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TÍTULO: A INFLUÊNCIA DO SOBREPESO E OBESIDADE MATERNA NO DESENVOLVIMENTO DO TRANSTORNO DO ESPECTRO AUTISTA

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A INFLUÊNCIA DO SOBREPESO E OBESIDADEMATERNA NO DESENVOLVIMENTO DO TRANSTORNO DO ESPECTRO AUTISTA

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Orientador: Profª. Drª Joana Margarida Navalho Gaspar

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Thiago Kucera Nunes

Título: A Influência do Sobrepeso e Obesidade Materna no Desenvolvimento do Transtorno doEspectro Autista

O presente trabalho em nível de mestrado foi avaliado e aprovado por banca examinadora compostapelos seguintes membros:

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“A imaginação, muitas vezes, nos leva a mundos que nunca existiram. Mas sem isso, não vamos a lugar algum” (SAGAN C., 1980)

RESUMO

O transtorno do espectro autista (TEA) é uma desordem deletéria na comunicação e interação social, que se manifesta no princípio da infância, e que apresenta diversas comorbidades associadas. A prevalência do TEA vem crescendo exponencialmente na população em diversas regiões do mundo, principalmente nas últimas três décadas, despertando a preocupação e o interesse científico. A etiologia do autismo ainda é desconhecida, porém, vários estudos conseguiram associar a influência do sobrepeso e obesidade materna a maiores riscos para o desenvolvimento deste transtorno. Como osprimeiros sintomas sempre estão presentes no início da infância, a hipótese de perturbações no neurodesenvolvimento, na vida intrauterina, é uma provável causa desse distúrbio. A vida intrauterina é um período sensível ao neurodesenvolvimento do feto, e a exposição a determinados fatores neste período apresenta efeitos negativos na prole. A obesidade é uma doença inflamatória crônica, e apresenta efeitos negativos à saúde das gestantes e dos filhos, incluindo o aumento do risco para o desenvolvimento do TEA. Perturbações oriundas da obesidade, como a alta glicemia e a inflamação crônica da gestante, podem afetar negativamente o neurodesenvolvimento dofeto, ocasionando a má formação e interconexão de estruturas cerebrais, e afetando a fisiologia do sistema nervoso central (SNC). Para melhor compreender tais efeitos no neurodesenvolvimento, estudos sobre os mecanismos bioquímicos da neuroinflamação e da disfunção mitocondrial no período da vida intrauterina são bastante convenientes, uma vez que são eventos comumente presentes em pacientes autistas, e que podem apresentar papel determinante na morfofisiologia do SNC e na etiologia do autismo. Nessa dissertação, o objetivo foi caracterizar o perfil metabólico de camundongos fêmeas alimentadas com uma dieta rica em gordura, e a revisão de dois artigos com os títulos “Neuroinflammation in Autism Spectrum Disorders: Exercise as a Pharmacological Tool” e “Obesity associated with Autism Spectrum Disorders: Mitochondrial Dysfunction and Neuroinflammation as a links”.
Essa dissertação conclui que a obesidade materna é um fator com grande probabilidade de gerar perturbações na vida intrauterina, aumentando o risco de neuroinflamação e disfunção mitocondrial na prole, e aumentando também a probabilidade do desencadeamento do autismo na prole de gestantes obesas. Portanto, mais estudos são necessários para atribuir a associação da neuroinflamação e da disfunção mitocondrial como eventos chave na etiologia e/ou sintomas do TEA.

Palavras-chave: autismo, obesidade materna, neuroinflamação, disfunção mitocondrial.

ABSTRACT

Autism spectrum disorders (ASD) are a complex neurodevelopmental disease that involves disturbances in communication and social interaction. ASD is manifested early in childhood and has several associatedcomorbidities. The prevalence of ASD has been growing all over the world,specially in the last three decades, raising concern and scientific interest. Theetiology of ASD is still unknown. However, several studies have associatedthe influence of overweight and obesity with higher risk for the ASDdevelopment. As the first symptoms are always present in early childhood, the hypothesis of neurodevelopmental disturbances in the intrauterine life isa probable cause of this disorder. Intrauterine life is a sensitive period for fetal neurodevelopment, and exposure to certain factors during this period has negative effects on the offspring. Obesity is a chronic inflammatory disease and has negative health effects on pregnant women and children, including an increased risk for ASD development. Disorders arising from obesity, such as hyperglicemia and chronic inflammation in pregnancy, can negatively affect fetus neurodevelopment, causing abnormal formation and interconnection of brain structures, affecting the physiology of the central nervous system (CNS). To better understand these deleterious effects, studies on the biochemical mechanisms of neuroinflammation and mitochondrial dysfunction during the period of intrauterine life are very convenient, since they are events commonly present in autistic patients, andthat can play a decisive role in the patophysiology of the CNS and in ASD etiology. Our objective was to characterize the metabolic profile of mice fedwith a high-fat diet, and to write two review articles entitled “Neuroinflammation in Autism Spectrum Disorders: Exercise as a Pharmacological Tool” and “Obesity associated with Autism Spectrum Disorders: Mitochondrial Dysfunction and Neuroinflammation as a links”.

We conclude that maternal obesity is a risk factor for generating disturbancesin intrauterine life, increasing the risk of neuroinflammation and mitochondrial dysfunction in the fetus, as key events in the etiology and/or symptoms of ASD.

Keywords: autism, maternal obesity, neuroinflammation, mitochondrialdysfunction

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LISTA DE ABREVIATURAS E SIGLAS

1H-MRS - espectroscopia de ressonância magnética de prótons

5HIAA - ácido hidroxi indol acético

ADI-R - entrevista revisitada para o diagnóstico de autismo

ADOS - cronograma de observação para o diagnóstico de autismo

ADP - adenosina difosfato

ATP - adenosina trifosfato

BDNF- fator neurotrófico derivado do cérebro

BLC - linfócito quimioatraente β

CCL2 - quimiocina ligante 2
CNVs - número de cópias variantes

CNTNAP2 - proteína 2 associada à contactina

CTE - cadeia transportadora de elétrons

DEA - desordens de espectro autista

DNA - ácido desoxirribonucleico

dnCNVs - número de cópias variantes com mutações *de novo*

dsRNA – RNA dupla fita
DSM-5 - manual de diagnóstico e estatística de desordens mentais, 5ª edição

EROs - espécies reativas de oxigênio

FGF-4 - fator de crescimento de fibroblasto - 4

FGF-9 - fator de crescimento de fibroblasto - 9

FMN - mononucletotídeo de flavina

GAD67 - ácido glutâmico descarboxilase 67

GFAP - proteína acídica fibrilar glial

GTT - teste de tolerância a glicose

GSH - glutationa reduzida

GR - glutationa redutase
GSH-PX - glutationa peroxidase

GSSG - glutationa oxidada

GWG - ganho excessivo de peso na gestação

H_2O - água

IGFBP3 - proteína ligante de fator de crescimento tipo insulina - 3

IGFBP4 - proteína ligante de fator de crescimento tipo insulina - 4

IFN-I - interferon classe I

IL-1RA - antagonista do receptor de interleucina - 1

IL-6 - interleucina - 6

IL-8 - interleucina - 8

IL-10 - interleucina - 10

IL-13 - interleucina - 13

IL-17 - interleucina - 17

IL-1β - interleucina 1 beta

IMC - índice de massa corporal

IP-10 - proteína indutora de interferon- γ 10

IRE-1 - proteína 1 requisidora de Inositol

LCR – líquido cefalorraquidiano

MCP1 - proteína quimiotática de monócitos 1

MCP3 - proteína quimiotática de monócitos 3

MDC - citocina derivada de macrófago

MIF - fator indutor de mesoderme

MIP1 β - proteína inflamatória de macrófago 1 β

mtDNA - DNA mitocondrial

NADP - nicotinamida adenina dinucletideo fosfato

NADPH - nicotinamida adenina dinucletideo fosfato hidrogenada

NAP2 - peptídeo ativador de neutrófilo 2

NRG1 - neuregulina 1

$ONOO^-$ - peroxinitrito

OXPHOS - fosforilação oxidativa

PRRs - receptor de reconhecimento padrão

PAPs - processos perissinápticos de astrócitos

PARC - quimiocina pulmonar regulada por ativação

Pi - fosfato inorgânico

RNA - ácido ribonucleico

SNC - sistema nervoso central

SCD - déficit de comunicação social

SNP - polimorfismo de nucleotídeo único

SNV - variante de nucleotídeo único

SOCS - supressor de sinalização de citocinas

SOD - superóxido dismutase

SOD2 - superóxido dismutase 2

TGF-β - fator de crescimento transformador β

TLR - receptor do tipo toll

TNF-α - fator de necrose tumoral α

UQ - ubiquinona

UQH – semiquinona

UV - ultravioleta

VEGF - fator de crescimento endotelial vascular

SUMÁRIO

1. INTRODUÇÃO

1.1 TRANSTORNO DO ESPECTRO AUTISTA

O Transtorno do Espectro Autista (TEA), comumente conhecido como autismo, é um grupo de desordens do neurodesenvolvimento caracterizado por danos na comunicação einteração social, interesses restritos e comportamentos repetitivos (Diagnostic Stat. Man.Ment. Disord. 5th Ed., 2013). Os primeiros sintomas clínicos do autismo podem aparecerentre 2 e 6 meses de idade (JONES;KLIN, 2013).

Os sintomas do TEA variam de leves a severos (MAYES et al., 2012) ecom relação às habilidades cognitivas, os portadores de TEA variam de extremamente dotados e ativos (GINN, 2011) a severamente prejudicados (RAO;LANDA, 2014), tornando a doença uma complexa condição com elevada heterogeneidade fenotípica (TOAL et al., 2010).

O termo autismo foi cunhado pelo psiquiatra suíço Eugen Bleuler, em 1911, quando se referiu a um núcleo de características entre pacientes com esquizofrenia (BLEULER, 1950). A inserção do termo autismo para a denominação de um grupo com característicasatípicas, foi feita pelo psiquiatra austríaco Leo Kanner, em 1943, após a observação de 11 crianças, as quais as caracterizou como sem predisposição à sociabilidade (KANNER,1943).

A prevalência global do TEA está estimada em cerca de 1.5% (LYALLet al., 2017). Umestudo da prevalência para o autismo no Brasil foi realizado emAtibaia (SP) em 2011, envolvendo 1470 crianças com idades entre 7 e 12 anos, e mostrou prevalência de 1/367 (PAULA et al., 2011). Já um estudo epidemiológico que utilizou dados secundários de órgãos de apoio a indivíduos autistas entre 2016 e 2017, relatou a prevalência estimada de 3.85 casos de autismo a cada 10 mil habitantes na região sul do Brasil. A cada 10 mil habitantes, o Estado do Rio Grande do Sul apresentou estimativa de 3.31, o Estado de Santa Catarina apresentou estimativa de 3.94, e o Estado do Paraná apresentou estimativade 4.32 pessoas com autismo. A maior prevalênciaficou entre indivíduos masculinos, 2.2vezes maior em comparação ao gênero feminino, e a faixa etária com maior prevalência foi entre 5 e 9 anos de idade (BECK, 2017).

A prevalência para o TEA tem aumentado significativamente na últimadécada nos Estados Unidos (aproximadamente 2.5 vezes), apresentando uma incidência 4 vezes maior no gênero masculino que no gênero feminino (MAENNER et al., 2020). Números da prevalência para o TEA nos EUA divulgados em 2020 apontam para 1/54 em criançascom idade de 8 anos de acordo com os critérios do Diagnostic and Statistic Manual of Mental Disorders,4th edição (DSM-4 e DSM-5) (MAENNER et al., 2020). Nos países europeus, aprevalência para o TEA varia bastante, alcançando 1/87 entre crianças de 7 a 9 anos de idade na Itália de acordo com os

critérios DSM-4 e CIE10 (Classificação Internacional de Enfermidades, 10ª edição, OMS) (NARZISI et al., 2018), 1/166 em crianças entre 6 e 11 anos de idade de acordo com os critérios CIE10 na Alemanha (BACHMANN; GERSTE; HOFFMANN, 2018), 1/125 entre todas as crianças nascidasna Noruega entre 1999 e 2009 de acordo com os critérios CIE9e CIE10, e na Espanha asprevalências foram de 1/64 em crianças com idades entre 3 e 4 anos e 1/100 em criançascom idade entre 10 e 11 anos (MORALES- HIDALGO et al., 2018). Um estudo feito na cidade de Okaia-shi no Japão, no qual aproximadamente 85% das crianças nascidas entre 2009 e 2012 foram avaliadas aos 6 anos de idade, apresentaram prevalência de 1/32 (SASAYAMA et al., 2020).

Poucos fármacos apresentam eficácia contra os sintomas do autismo, sendo os principais a Risperidona, e Aripiprazol, que tratam a irritabilidade, e Metilfenidato e Atomoxetina, que tratam o déficit de atenção e hiperatividade (CUKIER; BARRIOS, 2019).

1.1.1 Caracterização do TEA

De acordo com a quinta e última edição do DSM (Diagnostic and Statistic Manual of Mental Disorders, 5ª edição) o autismo é caracterizado por déficits persistentes em pelo menos 3 dos seguintes parâmetros: reciprocidade social e emocional; comportamentos de comunicação não verbais; desenvolvimento e manutenção de relações incomuns; falas e movimentos repetitivos; manutenção excessiva de rotinas e padrões de comportamentosverbais e não verbais ou excessiva recusa à mudanças; fixação em interesses incomuns ealtamente restritos; hiper ou hiporreatividade a estímulos sensoriais ou interesse extraordinário em aspectos apreciados do meio ambiente.

Ainda de acordo com o DSM-5, há diferentes níveis de severidade na manifestação do TEA entre indivíduos portadores desta desordem. Tais níveis, variam de 1 a 3 com relaçãoaos déficits na interação e comunicação social, e aos comportamentos repetitivos e interesses restritos. As principais características destes déficits nos três diferentes níveis de severidades que compõem o TEA estão resumidas a seguir na *tabela 1*.

	Severidade nível 1	Severidade nível 2	Severidade nível 3
Dficits na interação e comunicação social	Dificuldade iniciar e m interações sociais;	Déficits mais acentuados na conversação;	Graves prejuízos em situações que necessitam de interações sociais básicas;
	Respostas atípicas ou sem concessões para abertura social;	Limitado interesse em interações sociais;	Aberturas sociais muito limitadas e escassas.
	Interesse diminuído nas interações sociais;	Respostas atípicas com reduzido interesse em interações sociais.	
	Tentativa de amizades de forma estranha e malsucedida.		
Comportamentos repetitivos e restritivos	Funções significativamente comprometidas e dificuldades para alternar atividades;	Comportamentos atípicos mais frequentes e sensíveis a espectadores alheios;	Comportamentos atípicos interferindo negativamente de forma muito acentuada na qualidade de vida;
	Problemas com organização e planejamento.	Manifestações em vastos ambientes;	Dificuldade extrema em aceitar mudanças e mudar o foco ou ação.
		Grande dificuldade para mudar o foco ou ação.	

Tabela 1. Características do déficit na interação e comunicação social e dos comportamentos restritivos e repetitivos entre diferentes níveis de severidade do TEA (Diagnostic Stat. Man. Ment. Disord. 5th Ed., 2013).

1.1.2 Comorbidades associadas ao TEA

Além dos déficits comportamentais e sociais, o autismo está geralmente associado concomitantemente com outras disfunções neurológicas como epilepsia (EWEN et al., 2019), disfunções do sono (FLETCHER et al., 2020), deficit cognitivo (GABRIELSEN et al., 2018), transtorno do déficit de atenção e hiperatividade (GODOYet al., 2020), ansiedade (POSTORINO et al., 2017), esquizofrenia (ZHENG; ZHENG; ZOU, 2018) e transtorno obsessivo compulsivo (GADELKARIM et al., 2019). Somando-se a isso, grande parte dos indivíduos com TEA costumam apresentar outras condições clínicas, como problemas gastrointestinais (COURY et al., 2012), dificuldades motoras (AMENTet al., 2015), esclerose tuberosa (SPECCHIO et al., 2020), seletividade alimentar (CHISTOL et al., 2018), dislipidemia (LUO et al., 2020), obesidade (TOSCANO et al., 2019), dentre outras, sugerindo que possam haver mecanismos bioquímicos subjacentes sobrepostos no desencadeamento destas comorbidades.

Portanto, o autismo é uma condição debilitante nos âmbitos da interaçãoe comunicação social e de comportamentos repetitivos e estereotipados, que se manifesta logo no inícioda vida e apresenta diversas comorbidades associadas que podem prejudicar significativamente a qualidade de vida dos pacientes. O TEA ainda não possui cura, e estudos que abordem os mecanismos envolvidos com o desencadeamento e os sintomas desta desordem são fundamentais na busca de tratamentos que possam eliminar ou diminuir a severidade sintomáticaapresentada no autismo.

1.2 ETIOLOGIA DO TEA

A etiologia do autismo permanece em debate dentro da comunidade científica, havendo um crescente consenso de que seja uma condição desencadeada por complexas interações ambientais e genéticas (GHIRARDI et al., 2019; HEGARTY et al., 2020; SHARON et al., 2019; LE BELLE et al., 2014).

1.2.1 TEA, genética e epigenética

Alterações genéticas como o polimorfismo em base única (SNPs) (SANTINI et al., 2013),variações em um único nucleotídeo (SNVs) (DONG et al., 2014), variação no número decópias (CNVs) (SANDERS et al., 2015), e mutações *de novo* (aquelas presentes nos indivíduos mas não em seus progenitores) (FERNANDES et al., 2018), são comuns entreindivíduos autistas. Paralelamente a isso, a epigenética refere-se a mudanças na expressãogênica comausência de modificações na sequência do DNA, compreendendo uma maquinaria que regula a estrutura e a expressão do DNA. Os mecanismos epigenéticos

respondem a fatores ambientais (externos) e celulares (internos) (ROTHSTEIN; CAI; MARCHANT, 2009) e compreendem a metilação do DNA,modificações pós-tradução de RNA e histonas, que atuam regulando a expressão gênica. A interferência desse mecanismo regulatório na expressão gênica vem sendo associada a uma grande variedade de distúrbios (BRASA et al., 2016; HAERTLE et al., 2019; KUBOTA et al., 2019), incluindo o TEA (ANDARI et al., 2020).

A interferência da genética e epigenética na modificação e expressão degenes chave durante o neurodesenvolvimento, pode ter fundamental participação no desencadeamentodo fenótipo autista. A tabela (2) a seguir mostra exemplos dealterações genéticas, o localonde estas ocorrem, e as funções destes genes *in natura* ou em associação às mutações neles encontradas, as quais podem ser observadas em frequência aumentada em indivíduos autistas:

Tipo de Alteração Genética/Local	Transcritos	Função *in natura* ou alterações associadas com a mutação
SNP (variações no DNA em um único nucleotídeo) no gene EIF4E (SANTINI et al., 2013)	Promotor do gene EIF4E	Mutação associada com a desregulação do controle da tradução gênica (hipocampo, estriado e córtex pré-frontal). Aumento do receptor metabotrópico de glutamato (mGlur-LTD) no hipocampo e na densidade dos espinhos dendríticos nos neurônios piramidais do córtex pré-frontal. Aumento na frequência do potencial sináptico inibitório (mIPSC) (córtex pré-frontal, estriado e hipocampo) e de correntes excitatórias pós-sinápticas. Diminuição no volume de espinhos dendríticos e funcionamento anômalo na plasticidade neuronal. Fenótipo comportamental e social deficitário.
SNP no gene CNTNAP2 rs 7794745 (ZARE; MASHAYEKHI; BIDABADI, 2017)	Proteína Andaime CASPR2	Interações neurônio-glia e canais de cálcio em axônios mielinizados
SNP no gene DCC (LI et al., 2020)	Proteína receptora de Netrin-1	Desenvolvimento do Sistema Nervoso Central
SNV (variação em um único nucleotídeo) no gene KMT2E (DONG et al., 2014)	Proteína Lisina Metiltransferase 2E	Regulação da Cromatina e da regulação do Ciclo Celular
SNV no gene RIMS1 (DONG et al., 2014)	Proteína RIMS1	Regulação da exocitose da membrana sináptica e liberação de neurotransmissores
Mutações *de novo* com CNV (dnCNV) no gene GTF2I (SANDERS et al., 2015)	Fator de Transcrição Geral II-I e BAP-135	Regula negativamente a entrada de cálcio na célula, e regulação do sistema imune, respectivamente
Mutações *de novo* com CNV (dnCNV) no gene OXT (SEBAT et al., 2007)	Hormônio Ocitocina	Sensibilização social, lactação, contrações uterinas no parto
Mutações de novo com CNV (dnCNV) no gene GABRB3 (SANDERS et al., 2015)	Subunidade beta 3 do receptor GABA	Sensibilidade ao neurotransmissor GABA
Mutações de novo com CNV (dnCNV) no gene GTF2IRD2 (SANDERS et al., 2015)	Fator Geral de Transcrição II-I	Regulação de Transcrição
Mutações de novo com CNV (dnCNV) no gene WBSCR16 (SANDERS et al., 2015)	Proteína WBSCR16	Regulação de condensação de cromossomo 1
Mutações de novo com CNV (dnCNV) GABRA5 (SANDERS et al., 2015)	Receptor da subunidade alfa 5 do neurotransmissor GABA	Pode estar envolvido na montagem do receptor GABA-A e na imobilização e acúmulo do receptor GABA-A pela gefirina na sinapse
Mutações de novo com CNV (dnCNV) no gene GABRG3 (SANDERS et al., 2015)	Receptor da subunidade alfa 3 do neurotransmissor GABA	Sinalização de Akt e via de apoptose. Regula a atividade do canal de cloreto e a atividade do receptor GABA
Mutações de novo com CNV (dnCNV) no gene CDH13 (SANDERS et al., 2015)	Proteína CDH13	Proteína de adesão cálcio dependente. Regula o controle da migração celular, crescimento de neurônios e guia axônios. Variantes associadas ao comportamento agressivo
Mutações de novo no gene SETD5 (FERNANDES et al., 2018; GREEN; WILLOUGHBY; BALASUBRAMANIAN, 2017)	Proteína SETD5	Proteína reguladora da metilação da histona H3k36me, associada à regulação na proliferação neuronal e transmissão sináptica. Variantes associadas ao déficit intelectual

Tabela 2. Alterações genéticas e funções proteicas *in natura* ou associadas com os fenótipospolimórficos.

Com relação às alterações epigenéticas, a tabela (3) a seguir mostra exemplos que podemser observados em frequência aumentada em indivíduos autistas (ANDARI et al., 2020; NAKAGAWA et al., 2020), juntamente com o fenótipo associado a estas alterações:

Local da Alteração Epigenética	Fenótipo Associado à Alteração Epigenética
Metilação no sítio CpG 16 (intron 1 da região MT2) do gene receptor de ocitocina (OXTR) (ANDARI et al., 2020).	Déficits sociais, na linguagem e comunicação.
Metilação na região do exon 1 de OXTR (ANDARI et al., 2020).	Déficits na responsividade social e hiperconectividade entre áreas do estriado e córtex.
Altos níveis de metilação na região do intron 1 de OXTR (ANDARI et al., 2020).	Hiperconectividade entre regiões cerebrais córtico-corticais.
Metilação nas regiões CpG 16 e CpG 5,6 (exon 1 da região MT2) (ANDARI et al., 2020).	Negativamente associadas à conectividade funcional no estado de repouso entre áreas independentes, como córtex cingulado posterior e sulco temporal superior
Redução da acetilação na Lys^{16} da histona 4 (H4K16ac) (em casos em que há haploinsuficiência do gene SETD5) (NAKAGAWA et al., 2020).	Déficits na comunicação social, hiperatividade, danos na proliferação neuronal.

Tabela 3. Alterações epigenéticas encontradas em indivíduos autistas e fenótipos associados.

1.2.2 Fatores ambientais associados ao TEA

A vida intrauterina é extremamente sensível a fatores externos, e muitosdeles podem ter ação determinante no neurodesenvolvimento e nodesencadeamento do autismo. Dentre os fatores ambientais com manifestação navida intrauterina e que apresentam associação com o autismo estão a exposição materna à poluição atmosférica (FRYE et al., 2020), nutrição materna (LYALL et al., 2013), hipertensão materna (CORDERO et al., 2019), diabetes materno (XIANG et al., 2018), exposição materna a agrotóxicos (VONEHRENSTEIN et al., 2019), ativação do sistema imune materno (HOLINGUE et al., 2020; PATEL et al., 2020), uso de fármacos na maternidade (WIGGS et al., 2020) e obesidade materna (KRAKOWIAK et al., 2012).

Indivíduos com disfunção mitocondrial são mais suscetíveis a perturbações ambientais, muitas vezes resultando em regressão do neurodesenvolvimento (EDMONDS et al., 2002). Um estudo de meta- análise verificou que a regressão do neurodesenvolvimento émais comum em indivíduos que apresentavam autismo e disfunção mitocondrial que em indivíduos com autismo mas sem disfunção mitocondrial (ROSSIGNOL; FRYE, 2012).

Em casos de exposição pré-natal a altas concentrações de poluição atmosférica, representada por partículas no ar com diâmetro de 2.5µm ($PM_{2.5}$) de variadas fontes, tal

exposição foi associada a déficits comportamentais associados ao autismo e danos na respiração mitocondrial com déficit na produção de ATP (FRYE et al., 2020).

A exposição materna a dietas ricas em gordura, apresentaram maior associação com a presença do fenótipo autista nos filhos (LYALL et al., 2013). Foi verificado em estudo que a alta ingesta de gordura na maternidade alcançou maiores riscos para o desencadeamento do autismo na prole, porém, a alta ingestaconcomitante de gorduras poliinsaturadas, como o ω-3, apresentou efeito protetivo, reduzindo o risco para autismonos filhos em 53% (LYALL et al., 2013). O ácido α-linoleico também apresentou efeito protetivo, com a diminuiçãodo desfecho autista nos filhos chegando a 30% (LYALL et al., 2013).

