Mid-career Lead
Assistant Professor, UFRGS, Brazil

Giovanna Carello Collar

Giovanna Carello Collar, MSc
AAIC NeuroNext Early-career Lead
PhD student, UFRGS, Brazil

7.3. Certificado do comitê de ética de uso animal

Universidade de Brasília
Comissão de Ética no Uso Animal

Brasília, 18 de setembro de 2024.

DECLARAÇÃO

Declaramos que o projeto intitulado **"Efeito da dieta rica em gordura sobre desfechos metabólicos e do neurodesenvolvimento da prole do peixe-zebra"**, SEI nº 23106.075530/2024-12, sob responsabilidade do(a) pesquisador(a) **Jair Trapé Goulart**, **(jair.goulart@unb.br)**, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009 e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal – CONCEA.

Esse projeto foi avaliado e aprovado pela Comissão de Ética no Uso Animal (CEUA) da Universidade de Brasília na 222ª reunião ordinária, em **17/09/2024** para fins de **PESQUISA** e para utilização de ***Danio rerio*** **(Peixe-zebra, 6120 larvas – de 0 a 30 dias, 1536 juvenis – de 1 a 12 meses e 1024 adultos – de 3 a 12 meses, sendo estes últimos 512 machos e 512 fêmeas)** provenientes do(a) Biotério Aquático de peixe-zebra do Departamento de Genética e Morfologia do Instituto de Ciências Biológicas.

O presente certificado é válido pelo período de **18/09/2024** a **31/12/2030**.

Dr. Bruno Stéfano Lima Dallago
Coordenador da CEUA – UnB

Universidade de Brasília UnB

Comissão de Ética no Uso de Animais CEUA

*Este documento se restringe à avaliação ética do projeto supracitado e não substitui outras licenças e permissões que porventura se façam necessárias.