Disfunções gestacionais, como a hipertensão, apresentaram associação positiva com o fenótipo autista nos filhos em um estudo, com aumento do risco em 69% entre gestanteshipertensas ajustadas para parâmetros como idade, etnia, fumo, escolaridade, IMC, número de filhos, região residencial e diabetes, e aumento em 78% entre gestantes não ajustadas para os mesmos parâmetros (CORDERO et al., 2019).

Em um estudo sobre a exposição do diabetes tipo 1 na gestação, a cada 10 mil nascimentos, 4.4 indivíduos desenvolviam o autismo durante a infância (XIANG et al., 2018). A incidência do autismo para diabetes tipo 2 a cada 10 milnascimentos foi de 3.6, para diabetes gestacional foi de 2.9 e de 1.8 para gestaçõessem diabetes (XIANG et al., 2018). Neste estudo, os dados foram ajustados paraparâmetros como idade no parto, escolaridade, renda familiar, etnia e histórico de comorbidade (doença cardíaca, fígado, pulmão, rins e câncer) (XIANG et al.,2018).

Alguns compostos químicos, como os organoclorados (encontrados nosagrotóxicos), têmcapacidade para atravessar a placenta durante a gravidez (YINet al., 2019) e perturbar oneurodesenvolvimento com desregulação endócrina, e nos sistemas imunitário e nervoso (CHEVRIER et al., 2008; KIMURA-KURODA; NAGATA; KURODA, 2007). Um estudo mostrou que muitos tipos de agrotóxicos estão significativamenteassociados como aumento no risco do desencadeamento do autismo quando as gestantes são expostas a tais compostos (VON EHRENSTEIN et al., 2019). Esteestudo mostra que a exposição ao glifosato aumentou o risco para o desenvolvimento de autismo nos filhos em 11%, em 15% para exposição ao clorpirifos, 14% para o diazinon e para a avermectina, 10% para o acefato, 8% para o malathion, e 4% para o permetrin (VON EHRENSTEIN et al., 2019).

A ativação imune materna tem sido associada a problemas neuropsiquiátricos nos filhos, especialmente relacionados à esquizofrenia (comorbidade comumente associada ao TEA)(BROWN, 2006) e TEA (CHOI etal., 2016). Gestantes que foram expostas a rubéola

(*Rubella vírus*) tiveram alta expressão de interleucina 8 (IL-8) e TNF-α no soro, e prevalência de 20% no desencadeamento da esquizofrenia nos filhos, enquanto gestantesexpostas ao*Influenza vírus* apresentaram aumento no risco de desencadeamento da esquizofrenia nos filhos até sete vezes maior que a média da população geral (BROWN,2006). Dados de ativação imune materna em animais apresentaram maior incidência no desencadeamento do fenótipo tipo autista na prole a partir do estímulo imunitário em roedores gestantes através de injeções com LPS (lipopolissacarídeo constituinte de membrana de bactérias) no 15º dia gestacional(LOMBARDO et al., 2018). Este estudo mostrou também a alteração na expressão gênica nas gestantes (4h após injeções com LPS), apresentando 6923 genes com expressão diminuída e 4981 genes com expressão aumentada (LOMBARDO et al., 2018). Em outro estudo com modelos roedores de ativação imune materna inoculados intraperitonealmente com poly (I:C) (substância sintética análoga ao dsRNA, forte indutora de interferon e que mimetiza infecçãoviral), a prole apresentou maior probabilidade de desenvolver comportamentos tipo autistas (CHOI et al., 2016). As gestantes apresentaram elevada expressão de interleucina 17 (IL-17), interleucina 6 (IL-6), fator de necrose tumoral (TNF-α),interleucina 1-β (IL-1β), e interferon-β (IFN-β) no soro, e a prole apresentou elevada concentração de IL-17 no cérebro (CHOI et al., 2016).

Alguns medicamentos apresentam associação com o desencadeamento de autismo nos filhos quando utilizados durante a gestação. Um caso típico são os medicamentos anti-convulsão que apresentam em sua composição o ácido valpróico, que se mostrou forte indutor de autismo na prole (aumento em130% no risco) quando utilizado pelas mães noperíodo gestacional (WIGGS et al., 2020).

A obesidade materna também tem se mostrado um bom preditor para o desenvolvimentode autismo nos filhos, apresentando risco elevado para o TEA nos filhos (KRAKOWIAKet al., 2012), além de diversos aspectos negativos aosquais está associada. Estudos sobre obesidade materna e sua associação com o TEA mostram que a condição de obesidadeno período gestacional pode aumentaro risco para autismo nos filhos em até seis vezes (KONG et al., 2018).

1.3 ALTERAÇÕES MORFOFISIOLÓGICAS NO TEA

Indivíduos autistas (humanos e roedores) geralmente apresentam mudanças neurofisiológicas e neuroanatômicas em regiões do cérebro envolvidas na cognição, comportamento e emoções (KOJIMA et al., 2019; POSTEMA et al., 2019; STERNBERG, 2006; STOODLEY et al., 2017; WEGIEL et al., 2010;SCHOEN et al., 2019; OWEN et al.,

2018; AMARAL et al., 2017; SACCO; GABRIELE; PERSICO, 2015; SHAFRITZ et al., 2008; AZMITIA et al., 2011; MARKRAM; RINALDI; MARKRAM, 2007). Alguns exemplos dessas alterações podem ser observados natabela (4) a seguir:

Tipo de Alterações	Estruturas Cerebrais
Assimetria morfológica (POSTEMA et al., 2019).	Diminuição do córtex lateral esquerdo e aumento do córtex lateral direito.
Aumento no volume (SCHOEN et al., 2019; OWEN et al., 2018).	Tálamo, estriado, área ventricular e globo pálido.
Aumento na conectividade* (STOODLEY et al., 2017). (camundongos)*	RCrusI e: áreas corticais, matéria branca, lóbulo parietal inferior esquerdo.
Aumento na espessura/volume (POSTEMA et al., 2019; SACCO; GABRIELE; PERSICO, 2015; AMARAL et al., 2017).	Frontal superior, frontal médio rostral, orbitofrontal medial, temporal inferior, giro cingulado e giro fusiforme. Volume cerebral total.
Baixa ativação (SHAFRITZ et al., 2008).	Córtex parietal, frontal e estriado (associados à baixa capacidade cognitiva), córtex cingulado anterior e córtex parietal posterior (associado ao aumento na severidade nos comportamentos repetitivos.
Baixa densidade (Neurônios Piramidais e células Purkinje) (WEGIEL et al., 2010).	Ventrículos laterais, corno temporal e hipocampo (CA1) e cerebelo.
Camada de neurônios granulares fragmentada (migração neuronal anormal) (WEGIEL et al., 2010).	Giro denteado.
Displasia (WEGIEL et al., 2010).	Nódulos subependimários (ventrículos laterais), lóbulo flóculonodular (cerebelo), vermis (com desorganização de células Purkinje) e hipocampo (CA1).
Displasia multifocal (com perda de organização vertical e horizontal, anormal formação de camadas celulares e perda de orientação dos neurônios) (WEGIEL et al., 2010).	Neocortex, córtex entorrinal, corno Ammonis, e giro denteado.
Displasia flóculonodular (WEGIEL et al., 2010).	Cerebelo (com fina camada granular no labirinto).
Diminuição no volume (KOJIMA et al., 2019; OWEN et al., 2018).	Matéria cinza, córtex cingulado posterior, precuneus e matéria branca.
Espessamento (WEGIEL et al., 2010).	Camada de células subependimárias.
Hiperfunção (MARKRAM; RINALDI; MARKRAM, 2007).	Amígdala (associada a déficits no processamento de estímulos sensoriais.
Hipoplasia (WEGIEL et al., 2010).	Cerebelo (com baixa densidade de células Purkinje) e núcleo denteado (com convolução reduzida).
Heterotopia (migração neuronal anormal) (WEGIEL et al., 2010).	Cerebelo, hipocampo, região subcortical da matéria branca do giro cingulado anterior, matéria branca cerebelar, e vermis.
Inibição na atividade*. (STOODLEY et al., 2017). (camundongos)*	RCrusI.
Menor densidade de axônios mielinizados (LIU et al., 2020; OWEN et al., 2018).	Córtex orbitofrontal e corpo caloso.
Menor espessura (AZMITIA et al., 2011).	Neurônios da amígdala, hipocampo e córtex.
Neurônios pequenos e pobremente diferenciados (WEGIEL et al., 2010).	Núcleo caudado.
Numerosos nódulos (WEGIEL et al., 2010).	Camada de células subependimária.
Redução na densidade (WILLIAMS et al., 1980; LIU et al., 2020).	Dendritos apicais de neurônios piramidais no neocortex, corpo caloso, e axônios mielinizados no córtex orbitofrontal.

Tabela 4. Tipos de alterações morfológicas encontradas em cérebros de autistas.

Estas diversas alterações em estruturas cerebrais provavelmente têm íntima relação com a fisiologia cerebral e a severidade dos sintomas apresentadospor pacientes autistas, e talobservação pode ser de grande utilidade na busca por novos tratamentos terapêuticos quevisem atenuar os sintomas do autismo.

1.3.1 Alterações moleculares e fisiológicas em autistas

Alguns mecanismos moleculares também têm sido sugeridos como prováveis envolvidosna patofisiologia do autismo. Estes mecanismos incluem mudanças em proteínassinápticas (neuroliginas, neurexinas, proteínas scaffold pós sinápticas e proteínas deadesão) (CAST et al., 2020; ZHANG et al., 2018; HALI et al., 2020; WANG et al., 2018;BURROWS et al., 2017; AMAL et al., 2020; BOZDAGI et al., 2010; SCHOEN et al.,2019; HEISE et al., 2018; LAZARO et al., 2019; KIM et al., 2017), neurotrofinas(GHAFOURI-FARD et al., 2020; SKOGSTRAND et al., 2019), neurotransmissores e seus transportadores (DICARLO et al., 2019; GARBARINO et al., 2019), e neuroinflamação (VARGAS et al., 2005). A observação destas alterações pode auxiliarna busca por tratamentos terapêuticos dos sintomas do autismo, e algumas delas estãorepresentadas na tabela (5) a seguir:

Tipo de Alterações	Moléculas Envolvidas/ Alterações Associadas	Ocorrência
Aumento na concentração (ABDULAMIR; ABDUL-RASHEED; ABDULGHANI, 2018; GHAFOURI-FARD et al., 2020)	Serotonina (sangue) Transportadores de serotonina (sangue) BDNF, neurotrofina (sangue)	Humanos Humanos Humanos (crianças)
Diminuição na concentração (SKOGSTRAND et al., 2019)	BDNF (sangue), associada a alterações morfológicas no neurodesenvolvimento	Humanos (recém-nascidos)
Haploinsuficiência (BOZDAGI et al., 2010)	Shank 3 (proteína pós-sináptica que recruta componentes como mGlu e AMPA). Haploinsuficiência: associada à redução em sinais excitatórios no hipocampo, redução na transmissão mediada por AMPA, déficit na plasticidade e interações sociais.	Modelos roedores de autismo
Neuroinflamação (VARGAS et al., 2005)	Ativação de microglia e astroglia (córtex e cerebelo), aumento na expressão de citocinas no líquido cefalorraquidiano, córtex e cerebelo.	Humanos

Tabela 5. Tipos de alterações moleculares e fenotípicas encontradas em autistas.

É possível observar também alterações genéticas em indivíduos com autismo, e suas associações com alterações moleculares e fisiológicas em modelos animais tipo autistas geneticamente manipulados, conforme está demonstrado na tabela (6) a seguir:

Tipo de alteração	Proteínas afetadas e fenótipo associado	Ocorrência
Variantes genéticas (WANG et al., 2018; ZHANG et al., 2018; BURROWS et al., 2017; HEISE et al., 2018; AMAL et al., 2020; BOZDAGI et al., 2010; SCHOEN et al., 2019; HEISE et al., 2018; KIM et al., 2017; DICARLO et al., 2019).	Proteínas pré-sinápticas neurexinas, variantes 2 e 3 (NRXN2 rs12273892 e NRXN3 rs12879016) associadas com o fenótipo autista).	Humanos
	Proteínas pós-sinápticas neuroliginas, variante 3 (NL-3 R451C), associada a danos sociais no comportamento.	Humanos e modelos roedores de autismo
	Shank2-/- e Shank3 αβ-/- associadas com baixos níveis de receptores de glutamato no estrido e tálamo.	Modelo roedor de autismo
	Proteína pós-sináptica Shank-3 mutada levou ao aumento de fosforilação nas proteínas Synapsin1 e CREB, as quais afetam a mobilização de proteínas sinápticas e transcrição gênica, respectivamente.	Modelo roedor de autismo
	Cntn4-/- (nocautes para o gene da proteína contactina 4, proteína de adesão associada ao axônio, que modula a plasticidade sináptica), apresentaram baixos níveis de receptores de glutamato no córtex e hipocampo, e aumento destes no estriado. Redução de receptores GABA no hipocampo e tálamo.	Modelo roedor de autismo
	CNTNAP2 KO – severa redução na excitação e inibição sináptica em neurônios piramidais do córtex pré-frontal medial, aumento na conectividade entre córtex somatossensorial e córtex pré-frontal (disfunções na poda sináptica).	Modelo roedor de autismo
	Mutações em CNTNAP2 e Atg7 induzem alterações na conexão sináptica e na autofagia microglial, e estão associados com a diminuição de sinapses e de espinhos neurais, aumento da atividade inibitória em neurônios do córtex pré-frontal, aumento no número de espinhos dendríticos no córtex e desregulação na atividade de poda sináptica.	Modelo roedor de autismo
	Variante no transportador de dopamina (DAT T356) mostrou falha na recaptação de dopamina na fenda sináptica no estriado, aumento no peso corporal, comportamentos repetitivos, hiperativos e danos sociais.	Modelo roedor de autismo

Tabela 6. Variações genéticas e associações moleculares e fenotípicas encontradas em autistas.

Em conjunto, as alterações estruturais no cérebro e em mecanismos fisiológicos e moleculares estão possivelmente envolvidas na manifestação dos sintomas do autismo, as quais devido à complexidade em combinações possíveis, podem ser associadas com a heterogeneidade fenotípica dos sintomas do TEA.

1.4 NEUROINFLAMAÇÃO

A neuroinflamação está presente na maioria das condições patológicas do SNC, ecompreende um grande aspecto de respostas biológicas a perturbações, como danos cerebrais, infecções, e doenças neurodegenerativas. A neuroinflamação é caracterizada pela ativação de células imunes residentes responsáveis pela defesa e manutenção do SNC, microglia e astrócitos, que interpretam sinais biológicos de alerta e propagam respostas fisiológicas de defesa. Estes sinais podem ser oriundos de condições de obesidade (SAMARA et al., 2020), podendo ativar a neuroinflamação no feto em casos de obesidade gestacional, como pode ser observado em alguns modelos animais de obesidade gestacional (ERBAS et al., 2018; BILBO; TSANG, 2010).

Entre as principais moléculas sinalizadoras da neuroinflamação estão ascitocinas e as espécies reativas de oxigênio (EROs) (LI et al., 2018; YU et al., 2016; ISHIHARA et al.,2015). As principais fontes de EROs no SNC são a cadeia transportadora de elétrons, a NADPH oxidase, a lipooxigenase, a ciclooxigenase, e processos não enzimáticos como a auto-oxidação de noradrenalina e dopamina (LJUBISAVLJEVIC, 2016).

As citocinas, moléculas que promovem quadros inflamatórios, são proteínas pleiotrópicas que coordenam respostas fisiológicas de defesa nas células dos tecidos, incluindo o tecido nervoso (DEVERMAN; PATTERSON, 2009). Citocinas como TNF-α, IL-1β, IFN-I, e IL-17 são importantes na ativaçãode células como microglia e astrócitos (LI et al., 2018; YU et al., 2016; MENDIOLA; CARDONA, 2018; MUHAMMAD, 2020). Com a estimulação destas células imunes, ambas microglia e astrócitos alteram sua morfologia paraum fenótipo de ativação reativo, para combater osdanos locais e promover a proteção do SNC (SCUDERI et al., 2013).

Microglia são macrófagos nativos do SNC. Elas surgem do saco vitelino que coloniza o SNC em desenvolvimento, são de vida longa e autorrenováveis (GINHOUX et al., 2010; TAY et al., 2017). A microglia possui função de mielinização e manutenção das células progenitoras de oligodendrócitos (HAGEMEYER et al., 2017), também na poda sináptica(PAOLICELLI et al., 2011), e controla o crescimento e o posicionamento celular guiando circuitos neuronais durante o neurodesenvolvimento (SQUARZONI et al., 2014). A microglia tem um papel fundamental no SNC por responder a mudanças patológicas fagocitando e removendo detritos (VILHARDT, 2005), secretando citocinas, prostaglandinas, óxido nítrico, e ativando astrócitos para promover a inflamação e a manutenção do SNC (PASCUAL et al., 2012; BRÁS et al., 2020; QIU; WANG; CHEN, 2019). A microglia apresenta morfologia variada, dependendo da região, gênero, idade, tipo tecidual, tipo de estímulo ou dano local (ARCURI et

al., 2017). As células da microglia possuem o soma alongado na região da matéria branca cerebral, e o soma arredondado com ramificações radiais na matéria cinza. Em regiões circunventriculares e perivasculares, são mais compactas, forma na qual são mais vigilantes no monitoramento do SNC (NIMMERJAHN; KIRCHHOFF; HELMCHEN, 2005).

Quando ativada, a microglia muda para um formato ameboide e propaga o sinal neuroinflamatório através de citocinas como TNF-α, IL-6, IL-1β e IFN-γ para asdemais regiões do cérebro (*figura 1*), mediando respostas fisiológicas (KONSMAN; PARNET; DANTZER, 2002).

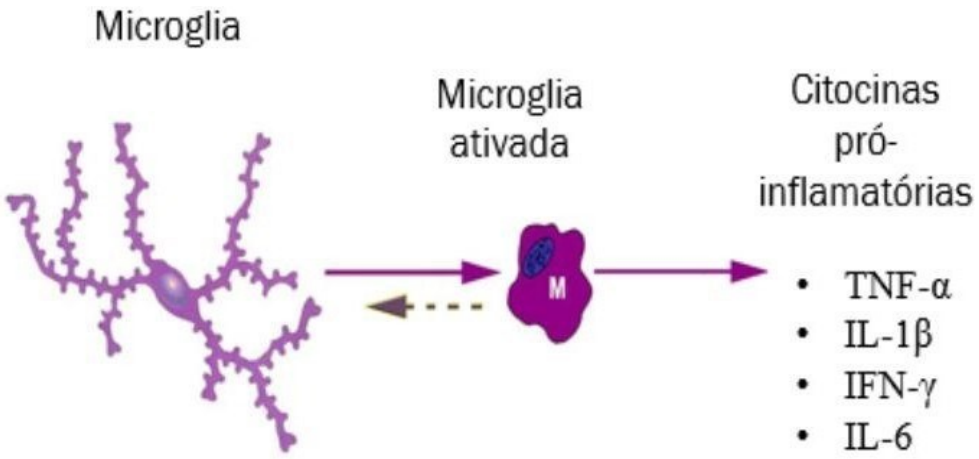

Figura 1. Ativação microglial em seu formato ameboide. Fonte: Adaptado do livro *The Secret Language of Cells*, de Jon Lieff.

Entretanto, a ativação microglial prolongada e exagerada pode levar a danos no tecido nervoso e causar perdas de conexões sinápticas (RODRIGUEZ;KERN, 2011), além de efeitos neurodegenerativos (FRAKES et al., 2014). Em razão desse alto poder em respostas imunológicas, as células da microglia estão sob forte regulação anti- inflamatória, feita por hormônios, neuropeptídeos e citocinas, como IL-10, TGF-β, proteínas ativadas por mitógenos (MAP) cinase fosfatase (MKPs) e supressor de sinalização de citocinas (SOCS), além de glicocorticoides secretados pelas glândulas adrenais (GLEZER; SIMARD; RIVEST, 2007; GLEZER; RIVEST, 2004). As células da microglia expressam inúmeros receptores de reconhecimento de padrão (PRRs), incluindo diversos membros da família dos Toll-like receptors (TLRs) (OLSON; MILLER, 2004), enquanto os astrócitos expressam um limitado repertório de PRRs, compoucos representantes dos receptores da família dos TLRs (FARINA et al., 2005), sendo portanto, células menos reativas que a microglia.

Astrócitos têm papel no controle da sinaptogênese (STOGSDILL et al.,2017), na

manutenção sináptica (CHUNG et al., 2013), no reparo neuronal (ENGLISH et al., 2020), guiam a migração e o desenvolvimento de axônios e neuroblastos (POWELL; GELLER, 1999), removem neurotransmissores (KHAKH; SOFRONIEW, 2015), participam da sinalização neuromodulatória para controlar a atividade e o comportamento dos circuitos neuronais (MA et al.,2016), além de promover sobrevivência e crescimento celular (BAUER; RAUSCHKA; LASSMANN, 2001). Em mamíferos, os astrócitos correspondem de 20 a 40% do número total de células no cérebro (HERCULANO- HOUZEL, 2014). Os astrócitos fibrosos e protoplasmáticos apresentam morfologias diferentes, e são os dois maiores tipos astrogliais encontrados na medula espinhale no cérebro, respectivamente (EMSLEY; MACKLIS, 2006; RODNIGHT; GOTTFRIED, 2013). Os processos astrogliais (prolongamentos celulares) são nomeados de acordo com seus tamanhos e localizações, como ramos, ramificações, processos perissinápticos de astrócitos (PAPs) e pés finais (KHAKH; SOFRONIEW, 2015). Astrócitos apresentam uma remodelagem estrutural nos PAPs que pode ser rápida (dentro de poucas horas), para proporcionar alteração na cobertura de neutrófilos em resposta a estímulos comportamentais, como estimulação osmótica, estresse e parto (THEODOSIS, 2002). Apesar de apresentarem menor repertório que a microglia de PRRs, os astrócitos também possuem atividade neuroinflamatória, podendo atingir a forma reativa, por exemplo, através de sinalização microglial (LIDDELOW et al., 2017), mediante fibrinólise e exposição ao plasminogênio (PONTECORVI et al., 2019), entre outras formas. A imunomarcação da proteína acídica fibrilar glial (GFAP) (que marca a presença do principal filamento dos astrócitos), é utilizada com frequência para detectar astrócitos reativos em modelos animais de patologias neurológicas (BURDA; SOFRONIEW, 2014; CEYZÉRIAT et al., 2018). Diversas doenças neurodegenerativas (BOOTH; HIRST; WADE- MARTINS, 2017; KHAKH et al., 2017; SIMPSON et al., 2010), infecções virais (PORTIS et al., 1995; VITKOVIC; DA CUNHA, 1995), doenças desmielinizantes inflamatórias (OHAMA et al., 1990), e danos cerebrais traumáticos (CERVÓS- NAVARRO; LAFUENTE, 1991) apresentam astrogliose reativa. Quando ativos, os astrócitos passam a secretar diversas citocinas pró-inflamatórias, como TNF-α, IL-1β, e quimiocina ligante 2 (CCL2)(PONTECORVI et al., 2019). Quando os astrócitos sofremdiferenciação para a forma reativa (após ativação) eles perdem suas habilidades benéficas e começam a expressar funções neurotóxicas, ativando sinalização que promove morte neuronal (LIDDELOW et al., 2017).

1.4.1 Neuroinflamação em indivíduos autistas

A neuroinflamação é um evento frequente entre indivíduos com TEA (TETREAULT

et al., 2012; VARGAS et al., 2005), e pode ter importante relevância no desencadeamento desta desordem. O cérebro é extremamente sensível a perturbações, e a presença da neuroinflamação em períodos críticos, como a vida intrauterina, pode gerar insultos com consequências deletérias relevantes no neurodesenvolvimento (GARAY; MCALLISTER, 2010; BILBO; TSANG, 2010).

A ativação de microglia e astrócitos foram observados em amostras post-mortem de cerebelo, giro cingulado, córtex, córtex pré-frontal, córtex pré- frontal medial, e hipocampo de indivíduos autistas (LAURENCE; FATEMI, 2005; MORGAN et al., 2010;VARGAS et al., 2005; TETREAULT et al., 2012; KERR et al., 2016). A alta expressão de citocinas, como a proteína quimiotática de monócitos 1 (MCP-1), interleucina-6 (IL- 6), interleucina 17 (IL-17), e fator de necrose tumoral alfa (TNF-α) foram observadas em amostras cerebrais de humanos e em modelos animais de autismo (FREYA; MANNELLAB, 2000; FRITHIOFF-BØJSØE et al., 2020; ITO et al., 2008; OHTOMO; IWATA; ARAI,2018; T.C.; D.; L., 2009; VARGAS et al., 2005; WEI et al., 2011). Análises post-mortem em cérebros de indivíduos autistas mostraram a ativação de astrócitos no cerebelo, córtex frontal medial e parietal, giro cingulado, córtex frontal e cerebelar (LAURENCE; FATEMI, 2005; VARGASet al., 2005). Astrogliose e microgliose na região do cerebelo foi intimamenteassociada com a presença de macrófagos e monócitos, e com a degeneração de células granulares, Purkinje e axônios (VARGAS et al., 2005). Altos níveis decitocinas pró-inflamatórias IL-6, IL-10, MCP-3, eotaxin, eotaxin2, MCP-1, citocina derivada de macrófago (MDC), quimiocina β8, peptídeo ativador de neutrófilo (NAP-2), linfócito quimioatraente-β (BLC), leptina e osteoprotegerina também foram encontrados no giro cingulado anterior (ACG) (VARGAS et al., 2005). A citocina anti-inflamatória TGF-β1 foi encontrada em altos níveis em tecidos de cerebelo e giro cingulado anterior de indivíduos autistas, e a ativação deastrócitos foi fortemente associada com o aumento nos níveis de IL-6 e MCP-1no cerebelo, giro cingulado anterior e giro frontal medial (VARGAS et al., 2005). Análises no líquido cefalorraquidiano (LCR) mostrou a presença de astrogliose reativa no SNC de crianças com autismo (ROSENGREN et al., 1992).Análises neste líquido em autistas também mostraram aumento nos níveis de citocinas pró-inflamatórias como IL- 6, IFN-γ, IL-8, proteína inflamatória de macrófago 1β (MIP1 β), NAP-2, proteína indutora de interferon- γ 10 (IP-10), e angiogenina, bem como do fator indutor de mesoderme (MIF), fator decrescimento endotelial vascular (VEGF), PARC, FGF-4, FGF-9, IGFBP3, IGFBP4 (VARGAS et al., 2005) e TNF-α (CHEZ et al., 2007).

Uma acentuada microgliose foi encontrada em amostras post-mortem de cerebelo de indivíduos autistas (VARGAS et al., 2005), e no córtex frontal a microgliose foi acompanhada

por um aumento na densidade e no volume microglial somal, com a massacerebral total negativamente associada com a densidade microglial no córtex pré-frontal (MORGAN et al., 2010).

O aumento na densidade microglial em indivíduos autistas foi relatado simultaneamentecom ativação microglial na região do córtex (TETREAULT et al., 2012).

Resultados contraditórios também foram encontrados, como os relatados por Fatemi e colaboradores, onde em análises post-mortem em cérebros de indivíduos autistas uma significante diminuição nos marcadores de ativação de astrócitos foi encontrada, emborasimultaneamente a um significativo aumentona astrogliose reativa no córtex frontal (FATEMI et al., 2008).

Modelos de roedores com indução da ativação do sistema imune no período gestacional, mostraram a ativação de linfócitos Th17, com alta expressão de citocinas pró-inflamatórias (IL17, IL6, IL1β, TNF-α) (CHOI et al., 2016). Essa ativação imune materna induziu processos neuroinflamatórios na prole e o aumento significativo do risco para o desenvolvimento de comportamentos tipo autista nos filhos (CHOI et al., 2016).

Portanto, estudos em humanos e modelos animais mostram a associaçãode perturbações na gestação, como a ativação imune materna, e a presença de neuroinflamação na prole. Há também a notável incidência de neuroinflamação em indivíduos autistas (SUZUKI etal., 2013; VARGAS et al., 2005), sugerindo que possa haver uma estreita relação entre o sistema imunitário e o desenvolvimento e/ou severidade do TEA. Mecanismos neuroinflamatórios com alta expressão de citocinas podem estar por trás do neurodesenvolvimento anormal, como sugerem Deverman e Patterson (DEVERMAN; PATTERSON, 2009). Com base nisso, é provável que a neuroinflamação esteja também presente no neurodesenvolvimento dos autistas, período que está situado majoritariamente na vida intrauterina e quando o sistema nervoso central é extremamente sensível a insultos (*figura 9)*. Uma melhor compreensão da influência da neuroinflamação e de seus principais atores no período do neurodesenvolvimento pode ajudar a esclarecer a etiologia e/ou sintomas do TEA.

1.5 MITOCÔNDRIA

A mitocôndria é composta por duas membranas (interna e externa), matriz mitocondrial, e cristas mitocondriais (*figura 2*). A membrana externa é permeável a pequenas moléculas e íons que circulam no citoplasma celular, através de canais transmembanares formados por proteínas integrais de membranas chamadas porinas. A membrana interna é praticamente impermeávele seletiva a maioria de moléculas e íons, incluindo prótons (H^+). Na

membrana interna estão localizadas as cristas mitocondriais, que são sequenciais dobramentos desta membrana onde estão localizados os cinco complexos mitocondriais (nomeados de I a V). São nestes complexos mitocondriais que ocorre o transporte de elétrons oriundos de processos metabólicos celulares que fornecem energia para a fosforilação do ADP em ATP. Encontram-se presentes na matriz mitocondrial as enzimas da β-oxidação de ácidos graxos, enzimas da oxidação de aminoácidos, enzimas do ciclo do ácido cítrico, o complexo piruvato desidrogenase, DNA mitocondrial, ribossomos, ADP, ATP, Pi e diversos intermediários inorgânicos (NELSON; COX, 2014).

Além de ser a mais importante fonte de ATP celular (molécula altamenteenergética), as mitocôndrias também são responsáveis por importantes funções celulares, como a homeostase do cálcio (Ca^{2+}) e a regulação da apoptose (KOWALTOWSKI et al., 2019; LEE et al., 2016).

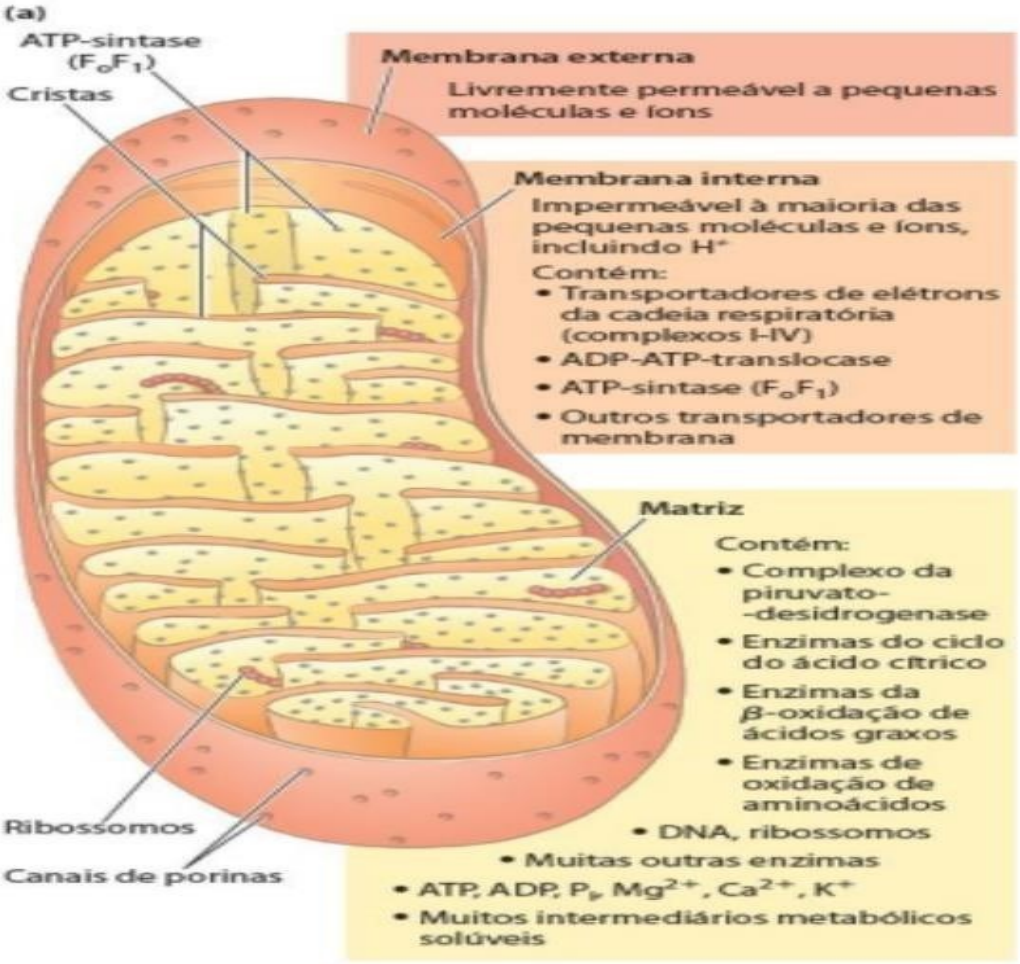

Figura 2. As estruturas mitocondriais (NELSON; COX, 2014).

O transporte de elétrons nos complexos mitocondriais, mecanismo denominado cadeia

transportadora de elétrons (CTE) (*figura 3*), é abastecido poraceptores universais de elétrons, nucleotídeos de nicotinamida (NAD^+ ou $NADP^+$) ou nucleotídeos de flavina (FMN ou FAD), que captam elétrons de vias catabólicas e os entregam para as desidrogenases dos complexos I e II (NELSON;COX, 2014). O complexo I, também chamado de NADH desidrogenase, catalisa a transferência de elétrons da coenzima NADH para a ubiquinona (UQ), forma oxidada da coenzima Q. O complexo II, também chamado de succinato desidrogenase, possui quatro subunidades proteicas, e é completamente codificado pelo DNA nuclear sem necessitar do DNA mitocondrial. Este complexo envolve a participação de um FAD ligado à succinato desidrogenase, dois centros ferro-enxofre, e um citocromo b650 (DI DONATO, 2000). Os elétrons da coenzima FADH2 (forma reduzida) são transferidos para a UQ. Em seguida, a ubiquinona transfere os elétrons dos complexos I e II para o complexo III, denominado citocromo c oxidorredutase. O complexo III apresenta onze subunidades, três das quais apresentam centros-redox para a transferência de elétrons. Estas três subunidades estão representadas pelo citocromo b, um citocromo c1, e um centro ferro-enxofre (SARASTE, 1990). A ubiquinona é então desprotonada a semiquinona (UQH•), sendo os elétrons transferidos do complexo III para o citocromo c, que flui no espaço intermembranas e cede os elétrons para o complexo IV (citocromo C oxidase), que possui quatorze subunidades. Estes elétrons são atraídos e entregues então ao oxigênio, formando H_2O (BIOENERGETICS 3 NICHOLLS, D. G., AND FERGUSON, S. J., ACADEMIC PRESS, LONDON, 2002).

Assim que os elétrons são transferidos do complexo I para a UQ, quatro H^+ sãobombeados para o espaço intermembranas. Este mecanismo se repete no complexo três, com quatro H^+ bombeados para o espaço intermembranas, seguido do bombeamento de apenas $2H^+$ do complexo IV para o espaço intermembranas quando os elétrons são transferidos da citocromo c oxidase parao oxigênio (NELSON; COX, 2014).

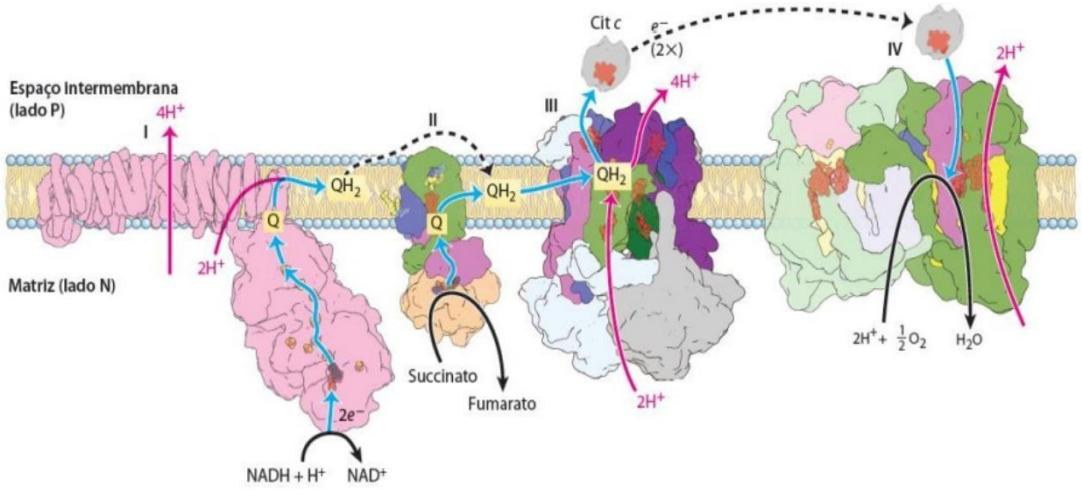

Figura 3. Os complexos mitocondriais. Os complexos I e III recebem elétrons e bombeiam para o espaço intermembranas 4 H^+, enquanto o complexo IV bombeia apenas $2H^+$. As coenzimas Q transportam os elétrons na membrana mitocondrial interna entre os complexosI e III, e a citocromo C carrega os elétronsentre os complexos III e IV (NELSON; COX, 2014).

1.5.1 Espécies reativas de oxigênio

As mitocôndrias são as maiores geradoras de espécies reativas de oxigênio (EROs), que são subprodutos da cadeia transportadora de elétrons (CTE), e síntese de ATP. As EROssão moléculas com radicais livres (elétrons desemparelhados no orbital de valência) nos átomos de oxigênio, que em excesso causam danos nos componentes celulares devido à avidez por estabilidade(emparelhamento dos elétrons no orbital de valência) (TURRENS, 2003). As EROs são provenientes em sua maioria da produção de energia apartir da glicose (BECERRA DEPABLOS; AREVALO GONZÁLEZ, 2000), e podem desencadear quadros neuroinflamatórios quando geradas em grandes quantidades (ISHIHARA et al., 2015). A formação de EROs acontece quando elétrons são transportados na cadeia respiratória para moléculas de oxigênio presentes na matriz, formando o superóxido ($O2^{\bullet -}$), o qual é considerado o precursor das demais EROS formadas nas células (ORRENIUS; GOGVADZE; ZHIVOTOVSKY, 2007). A grande maioria das EROs formadas pelas mitocôndrias se originam dos complexos I e III (*figura3*) da CTE (ORRENIUS;GOGVADZE; ZHIVOTOVSKY, 2007). Em condições fisiológicas normais, as EROs estão envolvidas com o metabolismo energético, fagocitose, sinalização celular, sobrevivência celular, crescimento celular, defesa e imunidade. Porém, quando em excesso, apresentam efeitos tóxicos como a peroxidação de

lipídios, danos em proteínas, membranas, carboidratos, DNA mitocondrial e DNA nuclear (MERTENS et al., 1995; TANG et al., 2007; CASTILHO et al., 1994; AMIN; BANO, 2018; LUNA; ESTÉVEZ, 2018; MÄKELÄ et al., 2017; URSAN; ODNOSHIVKINA; PETROV, 2020).

As EROs mais comuns são a hidroxila (HO•), o superóxido ($O_2^{\bullet -}$), a peroxila (ROO2•) a alcoxila (ROO•), o oxigênio singleto ($^1O_2$), o peróxido de hidrogênio (H_2O_2), e o ácido hipocloroso (HClO) (BARREIROS; DAVID; DAVID, 2006). Cerca de 1 a 2% do oxigênio total consumido pelas mitocôndrias são convertidos a superóxido ($O_2^{\bullet -}$) (CADENAS; DAVIES, 2000). O radical HO• é o mais tóxico ao organismo, uma vez quesua meia-vidaé muito curta e dificilmente pode ser sequestrado pelas defesas antioxidantes devido à sua alta reatividade. No DNA, por exemplo, as hidroxilas atacam tanto as bases nitrogenadas quanto a desoxirribose (*figura 4*). O ataque ao açúcar pode ser realizado por abstração de um dos átomos de hidrogênio, o que geralmente leva a ruptura da cadeia de DNA (HALLIWELL, 1999).

Já o peróxido de hidrogênio (H_2O_2) é gerado *in vivo* pela dismutação do ânion radical superóxido($O_2^{\bullet -}$) por enzimas oxidasesou pela β-oxidação de ácidos graxos.

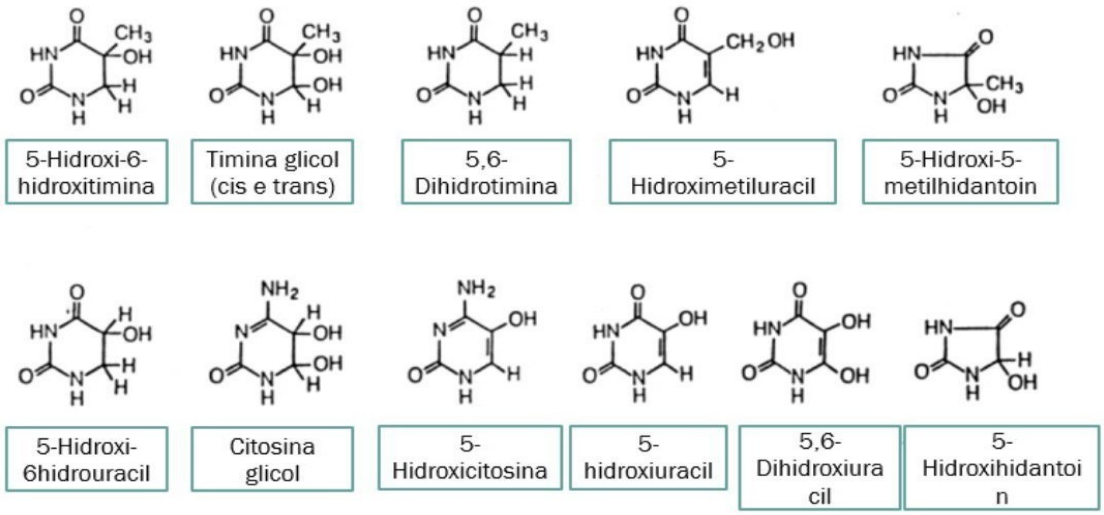

Figura 4. Estruturas químicas de alguns produtos de oxidação do DNA. Ataques por moléculas de hidroxila(HO•) (HALLIWELL, 1999).

Para combater e minimizar estes efeitos deletérios da oxidação causada por altos níveis de EROs, as células apresentam defesas antioxidantes, que são produzidas pelo corpo ouingeridas da dieta (BARREIROS; DAVID; DAVID, 2006). O desequilíbrio, onde as defesas

antioxidantes celulares são insuficientesfrente ao montante gerado de EROs, é chamado de estresse oxidativo. Um exemplo de defesa, são as enzimas superóxido dismutase (SOD), que convertem o $O2^{\bullet -}$ em peróxido de hidrogênio ($H2O2$). O $H2O2$ é uma espécie muito menos reativa, sendo eliminada em grande parte por enzimas antioxidantes como catalases e glutationas peroxidases (*figura 5*) (BARREIROS; DAVID; DAVID, 2006). As glutationas formam um grupo antioxidante, apresentando representantes como a glutationa (GSH), glutationas peroxidases (GSH-PX) e glutationas redutases (GR). A glutationa peroxidase apresenta selênio em seu domínio de cisteína, sendo este elemento essencial para o seu funcionamento enzimático (ROTRUCK et al., 1973). A glutationa redutase (GR) não age diretamente sobre radicais livres, e sim reduzindo as glutationas oxidadas (GSSG) em suas formas reduzidas novamente (GSH), utilizando NADPH (*figura5*) (MEISTER; ANDERSON, 1983).

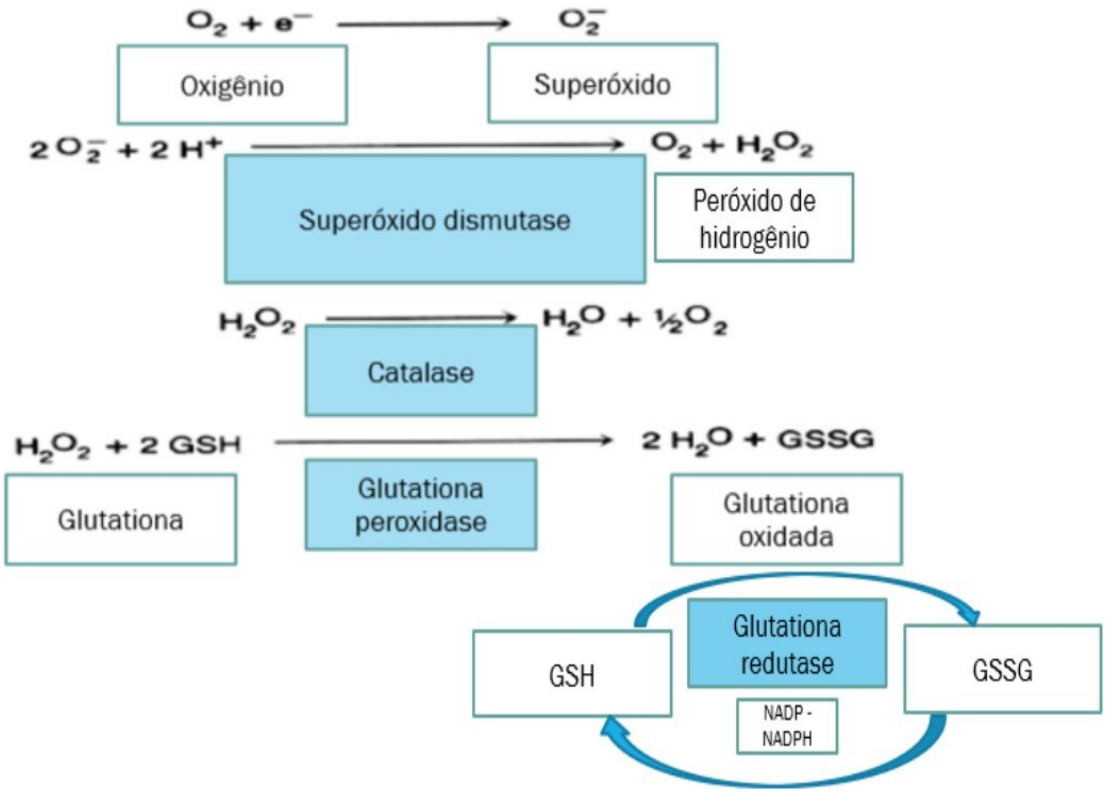

Figura 5. Modelo esquemático simplificado das enzimas antioxidantes SOD, catalase e glutationa (peroxidase e redutase). Tais enzimas metabolizam as espécies reativas de oxigênio, minimizando os efeitos deletérios que essas moléculas apresentam a nível mitocondrial e celularquando geradas em grandes quantidades. A glutationa redutase não age diretamente nas EROs, mas tem papel antioxidante reduzindoa GSSG à sua forma ativa, utilizando NADPH.

1.5.2 Disfunção mitocondrial

Em condições fisiológicas normais, a atividade mitocondrial gera EROs, que são importantes na sinalização de moléculas e na fisiologia celular, mas que em excesso geram toxicidade celular com o desbalanço redox (estresse oxidativo), característico da disfunção mitocondrial.

A primeira evidência de disfunção mitocondrial foi observada porErnster, em 1959, quando observou mitocôndrias de células musculares esqueléticas de indivíduos com taxas metabólicas basais muito elevadas (150 a 170%) (ERNSTER; IKKOS; LUFT, 1959). Disfunção mitocondrial resulta em diminuição na geração de ATP (ZHANG et al.,2019), com déficit na atividade dos complexos da cadeia respiratória (CHAUHAN et al., 2011), aumento na geração de EROs e na oxidação de componentes mitocondriais e celulares, os danificando (CHERAGHI et al., 2019).

O envelhecimento também é um importante fator para a disfunção mitocondrial, uma vezque causa redução na biogênese mitocondrial, que é uma etapa de renovação dasmitocôndrias, e redução também na mitofagia, etapa que elimina proteínas mitocondriais mutantes e com modificações estruturais, asquais prejudicam suas funções (CHISTIAKOV et al., 2014).

As membranas mitocondriais e o mtDNA são alvos recorrentes da ação oxidativa de EROs devido às suas proximidades com os sítios de geração de EROs e de fatores como o elevado conteúdo de ácidos graxos poliinsaturados nasmembranas mitocondriais (alvoscomuns de EROs) e a ausência de histonas no mtDNA. Além disso, a quantidade de mutações no mtDNA é maior em comparação ao DNA nuclear também devido ao menor número de mecanismos eficientes de reparo nas mitocôndrias (MANDAVILLI; SANTOS; VAN HOUTEN, 2002).

A exata razão pela qual ocorre a disfunção mitocondrial permanece desconhecida. Porém, alguns fatores estão fortemente associados com a disfunção mitocondrial, como obesidade (MA et al., 2014), dieta hipercalórica (GAO et al.,2010), raios UV (SU et al., 2018), xenobióticos (NIRANJAN; BHAT; AVADHANI, 1982), entre outros. Estes fatores, estão intimamente associados com mutações no mtDNA (TANG et al., 2007) ou DNA nuclear (DORISON et al., 2020), mutações em proteínas mitocondriais (CHEN et al., 2012), aumento de EROs e danos oxidativos em proteínas (AMIN; BANO, 2018; LUNA; ESTÉVEZ, 2018), carboidratos (MÄKELÄ et al., 2017), lipídios e membranas (CASTILHO et al., 1994; URSAN; ODNOSHIVKINA; PETROV, 2020), os quais levam a alterações na fisiologia celular. As principais disfunções mitocondriais estão relacionadas com a desregulação na

homeostase do cálcio (Ca^{2+}) (importante fator na geração do potencial de membrana) (KOWALTOWSKI et al., 2019), déficit energético (N.J. et al., 1993), aumento de EROs (estresse oxidativo) e apoptose (*figura 6*) (RIZWANet al., 2020).

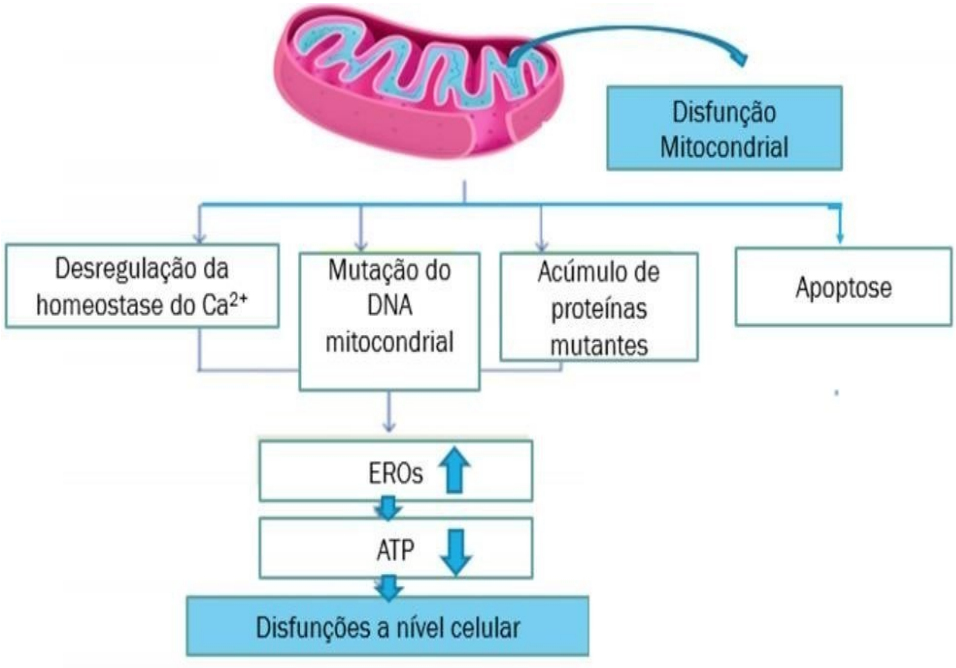

Figura 6. Esquema simplificado de disfunção mitocondrial. As condições mitocondriais como a desregulação da homeostase do cálcio, mutações no mtDNA e acúmulo deproteínas mutantes, levam a um aumento de EROs (estresse oxidativo) e déficit no aporte energético celular. Em conjunto, estes fatores podem levar a disfunções na fisiologia celular e apoptose celular.

Portanto, a disfunção mitocondrial apresenta como importantes características a diminuição na oferta de ATP, aumento de apoptose celular, e a geração excessiva de EROs. As EROS são oriundas principalmente da metabolização da glicose (principalmente quando há excesso de ingesta calórica)(BECERRA DEPABLOS; AREVALO GONZÁLEZ, 2000), e são substâncias

com alto potencial para causarem danos em estruturas e moléculas celulares, intimamente associadas a condições de obesidade (MA et al., 2014), e que podem desencadear quadros neuroinflamatórios (ISHIHARA et al., 2015).

1.5.3 Disfunção mitocondrial no cérebro de autistas

Perturbações nos sistemas mitocondriais desencadeiam diversos efeitos negativos, muitas vezes resultando em disfunções celulares. O cérebro é um tecido com altíssima demanda energética, e a presença de disfunção mitocondrial é crítica em seu funcionamento, estando associada a diversas doenças neurológicas e neurodegenerativas (CAMPUZANOet al., 1997; MORAIS et al., 2009; PEDRÓSet al., 2014).

A disfunção mitocondrial também está presente no cérebro de indivíduos autistas, e têm sido sugerida como tendo um papel na etiopatologia do autismo (GIULIVI et al., 2010). Foram relatadas alterações no metabolismo bioenergético, em proteínas implicadas na dinâmica mitocondrial, em proteínas dos complexos respiratórios mitocondriais, bem como em proteínas antioxidantes em amostras post-mortem de cerebelo, córtex pré- frontal, lobo frontal, e córtex temporal, parietal e occipital de indivíduos autistas (N.J. etal., 1993; CHUGANI et al., 1999; GU et al., 2013).

Evidências sugerem que a disfunção mitocondrial e o estresse oxidativopodem afetar o aporte energético do cérebro em desenvolvimento, e ativar uma cascata de eventos que podem contribuir com a etiologia do autismo (ROSE et al., 2012; CHAUHAN et al., 2011;CHAUHAN; AUDHYA; CHAUHAN, 2012).

A disfunção mitocondrial e a anormal bioenergética em indivíduos comautismo foi relatada há mais de 30 anos atrás, por Coleman e Blass (COLEMAN;BLASS, 1985). Depois disso, muitos estudos com indivíduos autistas também relataram a presença de disfunção mitocondrial (BENNURI; ROSE; FRYE, 2019; D.A.; R.E., 2012; GIULIVI etal., 2010; GOLDENTHAL et al., 2015; LEGIDO; JETHVA; GOLDENTHAL, 2013; YUI; SATO; IMATAKA, 2015).

Um estudo baseado num grande número amostral estimou que a disfunção mitocondrial pode estar presente em mais de 80% das crianças com autismo (GIULIVI et al., 2010). Outros autores reportam a prevalência dadisfunção mitocondrial em crianças com autismo, variando de 30 (ROSSIGNOL;FRYE, 2012) a até mais de 50% (FRYE, 2012). Estudos de espectroscopia de ressonância magnética têm mostrado alterações nos metabólitos relacionados à bioenergética no cérebro de pacientes com autismo (N.J. et al., 1993). O córtex frontal de indivíduos com autismo apresenta um estado energético hipermetabólico, o qual pode ser relacionado com anormalidades neurofisiológicas e neuropatológicas no autismo (N.J. et al., 1993). Foi observadauma redução nos níveis defosfocreatina no córtex pré-frontal de cérebros de autistas, indicando o alto uso deste composto para a geração de ATP, sugerindo falha na

geração de energia nos mecanismosda respiração mitocondrial (N.J. et al., 1993). Indivíduos com autismo apresentaram baixos níveis de N-acetil- aspartato no cerebelo, e altos níveis de lactato no plasma, que éconsistente com alterações metabólicas encontradas em crianças com autismo (CHUGANI et al.,1999). Uma revisão sistemática com meta-análise de espectroscopia de ressonância magnética de prótons (1H-MRS) em pacientes com autismo revelou a diminuição na concentração de N-acetil-aspartato na matéria cinza e matéria branca docérebro (IPSER et al., 2012). Altos níveis de creatina foram observados na matéria cinza de indivíduos com autismo, com aumento específico no lobo temporal (IPSER et al., 2012). Foi também observada a diminuição nos níveis decreatina no lobo occipital de crianças com autismo (IPSER et al., 2012). Não foram encontradas diferenças para N- acetil-aspartato ou creatina nos lobos frontais (IPSER et al., 2012).

Foi observado que as atividades dos complexos da cadeia transportadora de elétrons da mitocôndria, bem como a atividade da enzima piruvato desidrogenase (determinada pelaredução de NAD^+ a NADH) ficou reduzida no córtex frontal post-mortem de indivíduos com autismo (GU et al., 2013). Em crianças com autismo, foi observada a redução nos níveis de proteína doscomplexos III e V no cerebelo, do complexo I no córtex frontal, e dos complexos II, III e V no córtex temporal, enquanto nenhum dos cinco complexos da CTE ficou alterado nos córtices parietal e occipital (CHAUHAN et al., 2011). Estes dados sugerem que a expressão de complexos da cadeia transportadora de elétrons está diminuída nas regiões cerebrais frontal, temporal e no cerebelo de crianças com autismo, podendo levar a um metabolismo energético deficitário nestas regiões.

Foi também demonstrado um alto nível de estresse oxidativo no lobo temporal e área de Brodmann de indivíduos com autismo (PALMIERI et al., 2010). A expressão do carreador mitocondrial aspartato/glutamato e a atividade da citocromo c oxidase estavam ambas aumentadas em pacientes autistas, indicando uma ativação do metabolismo mitocondrial (PALMIERI et al., 2010).

Foi observado em crianças com autismo um aumento no mtDNA para os genes ND1, ND4 e CytB (que codificam subunidades dos complexos I e III).A taxa ND4/ND1 apresentou uma significante diminuição em 44% do grupo autista, e a taxa CytB/ND1 apresentou uma significante diminuição em 33% do grupo autista, mostrando deleções genéticas em indivíduos com autismo (GU etal., 2013).

Aumentos significativos nos radicais livres e nos níveis de hidroperóxidos lipídicos (resultantes da oxidação de ácidos graxos) foram detectados nos lobos parietal, occipital, no córtex frontal e no cerebelo de indivíduos com autismo (CHAUHAN et al., 2011).

Alterações em enzimas importantes para o metabolismo redox também têm sido encontradas em indivíduos com autismo. Glutationa peroxidase, Glutationa-S-transferase e glutamato cisteína ligase têm sido encontradas em concentrações diminuídas no córtex,na área de Brodmann 22 e no cerebelo de indivíduos com autismo (CHAUHAN; AUDHYA; CHAUHAN, 2012; ROSE etal., 2012). As atividades das enzimas superóxido dismutase (SOD) e superóxido dismutase 2 (SOD2) foram encontradas diminuídas e alto nível de estresse oxidativo foi observado no lobo temporal de indivíduoscom autismo (TANG et al., 2013).

Portanto, diversos estudos apontam para a presença de disfunção mitocondrial em diversas áreas do cérebro de indivíduos autistas (PALMIERI et al., 2010; GU et al., 2013;CHAUHAN et al., 2011). Estas alterações podem ser de alta relevância no neurodesenvolvimento, período no qual perturbações podem ter graves consequências a longo prazo em caráter morfofisiológico e neurológico. Evidências de disfunções mitocondriais no cérebro de indivíduos com TEA (*figura 7*) (PALMIERI et al., 2010; GUet al., 2013; CHAUHAN et al.,2011) colocam o foco para a importância de mais estudos sobre a disfunção mitocondrial no neurodesenvolvimento (vida intrauterina), período considerado chave no desencadeamento do TEA. Maiores estudos neste parâmetro serãode grande importância para tentar esclarecer as possíveis associações da disfunção mitocondrial na morfologia e fisiologia do cérebro em desenvolvimento, bem como a associação da disfunção mitocondrial cerebral com a etiologia, sintomas e severidade do autismo.

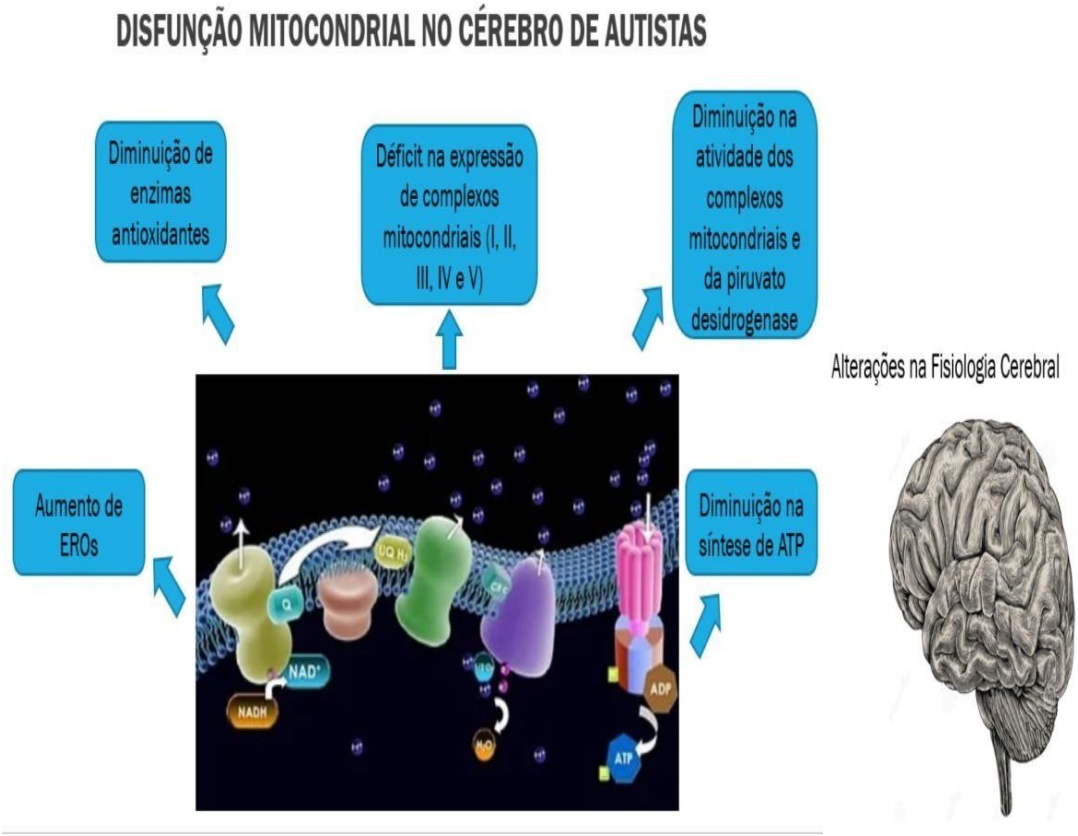

Figura 7. Disfunção mitocondrial associada a alterações na fisiologia cerebral no cérebro de autistas.

1.6 OBESIDADE

Obesidade é definida como um acúmulo anormal e excessivo de tecidoadiposo, que resulta de um balanço energético positivo continuado (WORLD, 2016). A obesidade é um fator de risco para muitas doenças, incluindo não apenasdoenças cardiovasculares e metabólicas, mas também doenças neurológicas e psiquiátricas (WORLD, 2016). A Organização Mundial de Saúde considera a obesidade como o maior problema de saúde pública do mundo devido às comorbidades associadas, cunhando o termo “globesidade” (DATA, 2011). A obesidade é classificada de acordo com o índice de massa corporal (IMC), uma razão entre o peso corporal (quilogramas) e a altura ao quadrado (metros). OIMC classifica o peso corporal em categorias de peso, como mostra a tabela (7) a seguir:

Abaixo do Peso	Peso Normal	Sobrepeso	Obesidade Classe 1	Obesidade Classe 2	Obesidade Classe 3
IMC < 18.5kg/m²	**18.5kg/m² ≤ IMC ≤ 24.9kg/m²**	**25kg/m² ≤ IMC ≤ 29.9kg/m²**	**30kg/m² ≤ IMC ≤ 34.9kg/m²**	**35kg/m² ≤ IMC ≤39.9kg/m²**	**IMC ≥ 40**

Tabela 7. Classificação do Índice de Massa Corporal (IMC) (WORLD, 2016).

Em 2016, foi estimado que mais de 1.9 bilhões de pessoas acima de 18 anos apresentavam sobrepeso, e destes, 650 milhões estavam obesos (WORLD, 2016). Ainda de acordo coma OMS, foi estimado que em 2019 cerca de 38 milhões de crianças estavam com sobrepeso ou obesidade. De acordo com o IBGE (Instituto Brasileiro de Geografia e Estatística), o Brasil atualmente contacom 60,3% da população adulta acima do peso (IMC ≥ 25).

A obesidade é considerada uma doença crônica de baixo grau inflamatório. O aumento do tecido adiposo ocorre tanto em número quanto no tamanho das células, com desregulação em sua função secretória e hormonal (JOet al., 2009). O aumento em adipócitos viscerais é fortemente associado com danos no metabolismo da glicose, hiperinsulinemia e resistência à insulina(*figura 8*) (HOFFSTEDT et al., 2010). Esse aumento no volume do tecido adiposo induz a liberação de citocinas pró-inflamatórias, gerando um estado inflamatório crônico responsável por alterações metabólicas (*figura 8*) (MCARDLE et al., 2013; STRASSER, 2017). Simultaneamente à expansão do tecido adiposo, ocorre o recrutamento e a infiltração de macrófagos, que são ativados liberandocitocinas pró-inflamatórias, as quais induzem a inflamação crônica (*figura 8*) (HAN et al., 2020).

A ativação dos macrófagos ocorre através de dois estados de polarização: M1 e M2, os quais dependem pricnipalmente de citocinas e das proteínas expressas nas membranas (VOGEL et al., 2014). A maturação no estado M1 ocorre através da exposição dos macrófagos a citocinas Th1, como IFN-γ, IL-2 e também LPS (GOERDT et al., 1999). Estes macrófagos M1 secretam citocinas pró-inflamatórias, sendo as principais: IL-6, TNF-α e IL-1β (GOERDT et al., 1999). A polarização dos macrófagos no estado M2 ocorre principalmente através de sua exposição a citocinas como IL-4, GM-CSF e IL- 13(BRAUNE et al., 2017). Macrófagos M2 têm função de reparo tecidual e secretam citocinas anti-inflamatórias como IL-10 e IL-1RA, além de secretarem fator de crescimento transformador β (TGF-β) (BOURLIER et al.,

2012). O recrutamento dos macrófagos e sua polarização fenotípica tem um forte papel noestado inflamatório, alémde regular a sensibilidade à insulina (JIA; MORGAN- BATHKE; JENSEN, 2020), a angiogênese (PANG et al., 2008), e a adipogênese(LEE; PETKOVA; GRANNEMAN, 2013).

Um estudo com modelos roedores de obesidade induzidos por dieta hiperlipídica, apontou que os reguladores transcricionais mais expressos nestesanimais foram o IFN-γ, o TLR-4, e o TNF-α (BAE et al., 2021). Algumas rotas apresentaram destaque nestes animais expostos a dietas hiperlipídicas, como a sinalização de extravasamento de leucócitos, sinalização de células Natural Killer, maturação de células dendríticas, sinalização de IL-10, produção de óxidonítrico e EROs em macrófagos, sinalização I3K em linfócitos B, sinalização Toll-like Receptor, e sinalização de receptor de células B (BAE et al., 2021).Entre os tipos celulares mais expressos em animais com exposição a dieta hiperlipídica, estiveram os macrófagos, células dendríticas, Natural Killer, e neutrófilos, sugerindo que a inflamação via IFN-γ, uma das moléculas mais expressas durante obesidade induzida por dieta hiperlipídica, está envolvida na resposta imune inata principalmente através do imunometabolismo dos macrófagos (BAE et al., 2021). Há também notória participação hormonal associada ao imunometabolismo em quadros de obesidade (PALHINHA et al., 2019).

A leptina é um hormônio secretado pelos adipócitos e codificada pelo gene da obesidade (OB), que tem receptores amplamente expressos no sistema cardiovascular em células endoteliais (WADA et al., 2014). A leptina estáassociada à desregulação metabólica, podendo causar adipogênese em célulaspré-adipócitos de uma maneira mTOR dependente (PALHINHA et al., 2019), alémde promover inflamação com alta expressão de proteína C reativa (a qual foisignificativamente atenuada com inibição da via ERK1/2) (SINGH et al., 2007)e aumento na expressão de citocinas pró-inflamatórias TNF-α e IL-6 em célulasestromais derivadas de tecido adiposo e em pré-adipócitos (PALHINHA et al., 2019). Um estudo em roedores demonstrou que a lipólise induzida por agentes inflamatórios ocorre em diferente via que a lipólise oriunda de sinais adrenérgicos ou hormonais em adipócitos (FOLEY et al., 2021). Bloqueando a atividade da quinase da proteína 1 requisidora de Inositol (IRE-1), foi suficiente para bloquear a lipólise em células adiposasdesencadeada por sinais de múltiplos ligantes inflamatórios, como componentes bacterianos e vários tipos de citocinas(FOLEY et al., 2021). Além disso, a lipólise via IRE-1 nos adipócitos foi independente de alterações na concentração de insulina, sugerindo que o estado inflamatório induz lipólise mesmo em células resistentes à insulina (FOLEY et al., 2021). A inibição da atividade da IRE-1 também foi capaz de bloquear a ativação da via NF-κB e a secreção de IL-6, expondo também estes pontos regulatórios do

imunometabolismo obesogênico (FOLEY et al., 2021).

O aumento exponencial na prevalência da obesidade mundial e as inúmeras comorbidadesassociadas a esta condição, tornou a obesidade o maior problema de saúde pública no mundo (WORLD, 2016).

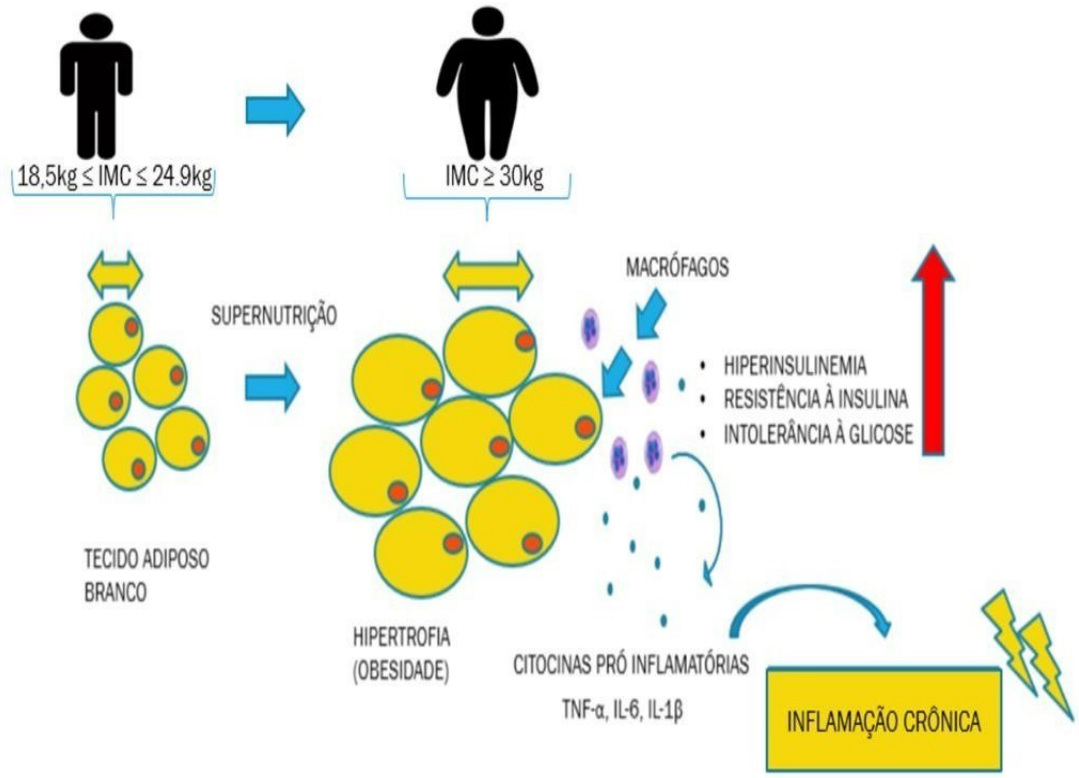

Figura 8. Obesidade e inflamação crônica. O excesso alimentar gera o aumento do tecido adiposo branco (obesidade) em tamanho e número de células. Quando isso ocorre, o tecidoadiposo branco sinaliza o recrutamento e ativação de macrófagos para o local, os quais secretam inúmeras citocinas pró inflamatórias,gerando um estado de inflamação crônica. Estas citocinas estão associadas à hiperinsulinemia, resistência à insulina e intolerância à glicose.

1.6.1 Obesidade materna

A obesidade e o sobrepeso na gravidez aumentaram significativamente,principalmente nas últimas três décadas, ganhando contornos pandêmicos e se tornando um dos principais fatores de risco evitáveis para diversos problemas de saúde (WORLD HEALTH ORGANIZATION, 2018). São preditores importantes para resultados adversos na saúde tanto em gestantes quanto na prole(WHO, 2015).

De acordo com a OMS, no ano de 2016 a média na Europa para obesidade (IMC ≥ 30) entre mulheres com 18 anos ou mais foi de 24.5% (WHO,2017). Ainda de acordo com a OMS,

países americanos apresentaram crescente alta nas prevalências para sobrepeso e obesidade em mulheres com 18 anos ou mais, obtendo médias de 30.4% na Costa Rica, 31% no Chile, 25.4% no Brasil, 32.8% no México, e 29% na Argentina no ano de 2016 (WHO, 2017).

De acordo com o Ministério da Saúde do Brasil, há poucos estudos sobrea prevalência do sobrepeso e obesidade no período gestacional. O mais recente, foi realizado entre os anos de 1991 e 1995, apontando prevalência de 19.2% para sobrepeso e 5.5% para obesidade (obesidade_sobrepeso_pre_gestacionais.pdf (saude.gov.br)).

No período entre 1990 e 2004, o Reino Unido aumentou em três vezes aprevalência parasobrepeso e obesidade no período pré-gestacional, apresentando18.9% das grávidas acima do peso (KANAGALINGAM et al., 2005). Portugal triplicou também a prevalência para mulheres com obesidade com 18 anos ou mais entre 1975 (6.8%) e 2016(21.2%) (WHO, 2017).

Muitos problemas durante a gestação e parto estão fortemente associados ao sobrepeso e obesidade da gestante, como a gravidez prolongada (gestações que atingem entre 40 e 42semanas) (USHA KIRAN et al., 2005), diabetes gestacional (YAO et al., 2020), complicações no parto (com aumento de internações, contrações irregulares ou ausentes) (ZHANG; TROENDLE, 2004), e aumento na incidência de cesariana (CHU et al., 2007). Entre as complicações pós-parto associadas às gestantes com sobrepeso ou obesidade, encontram-se ainda a pré-eclâmpsia (JÄÄSKELÄINEN et al., 2019), tromboembolismo (O'SHAUGHNESSY et al., 2019), infecção puerperal (OPØIEN et al., 2007), hipertensão (FERNÁNDEZ ALBA et al., 2018), problemas cardiovasculares (DIKAIOU et al., 2020), e hemorragia pós-parto (DALBYE et al., 2021). Em um estudo realizado no Brasil, mulheres com sobrepeso ou obesidade na gestação apresentaram 23% mais chances de desenvolverem depressão pós parto em comparação a mulheres com peso normal (FRAGA; THEME-FILHA, 2020).

Um estudo com meta-análise comparou gestações entre mulheres de peso normal com obesas, e mostrou que nas gestantes obesas as chances para anomalias congênitas estiveram aumentadas em 87% para defeitos no tubo neural, 224% para espinha bífida, 130% para defeitos cardiovasculares, 120% para anomalias no septo, 123% para fenda palatal, 120% para fenda lábio-palatal,148% para atresia ânus-retal, e 168% para hidrocefalia (STOTHARD et al., 2009). Outro estudo, analisou 1.2 milhões de filhos únicos de mães com diferentes classificações de peso, e mostrou que para quaisquer más formações congênitas, mães abaixo do peso e as de peso normal tiveram risco de 3.4%, com sobrepeso tiveram 3.5%, com obesidade classe I tiveram 3.8%, obesidade classeII tiveram 4.2%, e obesidade classe III tiveram 4.7% de risco. Entre as más formações congênitas observadas neste estudo, as mais frequentes foram a

cardíaca (1.6%), seguidapor órgão genital (0.5%), lábios (0.4%), sistema urinário(0.3%) e olhos (0.2%) (PERSSON et al., 2017).

A obesidade materna têm alto potencial para causar danos no sistema nervoso central daprole e causar déficits comportamentais (BILBO; TSANG, 2010). Crianças nascidas de gestações com sobrepeso ou obesidade materna apresentam maior risco para uma ou mais disfunções do neurodesenvolvimento, com incidência de 15% observada em um estudo realizado nos Estados Unidos (BITSKO et al., 2016). Além disso, sobrepeso e obesidadena gravidez estão relacionados com efeitos negativos na infância, como tristeza, medo, raiva (RODRIGUEZ, 2010), déficit em habilidades intelectuais (NEGGERS et al., 2003), dificuldades psicossociais (JO et al., 2015), transtorno do déficit deatenção e desordem de hiperatividade (RODRIGUEZ et al., 2008), atraso no desenvolvimento e problemas comportamentais (SANCHEZ et al., 2018). Sobrepeso e obesidade gestacional implicam em déficits neurológicos na prole, a qual apresenta disfunções cognitivas, comportamentais e emocionais, estando o autismo entre as desordens mais comuns associadas (SANCHEZ et al., 2018).

Krakowiac e colaboradores foram os primeiros a associarem as condições metabólicas dagestante que não apenas as restritas ao diabetes tipo 2 ediabetes gestacional, com o aumento do risco para o desenvolvimento de transtorno de espectro autista. Avaliaram também a influência da obesidade e hipertensão na gestação, e observaram o aumento do risco para autismo em filhosde mães obesas em 67% (KRAKOWIAK et al., 2012).

Um estudo com meta-análise, com base em 32 artigos incluindo regiões como Noruega, Holanda, Reino Unido, Dinamarca, Suécia, Austrália, e EstadosUnidos, encontrou associação significativamente positiva entre obesidade materna e o desenvolvimento do autismo (SANCHEZ et al., 2018). Este estudo, que contou com tamanho amostral variando de 62 a 333.057 crianças por estudo, mostrou que gestantes com sobrepeso ($25 \leq IMC < 30$) apresentaram 17% maior probabilidade de ter filhos com qualquer neurodesenvolvimento adverso em comparação a gestantes de peso normal (SANCHEZ et al., 2018). Esta probabilidade, aumentou para 51% quando analisou gestantes obesas ($IMC \geq 30$).Gestantes com sobrepeso ($25 \leq IMC < 30$) tiveram 10% mais chances de ter filhos autistas, e as gestantes obesas ($IMC \geq 30$) apresentaram 36% mais chances, ambas em comparação com gestantes de peso normal ($18.5 \leq IMC \leq 24.9$) (SANCHEZet al., 2018).

Em um estudo feito na Austrália entre 1989 e 1991, foram analisados os traços autistas nos filhos já adultos (entre 19 e 20 anos) de gestantes (VARCIN; NEWNHAM; WHITEHOUSE, 2019). As 2900 gestantes (com idades entre 16 e20 anos) foram agrupadas de

acordo com o IMC individual, que variou entre abaixo do peso, peso normal, sobrepeso, obesidade classe I e obesidade classe II. A avaliação dos traços autistas nos filhos destas gestantes foi feita utilizando o Quociente de Espectro Autista (AQ), um questionário que mensura os traços autistas na população geral. Através do questionário, o estudo apontou significativa associação do aumento do IMC das gestantes com a presença de traços autistas em seus filhos em idade adulta (VARCIN; NEWNHAM; WHITEHOUSE, 2019). Num outro estudo realizado na Finlândia, 23.747 gestantes que tiveram filhos entre 2004 e 2014, apresentavam no início da gravidez (entre as primeiras7 e 10 semanas) obesidade severa (IMC ≥ 35) (KONGet al., 2018). Elas tiveram chances aumentadas em mais de 6 vezes de ter filhos com autismo (6.49), em comparação a gestantes de peso normal (KONG et al., 2018).

O excessivo ganho de peso na gestação também tem se mostrado um fator de risco para o autismo. Um estudo chinês apontou associação do excesso deganho de peso gestacional com risco 32% maior de ter filhos com autismo (SHENet al., 2018). Este estudo utilizoucritérios pré-estabelecidos para classificar o ganho de peso gestacional normal de acordocom o respectivo IMC de cada gestante, sendo de 15 a 22kg para gestantes abaixo do peso, de 13 a 21kg para gestantes de peso normal, de 10 a 18kg para gestantes com sobrepeso, e de 9.5 a17kg para gestantes obesas. Ganhos de peso abaixo desses intervalos foram considerados insuficientes, e acima, excessivos. (SHEN et al., 2018).

Windham e colaboradores analisaram dois fatores, ganho de peso na gestação e IMC pré-gestacional (sobrepeso e obesidade materna), utilizando as diretrizes do Instituto de Medicina/Faculdade de Medicina de Obstetras e Ginecologistas para avaliar os ganhos de pesos individuais recomendados durante a gravidez. Ganhos de peso considerados normais para cada grupo de gestantes foram entre 12.6kg e 18.1kg para mulheres abaixodo peso (IMC < 18,5kg/m^2), entre 11.3kg e 15.8kg para mulheres de peso normal (18,5kg/m^2 ≤ IMC ≤ 24,9kg/m), entre 6.8kg e 11.3kg para mulheres com sobrepeso (25kg/m^2 ≤ IMC ≤ 29,9kg/m^2), e entre 4.9kg e 9kg para mulheres obesas (30kg/m^2 ≤ IMC) (COMMITTEE OPINION NO. 548: WEIGHT GAIN DURING PREGNANCY, 2013)**.** Entre gestantes com perfis ajustados para variáveis como idade, etnia, educação, paridade e fumo, o sobrepeso pré-gestacional foi associado com 25% mais chances de gerar filhos autistas que em gestantes de peso normal, e obesas pré-gestacionais tiveram 37% mais chances. Sem o ajuste para essas variáveis, o sobrepeso gestacional teve 40% maior probabilidade e a obesidade gestacional apresentou 72% maior probabilidade de gerar filhos com autismo (WINDHAM et al., 2019). Foi feita também a associação dicotômica para ganho de peso gestacional e grupos de IMC (normal, sobrepeso e obesidade) com a probabilidade de gerar filhos com autismo. Essa

dicotomia mostrou queasgestantes com sobrepeso e obesidade (IMC ≥ 25kg/m^2) mostraram maiores chances de terem filhos com autismo comparadas com as gestantes de IMC normal (18.5kg/m^2 ≤ IMC ≤ 24.9kg/m^2) (WINDHAM et al., 2019). As maiores probabilidades de gerar filhos com autismo estiveram presentes nas gestantes com sobrepeso ou obesas com ganhos depeso no intervalo de 15.8kg a 19.9kg e nas gestantes que ganharam acima de 19.9kg durante a gestação, com 117% e 90% de chances aumentadas respectivamente (WINDHAM et al., 2019). Foi observada também uma forte associação dos intervalos de ganho de peso gestacional de 15.8kg a 19.9kg e acima de 19.9kg, para filhos autistas sem disfunção intelectual, com probabilidades aumentadas em 162% e 200% respectivamente(WINDHAM et al., 2019). Além disso, foi também possível observar que a cada ganho de peso excedido em 2.25kg ao clinicamente recomendado, as chances de gerar filhos com autismo aumentavam em 6% (WINDHAM et al., 2019).

Em outro estudo com meta-análise, levantando dados de regiões como Estados Unidos (n= 83.201), Suécia (n= 333.057) e Noruega (n= 92.909), foi encontrada uma associação positiva entre sobrepeso e obesidade gestacional coma incidência de autismo nos filhos (WANG et al., 2016). O sobrepeso aumentouas chances de gerar filhos com autismo em 28%, e a obesidade em 36%. Foi encontrada também uma relação dose-resposta, onde cada incremento de 5kg/m^{2}no IMC materno aumentaram as chances da incidência do autismo nos filhos em16% (WANG et al., 2016).

Há também uma associação entre hiperglicemia intrauterina e o TEA, sendo que um estudo com meta-análise envolvendo 12 trabalhos de gestantes com diabetes mellitus, mostrou o aumento do risco em 50% para a presença do fenótipo autista nos filhos (XU et al., 2014).

A má nutrição na gravidez também é um fator que apresenta relação com o desenvolvimento do fenótipo tipo autista na prole, como pode ser observada em estudo com modelo roedor de gestação exposto à dieta hipoproteica (BATISTA; GIUSTI- PAIVA; VILELA, 2019). A prole de gestantes expostas à dieta hipoproteica na gestação apresentou na lactação menos comportamentos de vocalização, e na adolescência, danos no comportamento social e comportamentos estereotipados (BATISTA; GIUSTI- PAIVA; VILELA, 2019). Além disso, um estudo apontou que gestantes abaixo do peso (IMC < 18,5kg/m^2) apresentaram 43% maior probabilidade de terem filhos comautismo em relação a gestantes de peso normal (18,5 ≤ IMC < 24,95kg/m^2) (GETZ et al., 2016). Estes estudos mostram que há uma nítida associação do IMCmaterno (com destaque para o sobrepeso e obesidade), e também do ganho excessivo de peso gestacional e dahiperglicemia intrauterina com o aumento da probabilidade do desencadeamento dotranstorno de espectro autista nos filhos.

1.6.2 Obesidade materna e disfunção mitocondrial na prole

A disfunção mitocondrial é uma condição possível de ser herdada (transgeracional) em condições de estresse gestacional, como a obesidade materna e a alta ingesta calórica durante a maternidade (LETTIERI-BARBATO et al., 2017; MAREI et al., 2020; FEREY et al., 2019; SERAFIM et al., 2021).

A prole de camundongos (C57BL/6J) que recebeu dieta hipercalórica durante a gestação apresentou em células do tecido adiposo subcutâneo menor massa mitocondrial, com significante menor expressão de subunidades enzimáticas do ciclo do ácido cítrico e do mecanismo de fosforilação oxidativa (OxPHOS) (LETTIERI-BARBATO et al., 2017). Foi observada também nestes animais, a diminuição da enzima citrato sintase, bem como a diminuição na expressão de genes de mtDNA que codificam subunidades dos complexos I, IV e V, além da diminuição do consumo de oxigênio e da razão NAD^+/NADH (LETTIERI-BARBATO et al., 2017).

Camundongos com obesidade materna induzida por dieta hiperlipídica apresentaram disfunção mitocondrial nos oócitos, com anormalidades nas estruturas mitocondriais e alta concentração de espécies reativas de oxigênio (EROs) (MAREI et al., 2020). Foi observado também um aumento no conteúdo de mtDNA nas células, sugerindo falha na mitofagia (MAREI et al., 2020). Camundongos com obesidade induzida por dieta hiperlipídica e alto teor de sacarose tiveram filhotes com disfunção mitocondrial em células cardíacas (FEREY et al., 2019). Os oócitos destes animais apresentaram falhas na mitofagia e transmitiram mitocôndrias danificadas para seus filhotes (FEREY et al., 2019). As mitocôndrias cardíacas dos filhotes apresentaram diminuição no consumo de oxigênio (FEREY et al., 2019). Filhotes de ovelhas provenientes de mães obesas também apresentaram disfunção mitocondrial, com significativa diminuição nas atividades dos complexos mitocondriais I, II, III e IV em células hepáticas (SERAFIM et al., 2021).

Estes estudos (SERAFIM et al., 2021; FEREY et al., 2019; MAREI et al., 2020; LETTIERI-BARBATO et al., 2017) mostram que as condições metabólicas da gestante e a hiperglicemia intrauterina produzem efeitos deletérios na atividade mitocondrial de suas proles em tecidos periféricos e oócitos. É possível que disfunções mitocondriais sejam também encontradas no SNC da prole de mães obesas, e que estas possam ser correlacionadas com desordens neurológicas, como o TEA. Entretanto, mais estudos sobre a influência da obesidade materna no desencadeamento de disfunção mitocondrial no SNC da prole são necessários para estabelecer tal associação.

1.6.3 Obesidade materna e comportamentos tipo autista em modelos animais

Em um modelo animal (roedor) de disfunção metabólica gestacional induzido por alto consumo de frutose, foi possível observar mudanças morfofisiológicas e metabólicas nos animais gestantes e respectivas proles (ERBAS et al., 2018). Entre as mudanças, foram observados o fígado gorduroso nas gestantes, e o aumento significativo de colesterol e triglicerídeos no plasma de suas proles (ERBAS et al., 2018). Testes para análises de comportamentos tipo autistas na prole das gestantes expostas ao alto consumo de frutose na gestação, revelaram déficit na interação social e na atividade motora, e presença de comportamento estereotipado nos machos, sem prejuízos para as fêmeas (ERBAS et al.,2018). Entretanto, ambos os gêneros foram negativamente afetados em testes de aprendizagem e memória. Análises bioquímicas revelaram que ambos os gêneros também apresentaram altos níveis de TNF-α em relação aos controles, porém, apenas os filhotes machos apresentaram níveis diminuídos de fator de crescimento neuronal (NGF), neuregulin 1 (NRG1), ácido glutâmico descarboxilase 67 (GAD67), e ácido hidroxi indolacético (5HIAA) (ERBAS et al., 2018). A redução na densidade neuronal em neurônios piramidais (região CA1 do hipocampo) foi observada apenas em filhotes machos, embora uma importante astrogliose nas regiões CA1 e CA3 foi observada em ambos os gêneros (ERBAS et al., 2018). Estudos com roedores modelos de obesidade gestacional induzidopor dieta hipercalórica mostraram aumento do comportamento ansioso nesses animais através de testes clássicos de comportamento (BILBO; TSANG, 2010; TOZUKA et al., 2010; WHITE; PURPERA; MORRISON, 2009). Os animais também apresentaram expressão elevada de citocinas pró-inflamatórias no cérebro (BILBO; TSANG, 2010; WHITE; PURPERA; MORRISON, 2009) e alterações morfológicas nos neurônios do hipocampo, com diminuição no tamanho e número de dendritos (TOZUKA et al., 2010). A prole oriunda de gestantes com obesidade induzida por dieta hiperlipídica apresentou também ativaçãomicroglial e alta expressão deIL1β no hipocampo (BILBO; TSANG, 2010).

Estes estudos em modelos animais de obesidade gestacional e alta ingesta calórica (ERBAS et al., 2018;BILBO; TSANG, 2010; TOZUKA et al., 2010; WHITE; PURPERA; MORRISON, 2009), mostram que a obesidade e a hiperglicemia intrauterina são contextos de risco para o desencadeamento de neuroinflamação e de comportamentostipo autista na prole. É muito provável que o desencadeamento do TEA seja intermediadopor eventos neuroinflamatórios na prole ainda na vida intrauterina como evento de perturbação no neurodesenvolvimento, mediante estímulo da obesidade gestacional (*figura 9*) (ou outros

estímulos que possam perturbar a gestação).

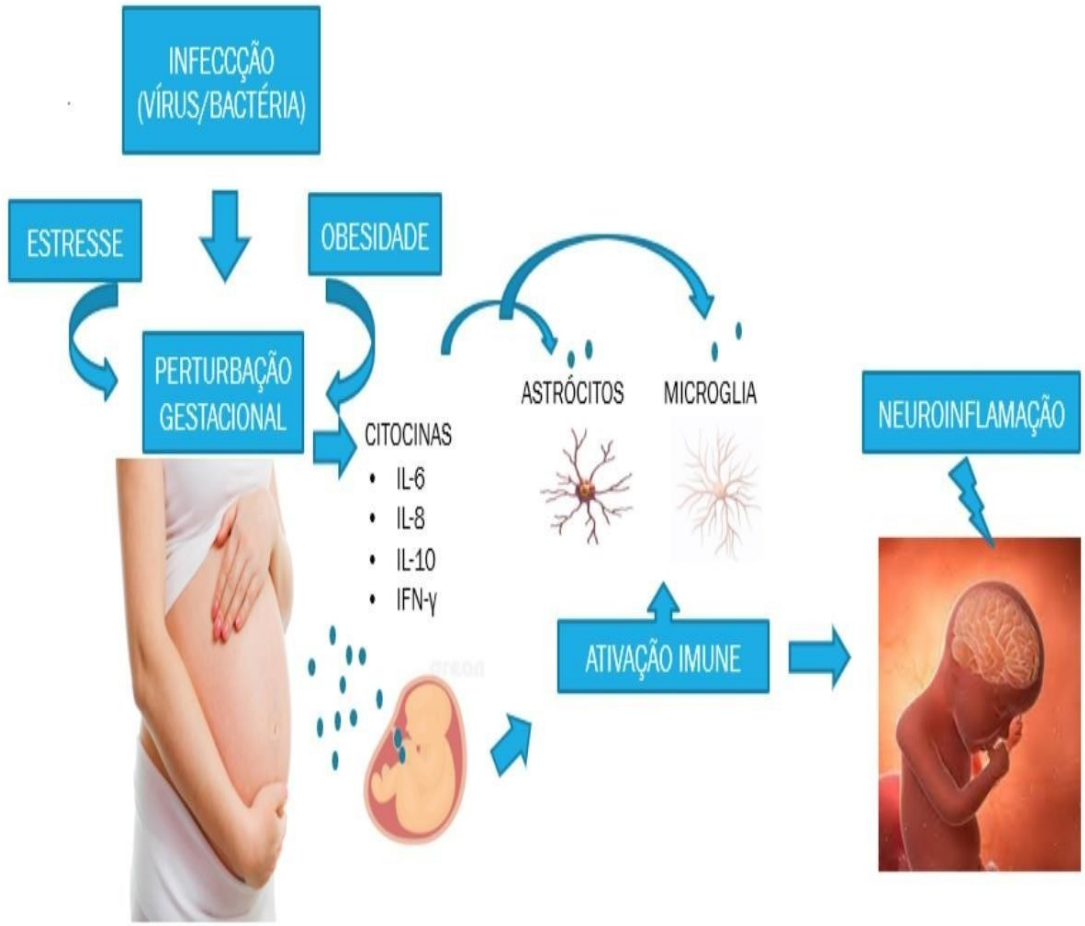

Figura 9. Neuroinflamação na vida intrauterina. Perturbações na gestação como infecções, estresse e obesidade podem desencadear a secreção de citocinas pró inflamatórias na gestante. Essas citocinas podemchegar ao feto via placenta, e ativar células imunes, desencadeando um estado neuroinflamatório no períododo neurodesenvolvimento.

Portanto, vários estudos em humanos (WINDHAM et al., 2019); (SHENet al., 2018); (VARCIN; NEWNHAM; WHITEHOUSE, 2019); (XU et al.,2014). foram capazes de associar significativamente o aumento do IMC pré-gestacional, o ganho excessivo de peso durante a gestação e a hiperglicemia intrauterina, com a maior incidência do fenótipo autista nos filhos destas gestantes. Em paralelo, estudos emanimais mostraram a associação positiva da obesidade materna e hiperglicemia intrauterina com a ativação do sistema imune materno e a presença de neuroinflamação na prole (ERBAS et al., 2018); (BILBO; TSANG, 2010; TOZUKA et al., 2010; WHITE; PURPERA; MORRISON, 2009), resultando no aumento da probabilidade para o desenvolvimento de comportamentos tipo autistas na prole.

Esta relação positiva do IMC materno e da hiperglicemia intrauterina com o desfecho autista nos filhos alerta para a importância do estado nutricionale manutenção de um IMC saudável (18,5kg/m^2 ≤ IMC < 25kg/m^2) durante a gestação como medida de prevenção ao desenvolvimento do transtorno de espectro autista nos filhos.

2. JUSTIFICATIVA

Além das deficiências nos âmbitos da comunicação e interação social, e dos comportamentos repetitivos e estereotipados, o autismo geralmente acomete os indivíduos simultaneamente a outras inúmeras disfunções que podem comprometer significativamente a qualidade de vida.

O transtorno do espectro autista apresentou um crescimento significativo em algumas regiões do mundo nas últimas décadas, e vem por essarazão, requerendo cada vez maioratenção da comunidade científica, seja para o diagnóstico dos casos ou para os possíveis mecanismos envolvidos com a etiologia e seus sintomas.

Como se manifesta logo nos primeiros meses de vida dos indivíduos, leva as crianças a atravessarem a infância com muitas dificuldades nas amizades, no círculo de convivênciasocial, na aprendizagem, na escola, na alimentação, e até em seus momentos individuais, quando acometidos por comportamentos repetitivos ou obsessivos compulsivos. É, portanto, uma desordem dissociativa no âmbito psicossocial. De acordo com a severidade manifestada, pode acarretar muitos prejuízos durante a infância, período no qual o indivíduo inicia a sua conexão com o mundo, constrói suas percepções de realidade, empatia, sociabilidade, respeito, e tantas outras que são essenciais para a formação da psique e da sociabilidade.

A obesidade é uma doença crônica cada vez mais comum, e apresenta uma associação com o desencadeamento do autismo quando presente durante a gestação. A crescente oferta em opções cotidianas que oferecem redução no esforço físico, otimização do tempoe/ou aumento do conforto (como elevadores,tele entregas; opções sedentárias de lazer - vídeo games, serviços de streaming;e a alimentação calórica de rápido preparo e baixo custo - ultraprocessados), tem agradado um número cada vez maior de pessoas que flertam com a comodidade,contribuindo com o aumento da prevalência do sobrepeso e obesidade. Os efeitos negativos do sobrepeso e obesidade comprometem a qualidade de vida das pessoas com comorbidades como diabetes, dislipidemia, doenças cardiovasculares, entre muitas outras.

O sobrepeso e a obesidade gestacional também tiveram suas frequências muito aumentadas nas últimas décadas, e têm relação com malefícios para a saúde de gestantese prole.

Dentre os efeitos negativos na prole, algumas podem ter impacto extremamente negativo na qualidade de vida, como as falhas na cognição, na comunicação, e interaçãosocial, repercutindo em muitos danos ao longo de toda a vida.

Portanto, o TEA tem etiologia desconhecida, e buscando elucidar essa e outras questões, o presente estudo procurou compreender melhor como a disfunção mitocondrial no SNCe a neuroinflamação possam estar envolvidas no desencadeamento do TEA. Foi ainda objetivo deste trabalho estabelecer a relação destes fatores com o sobrepeso/obesidade gestacional, contribuindo assim com uma visão mais apurada sobre a etiologia e/ou sintomas do TEA. A melhor compreensão da relação do sobrepeso/obesidade gestacional com mecanismos que possam aumentar a probabilidade do desenvolvimento do TEA (ouagravar sintomas) na prole, poderá direcionar a prevenção de hábitos que, no período gestacional, possam favorecer o desencadeamento do TEA.

3.HIPÓTESE

A obesidade e o sobrepeso são condições nutricionais associadas a mecanismos de neuroinflamação e de disfunção mitocondrial, os quais podem desencadear distúrbios neurológicos em períodos extremamente sensíveis para o neurodesenvolvimento, como operíodo gestacional. Procurando elucidar melhoros sintomas e/ou a etiologia do autismo, e objetivando também a busca de hábitos de prevenção do autismo, essa dissertação tevecomo foco a revisão de estudos envolvendo o sobrepeso e a obesidade gestacional como fatores desencadeadores na prole de mecanismos de neuroinflamação e disfunção mitocondrial (em especial no sistema nervoso central), possíveis mecanismos patológicos (ou mesmo, etiológicos) do autismo.

4. OBJETIVO GERAL

Caracterizar o modelo de obesidade materna induzida por dieta hiperlipídica relativamente ao ganho de massa corporal e desenvolvimento de tolerância à glicose.

Revisar a literatura com relação ao transtorno de espectro autista como distúrbio neuroinflamatório e de disfunção mitocondrial.

4.1 OBJETIVOS ESPECÍFICOS

- Estabelecer um modelo animal de obesidade gestacional (camundongos C57Bl/6J) induzida por dieta hiperlipídica;

- Verificar o contexto de obesidade das gestantes obesasatravés de acompanhamento glicêmico, peso corporal, mensuração da ingesta alimentar, e mensuração de triglicerídeos e tecido adiposo brancovisceral;
- Revisar a literatura com relação à obesidade materna e sua associação com o aumento do risco para TEA na prole associado a eventos neuroinflamatórios;
- Revisar a literatura com relação à disfunção mitocondrialno SNC comomecanismo neuropatológico do TEA.

5. MATERIAIS E MÉTODOS

Animais

Camundongos fêmeas C57Bl/6J, com 8 semanas de idade, obtidas do Biotério Central doCentro de Ciências Biológicas da Universidade Federal de Santa Catarina foram mantidos em ambiente controlado (22ºC ± 1ºC, ciclo claro/escuro de 12h) com água e comida *ad libitum.* Os animais foram alojados em caixas contendo cerca de 3cm de maravalha cobrindo todo o fundo, e a limpeza das caixas foi realizada semanalmente, sempre com atroca da maravalha. As garrafas de água também eram higienizadas com cloro diluído em água. Todos os protocolos experimentais foram aprovados pelo Comitê de Ética em PesquisaAnimal (CEUA 5004220419) da Universidade Federal de Santa Catarina (Brasil). Os experimentos foram realizados em conformidade com o Conselho das Comunidades Europeias, Diretiva de 24 de Novembro de 1986 (86/609 / CEE).

Para os experimentos com o objetivo de coleta de tecido e sangue, os animais foram anestesiados com pentobarbital sódico (50mg/kg de massa corporal, i.p.). Uma vez que aanestesia foi assegurada, os camundongos foram pesados e as amostras de sangue foramcoletadas. Os experimentos e extrações detecido sempre foram realizados entre 8 e 10 horas da manhã.

Modelo Experimental de Obesidade Induzida por Dieta

A partir de 8 semanas de idade os animais foram divididos em dois grupos aleatoriamente,o grupo controle foi alimentado com uma dieta padrão, e o grupo hiperlipídico foi alimentado com dieta hiperlipídica (HFD). A composição das dietas está representada na

tabela 8. Ambas as dietas foram disponibilizadas *ad libitum*. A dieta padrão (controle) era composta de 4% de lipídios, enquanto a dieta hiperlipídica apresentou 36% de lipídios, principalmente lipídeos saturados. A massa corporal dos animais, bem como a quantidadede ingestão de alimento foi mensurada (através de pesagem) antes do início da dieta, e semanalmente até o final do experimento.

	Dieta	Controle	Dieta	HFD
	g/100g	**%kcal**	**g/100g**	**%kcal**
Proteínas	20	80	20	80
Carboidratos	62	248	45	180
Lipídios	4	36	35	315
kcal/100g	-	364	-	575

Tabela 8. Composição de macronutrientes das dietas.

Acasalamento

Ao final das 12 semanas de exposição às dietas (padrão e hiperlipídica)os animais (fêmeas C57Bl/6J) foram alocados juntamente com machos C57Bl/6J, para que pudessemacasalar e gerar os filhotes, os quais seriam objeto de análises comportamentais e bioquímicas para este presente estudo. A alocação dos camundongos machos foi feita em caixas contendo duas fêmeas. Durante todo o acasalamento, o qual teve duração de 15 dias, os animais permaneceram recebendo o mesmo tipo dietético tal qual iniciaram o experimento. Após os 15 dias, os machos foram retirados das caixas contendo as fêmeas,para que estas pudessem gestar os filhotes com menor estresse.

Teste de tolerância à Glicose

A avaliação de tolerância à glicose foi medida pelo teste intraperitoneal de tolerância à glicose (ipGTT). No dia do teste ipGTT, os animais foram aclimatados na sala do experimento e mantidos em jejum por 6h, com livre acesso à água. A glicose sanguínea foi medida imediatamente antes, 15, 30, 45, 60 e 120minutos após uma injeção intraperitoneal de glicose (2,0 g / kg de massa corporal). A área sob a curva para os níveisde glicose foi calculada utilizando a regra trapezoidal para dados GTT. A medição da glicemia foi realizada com coleta de

sangue através de corte mínimo na extremidade finalda cauda do animal, e mensurada através de fitas reativas (Roche®) e aparato de leitura (Accu-check Active Roche, Mannheim, Alemanha).

Medição de Triglicerídeos Plasmáticos

O sangue para a mensuração dos triglicerídeos foi coletado após a decapitação dos animais, previamente anestesiados. Os níveis séricos de triglicerídeos foram determinados usando um kit comercial (Triglicerídio Labtest200ml, PRONTOLAB) seguindo as instruções do fabricante. A medida dos triglicerídeos foi realizada em triplicatas e a absorbância foi lida no equipamento MULTILEITORA INFINITE M200 TECAN do Laboratório Multiusuário de Estudos em Biologia da Universidade Federal de Santa Catarina (LAMEB/UFSC).

Análise Estatística

Os resultados foram expressos como média ± erro padrão da média. A análise estatísticados dados foi feita utilizando o software GraphPad Prism 5 ®, utilizando o teste *t* de *student*. As diferenças entre os grupos foram consideradas significativas quando $p \leq 0,05$.

6. RESULTADOS

O estudo tinha como objetivo inicial obter um modelo animal de obesidade gestacional (com camundongos C57Bl/6J), verificando a influência da obesidade gestacional no desencadeamento do autismo na prole. Testescomportamentais, comumente utilizados para análises de comportamentos tipo autistas em animais, seriam realizados na prole das gestantes magras e obesas, comparando os resultados que seriam obtidos entre os animais oriundos de gestantes em diferentes estados nutricionais quanto a parâmetros cognitivos, de ansiedade, comportamentos obsessivos compulsivos, e de interação social. Por fim, análises bioquímicas seriam realizadas nas gestantes e proles para verificar quais os possíveis mecanismos bioquímicos poderiam estar alterados nos animaisao longo dos experimentos. Estas análises envolveriam o acompanhamento glicêmico dos animais, mensuração de ativaçãoda microglia, a coleta do soro para mensuração de triglicerídeos, e a coleta de tecidos cerebrais dos animais (córtex, estriado e hipocampo) para a mensuração de monoaminas, citocinas, neurotrofinas (BDNF) e enzimas do metabolismo de neurotransmissores (comoa monoamina oxidase b). No entanto, devido à pandemia da covid19, apenas o manejo inicial e os primeiros testes nos animais foram realizados, e serão aqui reportados.

6.1 EVOLUÇÃO DO GANHO DE PESO CORPORAL E INGESTA ALIMENTAR

Semanalmente foi avaliado o ganho de massa corporal dos animais. Foiobservado que osanimais que foram alimentados com dieta hiperlipídica (HFD, n=6) têm maior tendência para ganhar maior massa corporal em comparação aosanimais tratados com dieta padrão(controles, n=7), demonstrado na *figura 10 (ae b).*

Relativamente à ingesta alimentar (kcal), não foram observadas diferenças estatísticas entre os grupos controle e HFD. Foi possível ainda observar que quando os animais são expostos pela primeira vez à dieta hiperlipídica há um grande aumento no consumo alimentar, no entanto após esseperíodo inicial, os animais consomem a mesma quantidade em comparação ao grupo tratado com dieta controle (*figura 10 c*).

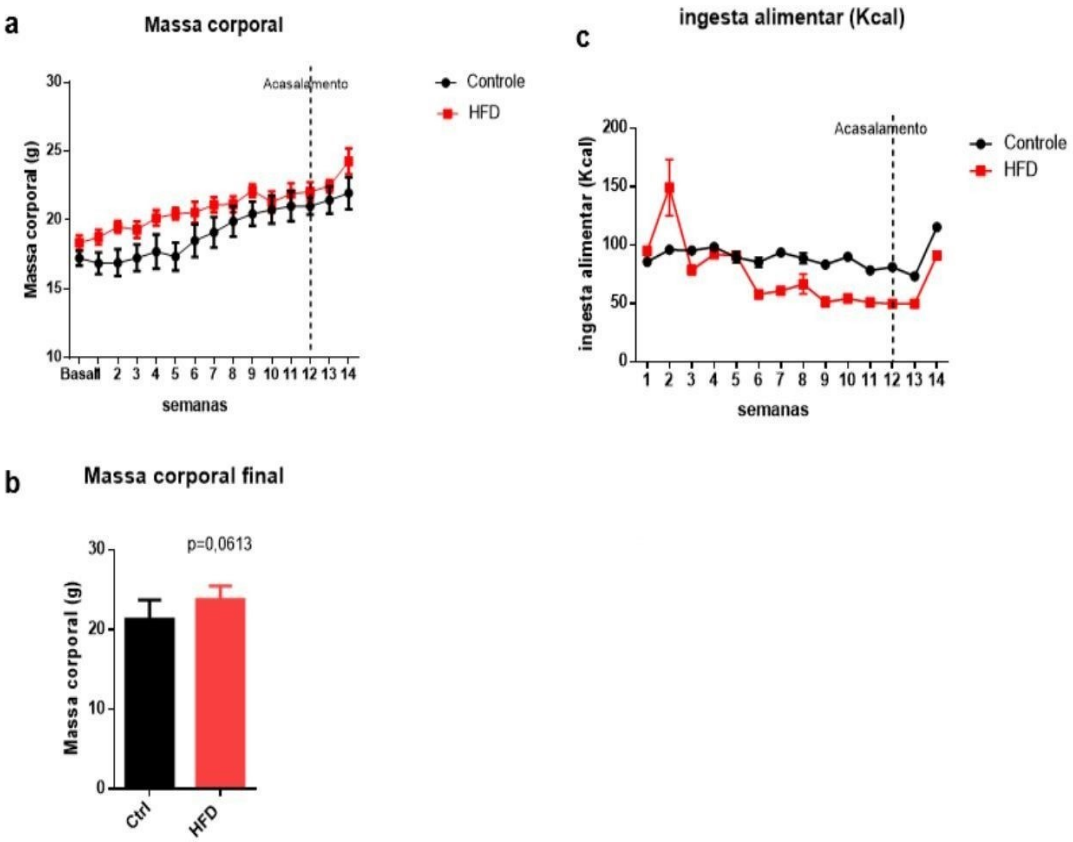

Figura 10. Mensuração da massa corporal e ingesta alimentar. a) Evolução semanal do ganho de peso dosanimais dos grupos controle (n=7) e HFD (n=6). b) Massa corporal final, referente aos dois grupos (controlee HFD) ao término das 12 semanas de tratamento dietético. c)Ingesta alimentar semanal (em kcal) dos animais controle e HFD.

6.2 EFEITO DA DIETA HIPERLIPÍDICA NO GANHO DO TECIDO ADIPOSO BRANCO VISCERAL E TECIDO ADIPOSO MARROM

A obesidade é definida como excesso de tecido adiposo. As diferenças individuais na relação entre a quantidade de massa magra (muscular) e de massagordurosa (tecido adiposo) torna muitas vezes o peso corporal dos animais um parâmetro pouco confiável para mensurar a obesidade nos animais. Assim, ao final do experimento (fim do período de amamentação), as mães foram sacrificadas e foi quantificado o tecido adiposo brancovisceral e o tecido adiposo marrom.

Observamos que os animais do grupo HFD (n=2) apresentaram, conforme mostra a *figura 11a*, maior teor de tecido adiposo branco, embora sem diferença estatística, do que os animais que receberam dieta controle (n=2). Os animais tratados com dieta hiperlipídica apresentaram uma tendência de aumento(p = 0,0685) no tecido adiposo marrom (*figura11b*).

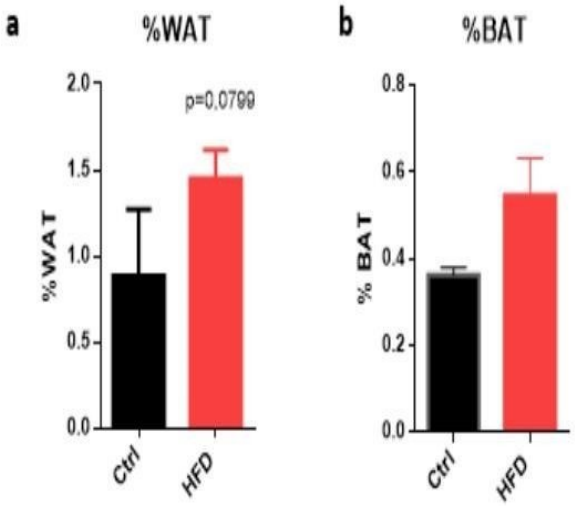

Figura 11. Quantificação de tecido adiposo branco visceral (WAT) (n=2) e marrom (BAT) (n=2). Tecidos coletados e mensurados após 17 semanas do início dos tratamentosdietéticos.

6.3 EFEITO DA DIETA HIPERLIPÍDICA NA INTOLERÂNCIA ÀGLICOSE E NOS TRIGLICERÍDEOS

O teste de tolerância à glicose é utilizado para verificar a disfunção metabólica nos animais, a qual está associada aos efeitos do consumo de dietas hipercalóricas como as dietas ricas em gorduras saturadas. Após 8 semanas de consumo de dieta hiperlipídica não houve

diferença significativa comparativamente aos animais do grupo controle em relação à tolerância à glicose (*figura 12a*). O mesmo foi observado ao fim de 12 semanas(*figura 12b*). No entanto, é possível observar ao fim de 12 semanas de consumo de dieta hiperlipídica que o decaimento glicêmico é mais demorado no grupo HFD (n=6)frente ao grupo controle (n=7) (*figura 12b*). Isso demonstra que o maior tempo de exposição dos animais à dieta hiperlipídica agrava a dificuldade celular nestes animais em assimilara glicose inoculada na corrente sanguínea, comprometendo o metabolismo de forma mais acentuada.

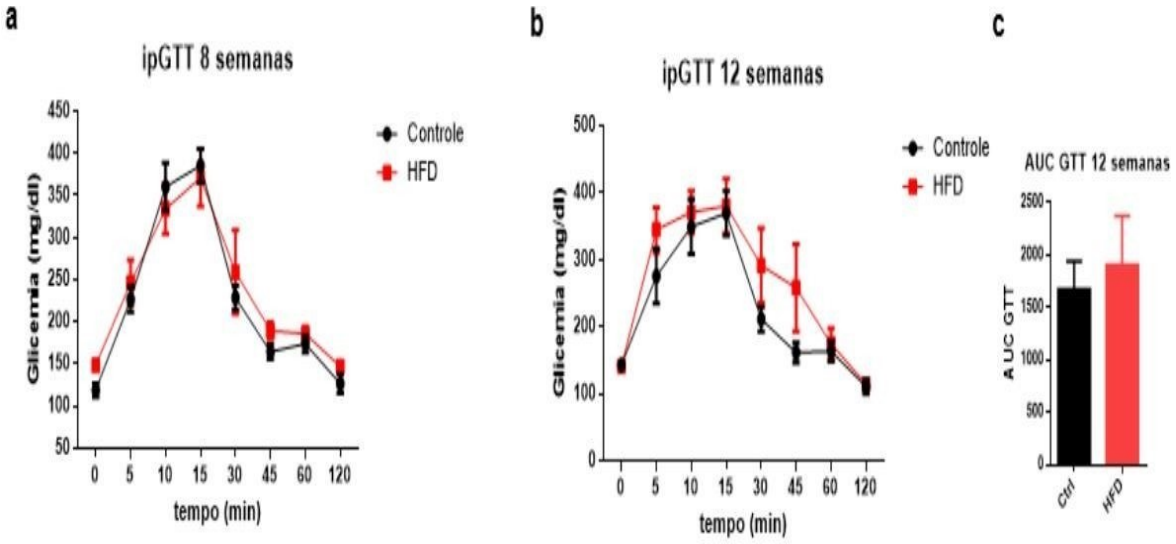

Figura 12. Teste de tolerância à glicose (ipGTT). Decaimento da glicose observados aos tempos: 0, 15, 30,45, 60 e 120 minutos em dois momentos distintos do experimento: *a*) ao final de 8 semanas de tratamentos dietéticos; e *b*) ao final de 12 semanas de tratamentos dietéticos. Grupo dieta HFD (n=6) representado emvermelho, e grupo dieta controle (n=7) representado em preto.

A avaliação da dislipidemia através da quantificação de triglicerídeos nosoro é um bom parâmetro bioquímico para analisar o perfil metabólico dos animais. Conforme mostra a *figura 13,* os animais que receberam a dieta hiperlipídica (n=2) apresentaram níveis superiores de triglicerídeos no soro em comparação aos animais de dieta controle (n=2), sugerindo uma disfunção metabólica nos animais que receberam dieta hiperlipídica. A hiperlipidemia é consequência do excesso de tecido adiposo (obesidade), que reflete a disfunção metabólica dos animais tratados com dieta hiperlipídica.

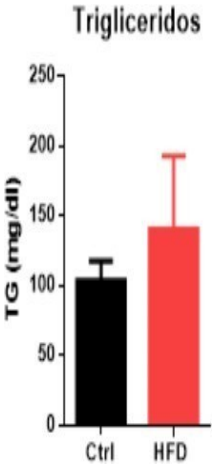

Figura 13. Quantificação de triglicerídeos. As absorbâncias (em preto, o grupo controle e vermelho, o grupo HFD) foram analisadas no multileitor de placas INFINITE M200 TECAN, utilizando o software integrado Magellan. Grupo dieta HFD (n=2) representado em vermelho, e grupo dieta controle (n=2) representado em preto.

7. CONCLUSÃO

Foi possível observar que os animais tratados com dieta hiperlipídica (HFD) apresentaram tendência maior para ganho de massa corporal frente aos animais tratados com dieta controle, bem como maior tendência para teor de tecido adiposo branco visceral e tecido adiposo marrom. Os animais HFD apresentaram também maior dificuldade na captação da glicose, verificada pelo decaimento glicêmico lento no ipGTT frente ao grupo controle, e maior teor de triglicerídeos no sangue, denotando a tendência para disfunção metabólica.

Em artigo anexo neste estudo "Neuroinflammation in Autism Spectrum Disorders: Exercise as a Pharmacological Tool" (TOSCANO et al., 2021) foi revisada a associação da neuroinflamação com a obesidade e a ativação do sistema imune periférico como mecanismos subjacentes envolvidos com o agravamento dos sintomas do TEA. Como medida terapêutica, foi proposta a inclusão de programas de atividades físicas aos pacientes autistas, as quais estão associadas em vasta literatura por melhoras em parâmetros do espectro autista, como as interações sociais, danos motores, cognição, ansiedade, e comportamentos repetitivos e estereotipados. Portanto, os exercícios físicos podem ser recomendados como alternativa terapêutica não invasiva, com boas perspectivas de resposta significativa na melhora da qualidade de vida dos pacientes com TEA.

O segundo artigo de revisão anexo neste estudo "Obesity associated with autism spectrum disorders: mitochondrial dysfunction and neuroinflammation as a links" (artigo em

fase de preparação para submissão), mostra que a obesidade e o consumo de dietas hiperlipídicas estão associadas ao desenvolvimento de processos neuroinflamatórios e dedisfunção mitocondrial no SNC em modelos animais e humanos. Tais processos neuroinflamatórios e de disfunção mitocondrial são frequentemente encontrados em indivíduos autistas, sugerindo que o estado nutricional obeso pode ser um agravante dos sintomas do autismo, propondo que a manutenção de um IMC saudável ($18,5 \leq IMC \leq 24,9$), bem como a baixa ingesta de dietas hiperlipídicas podem ser bons fatores na prevenção dos sintomas mais graves das comorbidades associadas ao autismo, bem como na atenuação de suas manifestações comportamentais típicas.

8. PERSPECTIVAS FUTURAS

A hipótese inicial deste estudo era a de que a obesidade materna, como fator de perturbação intrauterina, pudesse gerar na prole (camundongos C57BL/6J) a presença de comportamentos tipo autista em associação a mecanismos neuroinflamatórios (ativação de microglia e liberação de citocinas IL-17, IL-6 e IL-1β), além de alteração na expressão de monoaminas (serotoninae dopamina) e de enzimas de seu metabolismo (monoamina oxidase B), e no aumento da neurotrofina BDNF. Com o advento da pandemia Sars-Cov- 2 (covid19) e com a impossibilidade da realização de experimentos, o trabalho mudou seu foco para a revisão de mecanismos de neuroinflamação e disfunção mitocondrial, e suas associações no desencadeamento de comportamentos tipo autista na prole de gestantes obesas.

Portanto, como próximos passos para a continuidade deste estudo, poderão ser desenvolvidos os seguintes aspectos experimentais para a associaçãode parâmetros bioquímicos com o possível fenótipo autista que venha a ser desencadeado na prole:

- Avaliação bioquímica das gestantes logo após o parto e de metade da prole gerada,com a análise de estruturas cerebrais (hipocampo,estriado e córtex pré-frontal) com relação a ativação de microglia (anti- Iba1), e expressão de citocinas (IL-17, IL1β e IL-6);
- Testes comportamentais na prole com 12 semanas de vida (teste de cruz elevada,marble buryng, campo aberto, teste de interação social, *splash test*) para mensurarcomportamentos tipo autista (ansiedade, memória, transtorno obsessivo compulsivo, danos na interação social, déficit motor);
- Avaliação bioquímica da prole após testes comportamentaiscom relação à neuroinflamação (análise da ativação de microglia e expressão de citocinas IL-

17,IL-6 e IL1β no hipocampo, estriado e córtexpré-frontal;

- Análise da expressão de monoaminas (serotonina de dopamina), e da expressão de enzimas que metabolizam monoaminas (monoamina oxidase A e B);
- Análise da expressão de BDNF no cérebro (hipocampo, estriado e córtex pré- frontal) da prole antes dos testes comportamentais (metade da prole) e após os testes (a outra metade);
- Avaliação da função mitocondrial no cérebro da prole através da respirometria celular (hipocampo, estriado e córtex pré-frontal).

9. REFERÊNCIAS

AMAL, Haitham et al. Shank3 mutation in a mouse model of autism leads to changes inthe S-nitroso-proteome and affects key proteins involved in vesicle release and synaptic function. **Molecular Psychiatry**, [s. l.], 2020.

AMARAL, David G. et al. **In pursuit of neurophenotypes: Theconsequences of having autism and a big brain Autism Research**, 2017.

AMENT, Katarina et al. Evidence for Specificity of Motor Impairmentsin Catching and Balance in Children with Autism. **Journal of Autism and Developmental Disorders**, [s. l.], 2015.

AMIN, Fakhra; BANO, Bilqees. Damage of cystatin due to ROS- generation and radical-scavenging activity of antioxidants and associated compounds. **International Journal of Biological Macromolecules**, [s. l.], 2018.

ANDARI, Elissar et al. Epigenetic modification of the oxytocin receptor gene:implications for autism symptom severity and brain functional connectivity. **Neuropsychopharmacology**, [s. l.], 2020.

ARCURI, Cataldo et al. **The pathophysiological role of** microglia in dynamic surveillance, phagocytosis and structural remodeling of the developing CNSFrontiers in Molecular Neuroscience, 2017.

AZMITIA, Efrain C. et al. Dystrophic serotonin axons in postmortem brains from young autism patients. **Anatomical Record**, [s. l.], 2011.

BACHMANN, Christian J.; GERSTE, Bettina; HOFFMANN, Falk.Diagnoses of autism spectrum disorders in Germany: Time trends in administrative prevalence and diagnostic stability. **Autism**, [s. l.], 2018.

BAE, Heekyong R. et al. Ifnγ is a key link between obesity and th1- mediated

autoimmune diseases. **International Journal of MolecularSciences**, [s. l.], 2021.

BARREIROS, André L. B. S.; DAVID, Jorge M.; DAVID, Juceni P. Estresse oxidativo: relação entre geração de espéciesreativas e defesa do organismo. **Química Nova**, [s. l.], 2006.

BASTAKI, Kholoud N.; ALWAN, Sura; ZAHIR, Farah R. Maternal Prenatal Exposures in Pregnancy and Autism Spectrum Disorder: An Insight intothe Epigenetics of Drugs and Diet as Key Environmental Influences. In: **Advances in Neurobiology**. [s.l: s.n.].

BATISTA, Tatiane Helena; GIUSTI-PAIVA, Alexandre; VILELA, Fabiana Cardoso.Maternal protein malnutrition induces autism-like symptoms in rat offspring. **Nutritional Neuroscience**, [s. l.], 2019.

BAUER, Jan; RAUSCHKA, Helmut; LASSMANN, Hans. Inflammation in the nervous system: The human perspective. **GLIA**, [s. l.], 2001.

BECERRA DEPABLOS, Gladys; AREVALO GONZÁLEZ, Evelia. Envejecimientovs. radicales libres. **Rev. Fac. Farm. (Merida)**, [s. l.],2000.

BECK, Roberto Gaspari. **Estimativa do número de casos de Transtorno do EspectroAutista no Sul do Brasil**. 2017. [s. l.], 2017.

BENNURI, Sirish C.; ROSE, Shannon; FRYE, Richard E. Mitochondrial dysfunction is inducible in lymphoblastoid cell lines from children with autism and may involve the TORC1 pathway. **Frontiers in Psychiatry**, [s.l.], 2019.

BENTHAM, James et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. **The Lancet**, [s.l.], 2017.

BILBO, Staci D.; TSANG, Verne. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. **FASEB Journal**, [s. l.], 2010.

Bioenergetics 3 (Nicholls, D. G., and Ferguson, S. J.,

Academic Press, London, 2002). **Biochemistry (Moscow)**, [s. l.], 2004.

BITSKO, Rebecca H. et al. Health Care, Family, and Community Factors Associated with Mental, Behavioral, and Developmental Disorders in Early Childhood — United States, 2011–2012. **MMWR. Morbidity and Mortality Weekly Report**, [s. l.], 2016. BLEULER, E. **Dementia praecox or the group of schizophrenias (J. Zinkin, trans.)**.[s.l: s.n.].

BOOTH, Heather D. E.; HIRST, Warren D.; WADE- MARTINS, Richard. The Role of Astrocyte Dysfunction in Parkinson's Disease **Pathogenesis Trends in**

Neurosciences, 2017.

BOURLIER, Virginie et al. TGFbeta family members are key mediators in the induction of myofibroblast phenotype of human adipose tissue progenitor cells bymacrophages. **PLoS ONE**, [s. l.], 2012.

BOZDAGI, Ozlem et al. Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function, social interaction, and social communication. **Molecular Autism**, [s. l.], 2010.

BRÁS, João Paulo et al. TNF-alpha-induced microglia activation requires miR-342: impact on NF-kB signaling and neurotoxicity. **Cell Death andDisease**, [s. l.], 2020. BRASA, Sarah et al. Reciprocal changes in DNA methylation and hydroxymethylation and a broad repressive epigenetic switch characterize FMR1 transcriptional silencing in fragile X syndrome. **Clinical Epigenetics**, [s. l.], 2016.

BRAUNE, Julia et al. IL-6 Regulates M2 Polarization and Local Proliferation of Adipose Tissue Macrophages in Obesity. **The Journal of Immunology**, [s. l.], 2017. BROWN, Alan S. Prenatal infection as a risk factor for schizophrenia. **Schizophrenia Bulletin**, [s. l.], 2006.

BURDA, Joshua E.; SOFRONIEW, Michael V. **Reactive gliosis and the multicellular response to CNS damage and disease Neuron**, 2014.

BURROWS, E. L. et al. Social isolation alters social and mating behavior in the R451Cneuroligin mouse model of autism. **Neural Plasticity**, [s.l.], 2017.

CADENAS, Enrique; DAVIES, Kelvin J. A. Mitochondrial free radical generation,oxidative stress, and aging. **Free Radical Biology and Medicine**, [s.l.], 2000.

CAMPUZANO, Victoria et al. Frataxin is reduced in Friedreich ataxiapatients and is associated with mitochondrial membranes. **Human Molecular Genetics**, [s. l.], 1997.

CAST, Thomas et al. An Autism-Associated Mutation Impairs Neuroligin-4 Glycosylation and Enhances Excitatory Synaptic Transmission in Human Neurons. **TheJournal of Neuroscience**, [s. l.], 2020.

CASTILHO, Roger F. et al. Oxidative Damage of Mitochondria Induced by Fe(II)citrate is Potentiated by Ca2+ and Includes Lipid Peroxidation and Alterations in Membrane Proteins. **Archives of Biochemistry and Biophysics**, [s. l.], 1994.

CERVÓS-NAVARRO, J.; LAFUENTE, J. V. Traumatic brain injuries: structural changes. **Journal of the Neurological Sciences**, [s. l.], 1991.

CEYZÉRIAT, Kelly et al. Modulation of astrocyte reactivity improves functional

deficits in mouse models of Alzheimer's disease. **Actaneuropathologica communications**, [s. l.], 2018.

CHAUHAN, Abha et al. Brain region-specific deficit in mitochondrial electron transport chain complexes in children with autism. **Journal of Neurochemistry**, [s. l.],2011.

CHAUHAN, Abha; AUDHYA, Tapan; CHAUHAN, Ved. Brain region-specific glutathione redox imbalance in autism. **Neurochemical Research**, [s. l.], 2012. CHEN, L. et al. OPA1 mutation and late-onset cardiomyopathy: mitochondrial dysfunction and mtDNA instability. **Journal of the American Heart Association**, [s.l.], 2012.

CHERAGHI, Ghazale et al. High doses of sodium tungstate can promote mitochondrial dysfunction and oxidative stress in isolated mitochondria. **Journal of Biochemical and Molecular Toxicology**, [s. l.],2019.

CHEVRIER, Jonathan et al. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. **American Journal of Epidemiology**, [s. l.], 2008.

CHEZ, Michael G. et al. Elevation of Tumor Necrosis Factor-Alphain Cerebrospinal Fluid of Autistic Children. **Pediatric Neurology**, [s. l.], 2007.

CHISTIAKOV, Dimitry A. et al. **Mitochondrial aging and age- related dysfunction of mitochondria BioMed Research International**, 2014.

CHISTOL, Liem T. et al. Sensory Sensitivity and Food Selectivity in Children with Autism Spectrum Disorder. **Journal of Autism and Developmental Disorders**, [s. l.], 2018.

CHOI, Gloria B. et al. The maternal interleukin-17a pathwayin micepromotes autism-like phenotypes in offspring. **Science**, [s. l.], 2016.

CHU, S. Y. et al. Maternal obesity and risk of cesarean delivery: A meta-analysis. **Obesity Reviews**, [s. l.], 2007.

CHUGANI, Diane C. et al. Evidence of altered energy metabolism In autistic children.**Progress in Neuro-Psychopharmacology and Biological Psychiatry**, [s. l.], 1999.

CHUNG, Won Suk et al. Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. **Nature**, [s. l.], 2013.

CIEŚLIK, Magdalena et al. Maternal immune activation inducesneuroinflammation and cortical synaptic deficits in the adolescent rat offspring. **International Journal of Molecular Sciences**, [s. l.], 2020.

COLEMAN, Mary; BLASS, John P. Autism and lactic acidosis. **Journal of Autism and Developmental Disorders**, [s. l.], 1985. Committee opinion No. 548: Weight

gain during pregnancy. **Obstetrics and Gynecology**, 2013.

CORDERO, Christina et al. Maternal diabetes and hypertensive disorders in association with autism spectrum disorder. **Autism Research**, [s. l.],2019.

COURY, Daniel L. et al. **Gastrointestinal conditions in children with autism spectrum disorder: Developing a researchagenda Pediatrics**, 2012.

CRISTIANO, Claudia et al. Interplay between peripheral and centralinflammation in autism spectrum disorders: Possible nutritional and therapeutic strategies Frontiers in Physiology, 2018.

CUKIER, Sebastián; BARRIOS, Natalia. [Pharmacological interventions for intellectual disability and autism]. TT - Intervenciones farmacológicas en discapacidad intelectual y autismo. **Vertex**, [s. l.], 2019.

CURRAN, Eileen A. et al. Exposure to Hypertensive Disorders of Pregnancy Increases the Risk of Autism Spectrum Disorder in Affected Offspring. **Molecular Neurobiology**, [s. l.], 2018.

D.A., Rossignol; R.E., Frye. Mitochondrial dysfunction in autism spectrum disorders: A systematic review and meta-analysis. **Molecular Psychiatry**, [s. l.], 2012.

DALBYE, Rebecka et al. Maternal body mass index and risk of obstetric, maternal and neonatal outcomes: A cohort study of nulliparous womenwith spontaneous onset of labor. **Acta Obstetricia et Gynecologica Scandinavica**, [s. l.], 2021.

DATA, Detailed. WHO :: Global Database on Body Mass Index BMI classification WHO :: Global Database on Body Mass Index. **World Health**, [s. l.], 2011.

DEVERMAN, Benjamin E.; PATTERSON, Paul H. Cytokines and CNS DevelopmentNeuron, 2009.

DI DONATO, S. Disorders related to mitochondrial membranes: Pathology of therespiratory chain and neurodegeneration. In: JOURNAL OF INHERITED METABOLIC DISEASE 2000, **Anais**... [s.l: s.n.] **Diagnostic andStatistical Manual of Mental Disorders, 5th Edition**. [s.l: s.n.].

DICARLO, Gabriella E. et al. Autism-linked dopamine transporter mutation alters striatal dopamine neurotransmission and dopamine-dependent behaviors. **Journal of Clinical Investigation**, [s. l.], 2019.

DIKAIOU, Pigi et al. Obesity, overweight and risk for cardiovascular disease and mortality in young women. **European Journal of Preventive Cardiology**, [s. l.], 2020.DONG, Shan et al. De novo insertions and deletions of predominantly paternal origin

are associated with autism spectrum disorder. **CellReports**, [s. l.], 2014.

DORISON, Nathalie et al. Mitochondrial dysfunction caused by novel ATAD3Amutations. **Molecular Genetics and Metabolism**, [s. l.], 2020.

EDMONDS, Joseph L. et al. The otolaryngological manifestations of mitochondrial disease and the risk of neurodegeneration with infection. **Archives of Otolaryngology - Head and Neck Surgery**, [s. l.], 2002.

EMSLEY, Jason G.; MACKLIS, Jeffrey D. Astroglial heterogeneity closely reflects theneuronal-defined anatomy of the adult murine CNS. **NeuronGlia Biology**, [s. l.], 2006.

ENGLISH, Krystal et al. Astrocytes rescue neuronal health after cisplatin treatment through mitochondrial transfer. **Acta Neuropathologica Communications**, [s. l.], 2020.

ERBAS, Oytun et al. Neurobehavioral effects of long-term maternal fructose intake in rat offspring. **International Journal of DevelopmentalNeuroscience**, [s. l.], 2018.

ERNSTER, L.; IKKOS, D.; LUFT, R. Enzymic activities of human skeletal muscle mitochondria: A tool in clinical catabolic research. **Nature**, [s.l.], 1959.

EWEN, Joshua B. et al. Epilepsy and Autism Severity: A Study of 6,975 Children. **Autism Research**, [s. l.], 2019.

FANTUZZI, Giamila. **Adipose tissue, adipokines, and inflammation Journal of Allergy and Clinical Immunology**, 2005.

FARINA, Cinthia et al. Preferential expression and function of Toll-like receptor 3 inhuman astrocytes. **Journal of Neuroimmunology**, [s. l.], 2005.

FATEMI, S. Hossein et al. Expression of astrocytic markers aquaporin 4 and connexin43 is altered in brains of subjects with autism. **Synapse**, [s. l.], 2008.

FEREY, Jeremie L. A. et al. A maternal high-fat, high-sucrose diet induces transgenerational cardiac mitochondrial dysfunction independently of maternal mitochondrial inheritance. **American Journal of Physiology - Heart and Circulatory Physiology**, [s. l.], 2019.

FERNANDES, Isabella R. et al. **Genetic variations on SETD5 underlying autistic conditions Developmental Neurobiology**, 2018.

FERNÁNDEZ ALBA, Juan Jesús et al. Overweight and obesity at risk factors for hypertensive states of pregnancy: A retrospective cohort study. **Nutricion Hospitalaria**,[s. l.], 2018.

FLETCHER, Fay E. et al. Atypicalities in sleep and semantic consolidation in

autism. **Developmental Science**, [s. l.], 2020.

FOLEY, Kevin P. et al. Inflammation promotes adipocyte lipolysis via IRE1 kinase. **Journal of Biological Chemistry**, [s. l.], 2021.

FRAGA, Ana Claudia S. Amara.; THEME-FILHA, MarizaMiranda. Pregestational overweight and obesity and symptoms of postpartum depression:Data from the Birth in Brazil Study. **Journal of Affective Disorders**, [s. l.], 2020.

FRAKES, Ashley E. et al. Microglia induce motor neuron death via theclassical NF-κBpathway in amyotrophic lateral sclerosis. **Neuron**, [s. l.], 2014.

FREYA, Terrence G.; MANNELLAB, Carmen A. **The internal structure of mitochondria Trends in Biochemical Sciences**, 2000.

FRITHIOFF-BØJSØE, Christine et al. Leptin, adiponectin, and their ratio as markers of insulin resistance and cardiometabolic risk in childhood obesity. **Pediatric Diabetes**, [s.l.], 2020.

FRYE, Richard E. Biomarkers of Abnormal Energy Metabolism in Children with Autism Spectrum Disorder. **American Chinese Journal of Medicine and Science**, [s.l.], 2012.

FRYE, Richard E. et al. Prenatal air pollution influences neurodevelopment and behavior in autism spectrum disorder by modulating mitochondrial physiology. **Molecular Psychiatry**, [s. l.], 2020.

GABRIELSEN, Terisa P. et al. Functional MRI connectivity of children with autism and low verbal and cognitive performance. **Molecular Autism**, [s. l.], 2018.

GADELKARIM, W. et al. Overlap of obsessive–compulsive personality disorder and autism spectrum disorder traits among OCD outpatients: an exploratory study. **International Journal of Psychiatry in Clinical Practice**,[s. l.], 2019.

GAO, Chun Lin et al. Mitochondrial dysfunction is induced by high levels of glucose and free fatty acids in 3T3-L1 adipocytes. **Molecular and Cellular Endocrinology**, [s.l.], 2010.

GARAY, Paula A.; MCALLISTER, A. Kimberley. **Novel roles for immune molecules in neural development: Implications for neurodevelopmental disorders Frontiers in Synaptic Neuroscience**, 2010.

GARBARINO, Valentina R. et al. **Extreme enhancement or depletion of serotonin transporter function and serotonin availability in autism spectrum disorder Pharmacological Research**, 2019.

GETZ, Kelly D. et al. Maternal Pre-pregnancy Body Mass Index and Autism Spectrum Disorder among Offspring: A Population-Based Case– Control Study. **Paediatric and Perinatal Epidemiology**, [s. l.], 2016.

GHAFOURI-FARD, Soudeh et al. Expression Analysis of BDNF, BACE1, and Their Natural Occurring Antisenses in Autistic Patients. **Journal of Molecular Neuroscience**, [s. l.], 2020.

GHIRARDI, Laura et al. Genetic and environmental contribution to the overlapbetween ADHD and ASD trait dimensions in young adults: A twin study. **Psychological Medicine**, [s. l.], 2019.

GINHOUX, Florent et al. Fate mapping analysis reveals that adult microglia derivefrom primitive macrophages. **Science**, [s. l.], 2010.

GINN, S. Islands of Genius: The Bountiful Mind of the Autistic,Acquired, and SuddenSavant. **The British Journal of Psychiatry**, [s. l.], 2011.

GIULIVI, Cecilia et al. Mitochondrial dysfunction in autism. **JAMA**

- Journal of theAmerican Medical Association, [s. l.], 2010.

GLEZER, I.; SIMARD, A. R.; RIVEST, S. **Neuroprotectiverole of the innate immune system by microglia Neuroscience**, 2007.

GLEZER, Isaias; RIVEST, Serge. **Glucocorticoids: Protectors of the brain duringinnate immune responsesNeuroscientist**, 2004.

GODOY, Priscilla B. G. et al. Brief Report: Associations Between Cognitive Control Processes and Traits of Autism Spectrum Disorder (ASD), attention-Deficit/Hyperactivity Disorder (ADHD) and Anxiety in Children atElevated and Typical Familial Likelihood for ASD. **Journal of Autism and Developmental Disorders**, [s. l.], 2020.

GOERDT, Sergij et al. Alternative versus classical activation of macrophages. In:PATHOBIOLOGY 1999, **Anais**... [s.l: s.n.]

GOLDENTHAL, Michael J. et al. Mitochondrial enzyme dysfunction in autisms pectrum disorders; A novel biomarker revealed frombuccal swab analysis. **Biomarkers in Medicine**, [s. l.], 2015.

GREEN, Claire; WILLOUGHBY, Joshua; BALASUBRAMANIAN, Meena. De novo SETD5 loss-of-function variant as a cause for intellectual disability in a 10-year old boy with an aberrant blind ending bronchus. **AmericanJournal of Medical Genetics, Part A**, [s. l.], 2017.

GU, F. et al. Alterations in mitochondrial DNA copy number and the activities of electron transport chain complexes and pyruvate dehydrogenase in the frontal cortexfrom subjects with autism. **Translationalpsychiatry**, [s. l.], 2013.

HAERTLE, Larissa et al. Methylomic profiling in trisomy 21 identifies cognition- and Alzheimer's disease-related dysregulation. **Clinical Epigenetics**, [s. l.], 2019.

HAGEMEYER, Nora et al. Microglia contribute to normal myelinogenesis and to oligodendrocyte progenitor maintenance during adulthood. **Acta Neuropathologica**, [s.l.], 2017.

HALI, Sai et al. Modelling monogenic autism spectrum disorder using mouse cortical organoids. **Biochemical and Biophysical ResearchCommunications**, [s. l.], 2020. HALLIWELL, Barry. Oxygen and nitrogen are pro-carcinogens. Damage to DNA by reactive oxygen, chlorine and nitrogen species: Measurement, mechanism and the effects of nutrition. **Mutation Research - Genetic Toxicology and Environmental Mutagenesis**, [s. l.], 1999.

HAN, Myoung Sook et al. Regulation of adipose tissue inflammationby interleukin 6.**Proceedings of the National Academy of Sciences of the United States of America**,[s. l.], 2020.

HEGARTY, John P. et al. Genetic and environmental influences on corticostriatal circuits in twins with autism. **Journal of Psychiatry and Neuroscience**, [s. l.], 2020. HEISE, Christopher et al. Heterogeneity of cell surface glutamate and GABA receptor expression in shank and CNTN4 autism mouse models. **Frontiers in Molecular Neuroscience**, [s. l.], 2018.

HERCULANO-HOUZEL, Suzana. The glia/neuron ratio: How itvaries uniformly across brain structures and species and what that means for brain physiology and evolution GLIA, 2014.

HOFFSTEDT, J. et al. Regional impact of adipose tissue morphology on the metabolicprofile in morbid obesity. **Diabetologia**, [s. l.], 2010.

HOLINGUE, Calliope et al. Interaction between Maternal Immune Activation and Antibiotic Use during Pregnancy and Child Risk of Autism Spectrum Disorder. **Autism Research**, [s. l.], 2020.

ISHIHARA, Yasuhiro et al. Dual role of superoxide dismutase 2 induced in activated microglia: Oxidative stress tolerance and convergence of inflammatory responses. **Journal of Biological Chemistry**, [s. l.], 2015.

ITO, Ayaka et al. Role of CC chemokine receptor 2 in bone marrow cells in the recruitment of macrophages into obese adipose tissue. **Journal of Biological Chemistry**, [s. l.], 2008.

JÄÄSKELÄINEN, Tiina et al. Impact of obesity on angiogenic and inflammatory markers in the Finnish Genetics of Pre-eclampsia Consortium (FINNPEC) cohort.**International Journal of Obesity**, [s. l.],2019.

JIA, Qingyi; MORGAN-BATHKE, Maria E.; JENSEN, Michael D. Adipose tissue macrophage burden, systemic inflammation, and insulin resistance. **American Journal of Physiology - Endocrinology and Metabolism**, [s. l.], 2020.

JO, Heejoo et al. Maternal prepregnancy body mass index and child psychosocialdevelopment at 6 years of age. **Pediatrics**, [s. l.], 2015.

JO, Junghyo et al. Hypertrophy and/or hyperplasia: Dynamics of adipose tissue growth. **PLoS Computational Biology**, [s. l.], 2009.

JONES, Warren; KLIN, Ami. Attention to eyes is present but in decline in 2-6-month-old infants later diagnosed with autism. **Nature**, [s. l.], 2013.

JUGE-AUBRY, Cristiana E. et al. Adipose tissue is a major source ofinterleukin-1 receptor antagonist: Upregulation in obesity and inflammation. **Diabetes**, [s. l.], 2003. KANAGALINGAM, Malini G. et al. Changes in booking body mass index over a decade: Retrospective analysis from a Glasgow Maternity Hospital. **BJOG: An International Journal of Obstetricsand Gynaecology**, [s. l.], 2005.

KANNER, Leo. Autistic disturbances of affective contact. Nervous child. **NervousChild**, [s. l.], 1943.

KERR, Daniel J. et al. Aberrant hippocampal Atp8a1 levels are associated with altered synaptic strength, electrical activity, and autistic-like behavior. **Biochimica et Biophysica Acta - Molecular Basis of Disease**, [s. l.],2016.

KHAKH, Baljit S. et al. **Unravelling and Exploiting AstrocyteDysfunction in Huntington's Disease Trends in Neurosciences**, 2017.

KHAKH, Baljit S.; SOFRONIEW, Michael V. Diversity of astrocytefunctions and phenotypes in neural circuits. **Nature Neuroscience**, [s. l.], 2015.

KIM, H. J. et al. Deficient autophagy in microglia impairs synapticpruning and causes social behavioral defects. **Molecular Psychiatry**, [s. l.], 2017.

KIMURA-KURODA, Junko; NAGATA, Isao; KURODA, Yoichiro. Disrupting effects of hydroxy-polychlorinated biphenyl (PCB) congeners on neuronal development of

cerebellar Purkinje cells: A possible causal factor for developmental brain disorders? **Chemosphere**, [s. l.], 2007.

KOJIMA, Masaki et al. Neuroanatomical Correlates of Advanced Paternal and Maternal Age at Birth in Autism Spectrum Disorder. **Cerebral Cortex**, [s. l.], 2019.

KONG, Linghua et al. The risk of offspring psychiatric disorders in thesetting of maternal obesity and diabetes. **Pediatrics**, [s. l.], 2018.

KONSMAN, Jan Pieter; PARNET, Patricia; DANTZER, Robert. **Cytokine-induced sickness behaviour: Mechanisms and implicationsTrends in Neurosciences**, 2002.

KOWALTOWSKI, Alicia et al. Mitochondrial Morphology Regulates Organellar Ca 2+Uptake and Changes Cellular Ca 2+ Homeostasis. **The FASEB Journal**, [s. l.], 2019.

KRAKOWIAK, Paula et al. Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. **Pediatrics**, [s. l.], 2012.

KUBOTA, Yasuo et al. Integrated genetic and epigenetic analysis revealed heterogeneity of acute lymphoblastic leukemia in Down syndrome. **Cancer Science**, [s.l.], 2019.

LAURENCE, J. A.; FATEMI, S. H. Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. **Cerebellum**, [s. l.], 2005.

LAZARO, Maria T. et al. Reduced Prefrontal Synaptic Connectivity and Disturbed Oscillatory Population Dynamics in the CNTNAP2 Model of Autism. **Cell Reports**, [s.l.], 2019.

LE BELLE, Janel E. et al. Maternal inflammation contributes to brainovergrowth and autism-associated behaviors through altered redox signaling instem and progenitor cells. **Stem Cell Reports**, [s. l.], 2014.

LEE, Wen Chin et al. Mitochondrial fission increases apoptosis and decreases autophagy in renal proximal tubular epithelial cells treated with high glucose. **DNA andCell Biology**, [s. l.], 2016.

LEE, Yun Hee; PETKOVA, Anelia P.; GRANNEMAN, James G. Identification of an adipogenic niche for adipose tissue remodeling And restoration. **Cell Metabolism**, [s. l.], 2013.

LEGIDO, Agustín; JETHVA, Reena; GOLDENTHAL, MichaelJ. Mitochondrial dysfunction in autism.**Seminars in Pediatric Neurology**, [s. l.], 2013.

LETTIERI-BARBATO, Daniele et al. Maternal high calorie diet induces

mitochondrial dysfunction and senescence phenotype in subcutaneous fatof newborn mice. **Oncotarget**, [s. l.], 2017.

LI, Wen et al. Microglia have a more extensive and divergent response to interferon-αcompared with astrocytes. **GLIA**, [s. l.], 2018.

LI, Yan et al. Association Between DCC Polymorphisms and Susceptibility to Autism Spectrum Disorder. **Journal of Autism and Developmental Disorders**, [s. l.], 2020.

LIDDELOW, Shane A. et al. Neurotoxic reactive astrocytes are induced by activated microglia. **Nature**, [s. l.], 2017.

LJUBISAVLJEVIC, Srdjan. **Oxidative Stress and Neurobiology of Demyelination Molecular Neurobiology**,2016.

LOMBARDO, M. V. et al. Maternal immune activation dysregulationof the fetal brain transcriptome and relevance to the pathophysiology of autism spectrum disorder. **Molecular Psychiatry**, [s. l.], 2018.

LUCCHINA, Luciana; DEPINO, Amaicha Mara. Altered Peripheral and Central Inflammatory Responses in a Mouse Model of Autism. **Autism Research**, [s. l.], 2014.LUNA, Carolina; ESTÉVEZ, Mario. Oxidative damage to food and human serum proteins: Radical-mediated oxidation vs. glyco- oxidation. **Food Chemistry**, [s. l.], 2018.

LUO, Yuan et al. A multidimensional precision medicine approach identifies an autism subtype characterized by dyslipidemia. **Nature Medicine**, [s. l.], 2020.

LYALL, Kristen et al. Maternal dietary fat intake in association with autism spectrum disorders. **American Journal of Epidemiology**, [s. l.], 2013.

LYALL, Kristen et al. The Changing Epidemiology of Autism Spectrum Disorders. **Annual Review of Public Health**, [s. l.], 2017.

MA, Weiwei et al. Mitochondrial dysfunction and oxidative damage inthe brain of diet-induced obese rats but not in diet-resistant rats. **Life Sciences**, [s. l.], 2014.

MA, Zhiguo et al. Neuromodulators signal through astrocytes to alter neural circuitactivity and behaviour. **Nature**, [s. l.], 2016.

MAENNER, Matthew J. et al. Prevalence of autism spectrum disorder among children aged 8 Years-Autism and developmental disabilities monitoring network, 11 Sites, United States, 2016. **MMWR Surveillance Summaries**, [s. l.], 2020.

MÄKELÄ, Noora et al. Reaction pathways during oxidation of cereal β-glucans. **Carbohydrate Polymers**, [s. l.], 2017.

MANDAVILLI, Bhaskar S.; SANTOS, Janine H.; VAN HOUTEN, Bennett.

Mitochondrial DNA repair and aging. **Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis**, [s. l.], 2002.

MAREI, Waleed F. A. et al. Differential effects of high fat diet- induced obesity on oocyte mitochondrial functions in inbred and outbred mice.**Scientific Reports**, [s. l.],2020.

MARKRAM, Henry; RINALDI, Tania; MARKRAM, Kamila. **The Intense World Syndrome – an Alternative Hypothesis for Autism Frontiers in Neuroscience**, 2007.MAYES, Susan Dickerson et al. Use of the Childhood Autism Rating Scale (CARS) for children with high functioning autism or Asperger syndrome. **Focus on Autism and Other Developmental Disabilities**,[s. l.], 2012.

MCARDLE, Maeve A. et al. Mechanisms of obesity-induced inflammation and insulin resistance: Insights into the emerging role of nutritional strategie sFrontiers in Endocrinology, 2013.

MCGUINN, Laura A. et al. Early Life Exposure to Air Pollution and Autism Spectrum Disorder: Findings from a Multisite Case-Control Study. **Epidemiology (Cambridge, Mass.)**, [s. l.], 2020.

MEISTER, A.; ANDERSON, M. E. Glutathione. **Annual Review of Biochemistry**, [s.l.], 1983.

MERTENS, Jos J. W. M. et al. Reactive oxygen species and DNA damage in 2-Bromo- (glutathione-S-yl) hydroquinone-mediated cytotoxicity.**Archives of Biochemistry and Biophysics**, [s. l.], 1995.

MORAIS, Vanessa A. et al. Parkinson's disease mutations in PINK1 result in decreased Complex I activity and deficient synaptic function. **EMBO Molecular Medicine**, [s. l.],2009.

MORALES-HIDALGO, Paula et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Spanish School-Age Children. **Journal of Autism and Developmental Disorders**, [s. l.], 2018.

MORGAN, John T. et al. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. **Biological Psychiatry**, [s. l.],2010.

N.J., Minshew et al. A preliminary 31P MRS study of autism: Evidence for undersynthesis and increased degradation of brain membranes. **Biological Psychiatry**,[s. l.], 1993.

NADEEM, Ahmed et al. Oxidative and inflammatory mediators are upregulated in neutrophils of autistic children: Role of IL-17A receptor signaling. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, [s. l.], 2019.

NAKAGAWA, Tadashi et al. The Autism-Related Protein SETD5Controls Neural Cell Proliferation through Epigenetic Regulation of rDNA Expression. **iScience**, [s. l.], 2020.NARZISI, A. et al. Prevalence of Autism Spectrum Disorder in a large Italian catchment area: A school-based populationstudy within the ASDEU project. **Epidemiology and Psychiatric Sciences**, [s. l.], 2018.

NEGGERS, Yasmin H. et al. Maternal prepregnancy body mass index and psychomotordevelopment in children. **Acta Obstetricia et Gynecologica Scandinavica**, [s. l.], 2003.

NELSON, David L.; COX, Michael M. **Principos De Bioquimica (6thEd.)**. [s.l: s.n.].

NIMMERJAHN, A.; KIRCHHOFF, Frank; HELMCHEN, F.Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. **Neuroforum**, [s. l.], 2005.

NIRANJAN, B. G.; BHAT, N. K.; AVADHANI, N. G. Preferential attack of mitochondrial DNA by aflatoxin B1 during hepatocarcinogenesis. **Science**, [s. l.], 1982.

O'SHAUGHNESSY, Fergal et al. Prevalence of postpartum venous thromboembolism risk factors in an Irish urban obstetric population. **Journal of Thrombosis and Haemostasis**, [s. l.], 2019.

OHAMA, E. et al. Demyelination and remyelination in spinal cord lesions of human lymphotropic virus type I-associated myelopathy. **Acta Neuropathologica**, [s. l.], 1990.

OHTOMO, Ryo; IWATA, Atsushi; ARAI, Ken. **Molecular mechanisms of oligodendrocyte regeneration in white matter-related diseases International Journal of Molecular Sciences**, 2018.

OLSON, Julie K.; MILLER, Stephen D. Microglia Initiate Central Nervous System Innate and Adaptive Immune Responses through Multiple TLRs. **The Journal of Immunology**, [s. l.], 2004.

OPØIEN, Hans Kristian et al. Post-cesarean surgical site infections according to CDC standards: Rates and risk factors. A prospective cohort study. **Acta Obstetricia et Gynecologica Scandinavica**, [s. l.], 2007.

ORGANIZATION, World Health. **Obesity and Overweight factsheet from theWHO**. 2018.

ORRENIUS, Sten; GOGVADZE, Vladimir; ZHIVOTOVSKY, Boris. Mitochondrialoxidative stress: Implications for cell deathAnnual Review of Pharmacology and Toxicology, 2007.

OWEN, Julia P. et al. Brain MR imaging findings and associated outcomes in carriers of the reciprocal copy number variation at 16p11.2. **Radiology**, [s. l.], 2018.

PALHINHA, Lohanna et al. Leptin Induces Proadipogenic and Proinflammatory Signaling in Adipocytes. **Frontiers in Endocrinology**, [s. l.], 2019.

PALMIERI, L. et al. Altered calcium homeostasis in autism- spectrum disorders: Evidence from biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier AGC1. **Molecular Psychiatry**, [s. l.], 2010.

PANG, Can et al. Macrophage infiltration into adipose tissue may promote angiogenesis for adipose tissue remodeling in obesity. **American Journal of Physiology - Endocrinology and Metabolism**, [s. l.], 2008.

PAOLICELLI, Rosa C. et al. Synaptic pruning by microglia is necessaryfor normal brain development. **Science**, [s. l.], 2011.

PASCUAL, Olivier et al. Microglia activation triggers astrocyte- mediated modulation of excitatory neurotransmission. **Proceedings of the National Academy of Sciences of the United States of America**, [s. l.], 2012.

PATEL, Shrujna et al. Maternal immune conditions are increased in males with autism spectrum disorders and are associated with behavioural and emotional but not cognitive co-morbidity. **Translational Psychiatry**, [s. l.], 2020.

PAULA, Cristiane S. et al. Brief report: Prevalence of pervasive developmental disorder in Brazil: A pilot study. **Journal of Autism and Developmental Disorders**, [s. l.], 2011.

PEDRÓS, Ignacio et al. Early alterations in energy metabolism in the hippocampus of APPswe/PS1dE9 mouse model of Alzheimer's disease. **Biochimica et Biophysica Acta-Molecular Basis of Disease**, [s. l.], 2014.

PELPHREY, Kevin A. et al. Research review: Constraining heterogeneity: Thesocial brain and its development in autism spectrumdisorderJournal of Child Psychology and Psychiatry and Allied Disciplines, 2011.

PERSSON, Martina et al. Risk of major congenital malformations in relation to maternal overweight and obesity severity: Cohort study of 1.2 million singletons. **BMJ (Online)**, [s. l.], 2017.

PIZZARELLI, Rocco; CHERUBINI, Enrico. **Alterations of GABAergic signaling in autism spectrum disorders Neural Plasticity**, 2011.

PONTECORVI, Paola et al. Fibrinolysis protease receptors promote activation of astrocytes

to express pro-inflammatory cytokines. **Journal of Neuroinflammation**, [s.l.], 2019.

PORTIS, J. L. et al. Characterization of a neurologic disease induced bya polytropic murine retrovirus: evidence for differential targeting of ecotropic andpolytropic virusesin the brain. **Journal of Virology**, [s. l.], 1995.

POSTEMA, Merel C. et al. Altered structural brain asymmetry inautism spectrum disorder in a study of 54 datasets. **Nature Communications**, [s. l.], 2019.

POSTORINO, Valentina et al. **Anxiety Disorders and Obsessive- Compulsive Disorder in Individuals with Autism Spectrum Disorder Current Psychiatry Reports**, 2017.

POWELL, Elizabeth M.; GELLER, Herbert M. Dissection ofastrocyte-mediated cuesin neuronal guidance and process extension. **GLIA**, [s. l.], 1999.

QIU, Zhenmin; WANG, Yi; CHEN, Jie. Perinatal exposure to nonylphenol induces microglia-mediated nitric oxide and prostaglandin E2 production in offspring hippocampus. **Toxicology Letters**, [s. l.], 2019.

RAO, Patricia A.; LANDA, Rebecca J. Association between severity ofbehavioral phenotype and comorbid attention deficit hyperactivity disorder symptoms in children with autism spectrum disorders. **Autism**, [s. l.], 2014.

RIZWAN, Huma et al. High glucose augments ROS generation regulates mitochondrial dysfunction and apoptosis via stress signalling cascades in keratinocytes. **Life Sciences**, [s. l.], 2020.

RODNIGHT, Richard Burnard; GOTTFRIED, Carmem. **Morphological plasticity of rodent astroglia Journal of Neurochemistry**, 2013.

RODRIGUEZ, A. et al. Maternal adiposity prior to pregnancy is associatedwith ADHD symptoms in offspring: Evidence from three prospective pregnancy cohorts. **International Journal of Obesity**, [s. l.], 2008.

RODRIGUEZ, Alina. Maternal pre-pregnancy obesity and risk forinattention and negative emotionality in children. **Journal of Child Psychologyand Psychiatry and Allied Disciplines**, [s. l.], 2010.

RODRIGUEZ, Juan I.; KERN, Janet K. Evidence of microglial activation in autism and its possible role in brain underconnectivity. **Neuron GliaBiology**, [s. l.], 2011.

ROSE, S. et al. Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. **Translational Psychiatry**, [s. l.], 2012.ROSENGREN, Lars E. et al. A sensitive ELISA for glial fibrillary acidic protein:

application in CSF of children. **Journal of Neuroscience Methods**, [s. l.], 1992.

ROSSIGNOL, D. A.; FRYE, R. E. **Mitochondrial dysfunction in autism spectrum disorders: A systematic review and meta- analysis Molecular Psychiatry**, 2012.

ROTHSTEIN, Mark A.; CAI, Y.; MARCHANT, Gary E. The ghost in our genes: legal and ethical implications of epigenetics. **Health matrix (Cleveland, Ohio : 1991)**, [s. l.],2009.

ROTRUCK, J. T. et al. Selenium: Biochemical role as a component of glutathione peroxidase. **Science**, [s. l.], 1973.

SACCO, Roberto; GABRIELE, Stefano; PERSICO, Antonio M. Head circumference and brain size in autism spectrum disorder: A systematic review and meta-analysis. **Psychiatry Research - Neuroimaging**, [s. l.], 2015.

SAMARA, Amjad et al. Neuroinflammation and White Matter Alterations in Obesity Assessed by Diffusion Basis Spectrum Imaging. **Frontiersin Human Neuroscience** [s. l.], 2020.

SANCHEZ, C. E. et al. Maternal pre-pregnancy obesity and child neurodevelopmental outcomes: a meta-analysis International Journal of Obesity,2018.

SANDERS, Stephan J. et al. Insights into Autism Spectrum DisorderGenomic Architecture and Biology from 71 Risk Loci. **Neuron**, [s. l.], 2015.

SANTINI, Emanuela et al. Exaggerated translation causes synaptic and behaviour al aberrations associated with autism. **Nature**, [s. l.], 2013.

SARASTE, Matti. Structural features of cytochrome oxidase.**Quarterly Reviews of Biophysics**, [s. l.], 1990.

SARDINHA, Luís B. et al. Prevalence of Overweight, Obesity, and Abdominal Obesity in a Representative Sample of Portuguese Adults. **PLoS ONE**, [s. l.], 2012.

SASAYAMA, Daimei et al. Brief Report: Cumulative Incidence of Autism Spectrum Disorder Before School Entry in a Thoroughly ScreenedPopulation. **Journal of Autism and Developmental Disorders**, [s. l.], 2020.

SCHOEN, Michael et al. Shank3 transgenic and prenatal zinc- deficient autism mouse models show convergent and individual alterations of brain structures in MRI. **Frontiers in Neural Circuits**, [s. l.], 2019.

SCUDERI, Caterina et al. **Role of astrocytes in major neurologicaldisorders: The evidence and implications IUBMB Life**, 2013.

SEBAT, Jonathan et al. Strong association of de novo copynumber mutations with autism. **Science**, [s. l.], 2007.

SERAFIM, Teresa L. et al. Maternal obesity in sheep impairs foetal

hepatic mitochondrial respiratory chain capacity. **European Journal of Clinical Investigation**, [s. l.],2021.

SHAFRITZ, Keith M. et al. The Neural Circuitry Mediating Shifts inBehavioral Response and Cognitive Set in Autism. **Biological Psychiatry**, [s. l.], 2008.

SHARON, Gil et al. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. **Cell**, [s. l.], 2019.

SHEN, Yidong et al. Associations among maternal pre-pregnancy body mass index, gestational weight gain and risk of autism in the Han Chinese population. **BMC Psychiatry**, [s. l.], 2018.

SIMPSON, J. E. et al. Astrocyte phenotype in relation to Alzheimer-type pathology inthe ageing brain. **Neurobiology of Aging**, [s. l.], 2010.

SINGH, Prachi et al. Leptin induces C-reactive protein expression in vascular endothelial cells. **Arteriosclerosis, Thrombosis, and Vascular Biology**, [s. l.], 2007.SKOGSTRAND, Kristin et al. Reduced neonatal brain- derived neurotrophic factor is associated with autism spectrum disorders. **Translational Psychiatry**, [s. l.], 2019.

SPECCHIO, Nicola et al. Autism and Epilepsy in Patients WithTuberous Sclerosis Complex. **Frontiers in Neurology**, [s. l.], 2020.

SQUARZONI, Paola et al. Microglia Modulate Wiring of the Embryonic Forebrain. **Cell Reports**, [s. l.], 2014.

STERNBERG, Esther M. Neural regulation of innate immunity: A coordinated nonspecific host response to pathogens Nature Reviews Immunology, 2006.

STOGSDILL, Jeff A. et al. Astrocytic neuroligins control

astrocyte morphogenesis and synaptogenesis. **Nature**, [s. l.], 2017.

STOODLEY, Catherine J. et al. Altered cerebellar connectivity in autism and cerebellar-mediated rescue of autism-related behaviors in mice. **Nature Neuroscience**, [s. l.], 2017.

STOTHARD, Katherine J. et al. Maternal overweight and obesityand the risk of congenital anomalies: A systematic review and meta- analysis JAMA - Journal of the American Medical Association, 2009.

STRASSER, Barbara. **Immune-mediated inflammation as a driver of obesity**

and comorbid conditions Obesity, 2017.

SU, Li et al. Ultraviolet-Ray-Induced Sea Cucumber (Stichopus japonicus) Melting Is Mediated by the Caspase-Dependent Mitochondrial Apoptotic Pathway. **Journal of Agricultural and Food Chemistry**, [s. l.],2018.

SUZUKI, Katsuaki et al. Microglial activation in young adults With autism spectrum disorder. **Archives of General Psychiatry**, [s. l.], 2013.

T.C., Theoharides; D., Kempuraj; L., Redwood. **Autism: An emerging "neuroimmune disorder" in search of therapy Expert Opinion on Pharmacotherapy**, 2009.

TANG, Guomei et al. Mitochondrial abnormalities in temporal lobe of autistic brain. **Neurobiology of Disease**, [s. l.], 2013.

TANG, Min et al. Mitochondrial DNA damage induced by oxidative stress in retinal pigment epithelial cells. **Chinese Ophthalmic Research**, [s. l.], 2007.

TAY, Tuan Leng et al. A new fate mapping system reveals context- dependent randomor clonal expansion of microglia. **Nature Neuroscience**, [s.l.], 2017.

TETREAULT, Nicole A. et al. Microglia in the cerebral cortex in autism. **Journal of Autism and Developmental Disorders**, [s. l.], 2012.

THEODOSIS, Dionysia T. Oxytocin-secreting neurons: A physiological model of morphological neuronal and glial plasticity in the adult hypothalamus. **Frontiers in Neuroendocrinology**, [s. l.], 2002.

TOAL, F. et al. Clinical and anatomical heterogeneity in autistic spectrum disorder: A structural MRI study. **Psychological Medicine**, [s. l.], 2010.

TOSCANO, Chrystiane V. A. et al. Growth and weight status of Brazilian children withautism spectrum disorders: A mixed longitudinal study. **Jornal de Pediatria (Versão em Português)**, [s. l.], 2019.

TOSCANO, Chrystiane V. A. et al. Neuroinflammation in autism spectrum disorders: Exercise as a "pharmacological" tool. **Neuroscience & Biobehavioral Reviews**, [s. l.],2021.

TOZUKA, Yusuke et al. Maternal obesity impairs hippocampal BDNF production andspatial learning performance in young mouse offspring. **Neurochemistry International**, [s. l.], 2010.

TURRENS, Julio F. **Mitochondrial formation of reactive oxygen speciesJournal ofPhysiology**, 2003.

URSAN, Roman; ODNOSHIVKINA, Ulia G.; PETROV, Alexey M. Membrane cholesterol oxidation downregulates atrial β-adrenergic responses in ROS-dependentmanner. **Cellular Signalling**, [s. l.], 2020.

USHA KIRAN, T. S. et al. Outcome of pregnancy in a woman with anincreased body mass index. **BJOG: An International Journal of Obstetrics and Gynaecology**, [s. l.],2005.

VARCIN, Kandice J.; NEWNHAM, John P.; WHITEHOUSE, Andrew J. O. Maternal pre-pregnancy weight and autistic-like traits amongoffspring in the general population.**Autism Research**, [s. l.], 2019.

VARGAS, Diana L. et al. Neuroglial activation and neuroinflammationin the brain ofpatients with autism. **Annals of Neurology**, [s. l.], 2005.

VILHARDT, Frederik. **Microglia: Phagocyte And glia cell International Journal of Biochemistry and Cell Biology**, 2005.

VITKOVIC, L.; DA CUNHA, A. Role for astrocytosis in HIV-1-associated dementia. In: CURRENT TOPICS IN MICROBIOLOGY AND IMMUNOLOGY 1995, **Anais**... [s.l: s.n.]

VOGEL, Daphne Y. S. et al. Human macrophage polarization in vitro:Maturation andactivation methods compared. **Immunobiology**, [s. l.], 2014.

VON EHRENSTEIN, Ondine S. et al. Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: Population based case-controlstudy. **The BMJ**, [s. l.], 2019.

WADA, Nobuhiro et al. Leptin and its receptors. **Journal of Chemical Neuroanatomy**,[s. l.], 2014.

WANG, Jia et al. Neurexin gene family variants as risk factors for autism spectrumdisorder. **Autism Research**, [s. l.], 2018.

WANG, Ying et al. Maternal body mass index and risk of autism spectrum disorders inoffspring: A meta-analysis. **Scientific Reports**, [s. l.], 2016.

WANG, Ying et al. Probiotics and fructo-oligosaccharide interventionmodulate the microbiota-gut brain axis to improve autism spectrum reducing also the hyper- serotonergic state and the dopamine metabolism disorder.**Pharmacological Research**,[s. l.], 2020.

WEGIEL, Jerzy et al. The neuropathology of autism: Defects of neurogenesis and neuronal migration, and dysplastic changes. **Acta Neuropathologica**, [s. l.], 2010. WEI, Hongen et al. IL-6 is increased in the cerebellum of autistic brain and alters neural cell

adhesion, migration andsynaptic formation. **Journal of Neuroinflammation**, [s. l.], 2011.

WHITE, Christy L.; PURPERA, Megan N.; MORRISON, Christopher D. Maternal obesity is necessary for programming effect of high-fat diet on offspring. **American** Journal of Physiology - Regulatory Integrative and Comparative Physiology, [s. l.],2009.

WHO. State of Inequality Reproductive, maternal, newborn and child healthWorldHealth Organisation. [s.l: s.n.].

WHO. Prevalence of obesity among adults, BMI ≥ 30, age-standardized - Estimated byWHO region. **WHO**, [s. l.], 2017.

WIGGS, Kelsey K. et al. Anti-seizure medication use duringpregnancy and risk ofASD and ADHD in children. **Neurology**, [s. l.], 2020.

WINDHAM, Gayle C. et al. Maternal Pre-pregnancy Body Mass Index and GestationalWeight Gain in Relation to Autism Spectrum Disorder and otherDevelopmental Disorders in Offspring. **Autism Research**, [s. l.], 2019.

WORLD, Organization Health. World Health Organisation Obesity and OverweightFact Sheet. **Who**, [s. l.], 2016.

XIANG, Anny H. et al. **Maternal type 1 diabetes and risk of autism in offspringJAMA - Journal of the American Medical Association**, 2018.

XU, Guifeng et al. Maternal diabetes and the risk of autism spectrumdisorders inthe offspring: A systematic review and meta-analysisJournal ofAutism and Developmental Disorders, 2014.

YAO, Da et al. **Relationship between Maternal Central Obesity and the Risk of Gestational Diabetes Mellitus:A Systematic Review and Meta-Analysis of Cohort Studies Journal of Diabetes Research**, 2020.

YIN, Shanshan et al. Transplacental transfer of organochlorine pesticides: Concentration ratio and chiral properties. **Environment International**, [s. l.], 2019.YU, Anyong et al. IL-17A promotes microglial activation and neuroinflammation in mouse models of intracerebral haemorrhage. **Molecular Immunology**, [s. l.], 2016.

YUI, Kunio; SATO, Atsushi; IMATAKA, George. Mitochondrial Dysfunction and Its Relationship with mTOR Signaling and Oxidative Damage in Autism Spectrum Disorders. **Mini-Reviews in Medicinal Chemistry**, [s. l.], 2015.

ZARE, Sahar; MASHAYEKHI, Farhad; BIDABADI, Elham. The association of CNTNAP2 rs7794745 gene polymorphism and autism in Iranian population. **Journal of Clinical Neuroscience**, [s. l.], 2017.

ZHANG, Bo et al. Autism-associated neuroligin-4 mutation selectively impairs glycinergic synaptic transmission in mouse brainstem synapses. **Journal of Experimental Medicine**, [s. l.], 2018.

ZHANG, Jun; TROENDLE, James F. Maternal prepregnancy overweight and obesityand the pattern of labor progression in term nulliparous women. **Obstetrics and Gynecology**, [s. l.], 2004.

ZHANG, Mengli et al. SIRT5 deficiency suppresses mitochondrial ATPproduction andpromotes AMPK activation in response to energy stress. **PLoS ONE**, [s. l.], 2019.

ZHENG, Zhen; ZHENG, Peng; ZOU, Xiaobing. Association between schizophreniaand autism spectrum disorder: A systematic review and meta-analysis. **Autism Research**, [s. l.], 2018.

10. ANEXOS

NEUBIOREV-D-21-0

0147_R1 revised (1).

Neuroscience and Biobehavioral Reviews **Neuroinflammation in Autism Spectrum Disorders: Exercise as a "Pharmacological"Tool --Manuscript Draft--**

Manuscript Number:	NEUBIOREV-D-21-00147R1
Article Type:	Review Article
Keywords:	Autism Spectrum Disorders; neuroinflammation; microglia; Metabolic Disorders; Exercise; Cytokines
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Abstract:	The worldwide prevalence of ASD is around 1%. Although the pathogenesis of ASD is not entirely understood, it is recognized that a combination of genetic, epigenetics, environmental factors and immune system dysfunction can play an essential role in its development. It has been suggested that autism results from the central nervous system derangements due to low-grade chronic inflammatory reactions associated withthe immune system activation. ASD individuals have increased microglial activation, density, and increased proinflammatory cytokines in the several brain regions. Autism has no available pharmacological treatments, however there are pedagogical and psychotherapeutic therapies, and pharmacological treatment, that help to control behavioral symptoms. Recent data indicate that exercise intervention programs may improve cognitive and behavioral symptoms in children with ASD. Exercise can also modify inflammatory profiles that will ameliorate associated metabolic disorders. This review highlights the involvement of neuroinflammation in ASD and the beneficial effects of physical exercise on managing ASD symptoms and associated comorbidities.
Suggested Reviewers:	Marie-Ève Tremblay University of Victoria evetremblay@uvic.ca
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Response to Reviewers:	

Cover Letter

Dear Professor Giovanni Laviola

We are pleased to submit the revised manuscript "**Neuroinflammation in Autism Spectrum Disorders: Exercise as a "Pharmacological" Tool**", for consideration on Neuroscience & Biobehavioral Reviews.

We are very grateful for the positive, constructive, and helpful comments of the reviewers. The reviewers pointed out some issues that were not clearly explained in the previous version of our manuscript, and new topics were added to improve the manuscript. The manuscript is now corrected in the present revised version.

We acknowledge the reviewer's positive feedback, and the comments rose, which allow the improvement of the manuscript. We will answer all comments raised, and we addressed them in the manuscript accordingly and performed the changes required.

This manuscript contains material that is original and not previously published in text or on the Internet, nor is it being considered elsewhere until a decision is made as to its acceptability by the Neuroscience & Biobehavioral Reviews Editorial Review Board.

All authors have contributed to the project and manuscript, and all agree with the content of the manuscript, approve submission of this version of the manuscript, and take full responsibility for its content.

The authors transfer the publication rights for Neuroscience & Biobehavioral Reviews.

Hope the manuscript is in good order and meets the criteria of the Journal

Yours sincerely,Joana M. Gaspar

Response to Reviewers

Manuscript Number: NEUBIOREV-D-21-00147

Neuroinflammation in Autism Spectrum Disorders: Exercise as a "Pharmacological"Tool

Chrystiane V. A. Toscano, Leonardo Barros, Ahlan B. Lima, Thiago Nunes, Humberto

M. Carvalho, Joana M. Gaspar

We are very grateful for the positive, constructive, and helpful comments of the reviewers. The reviewers pointed out some issues that were not clearly explained in the previous version of our manuscript, and new topics were added to improve the manuscript. The manuscript is now corrected in the present revised version. Hopefully, we addressed all the reviewers´ concerns.

Reviewers' comments:

Reviewer #1: This manuscript reviews various aspects of autism spectrum disorder (ASD), including neuroinflammation and exercise interventions. The topic is interesting and highly relevant to this journal. Whilst generally clearly written, there are aspects that could be improved, as detailed below.

1. A bit more in-depth discussion could be added to more substantially address the roleof neuroinflammation in the pathogenesis of ASD and the therapeutic potential of exercise and physical activity.

R: We agreed with the reviewer's comment, and we added a more detailed discussion inthe revised manuscript.

2. It would be more helpful if they can be more precise when they were describing particular topics and issues. Examples include: "decrease in stereotypical

behavior" - to what extent? "Improvement of social interaction" - in what kind of scenarios and with which measures?

R: We agree with the reviewer's comment, and added a more detailed explanation in therevised manuscript.

3. The authors mentioned that maternal immune activation could increase likelihood of ASD in offspring. Is there any published research showing children from exercised mothers showed less chance of having ASD? This would be one way to better tie the neuroinflammation and exercise sections.

R: This is a very interesting point raised by the reviewer. Unfortunately, no published research showing children from exercised mothers showed less chance of having ASD toour best knowledge. However, parental health and lifestyle influence embryonic development and susceptibility to disease in the offspring by transgenerational epigenetic mechanisms (Goli and Yazdi, 2021; Yeshurun and Hannan, 2019). Non-genetic inheritance of acquired traits associated with parental environmental exposures, mainly this transgenerational modulation of phenotypic traits, is directly relevant to psychiatric disorders. A positive environment, such as physical exercise, environmental enrichment, or other enhanced motor, sensory, and cognitive stimulation. Three months of exercise training in humans was found to modify the DNA methylation profile in their sperm, specifically in genes that are related to several brain disorders, including autism, schizophrenia, and Parkinson's disease, suggesting that the effects of exercise can be epigenetically inherited (Denham et al., 2015)

Regarding paternal influence, it was demonstrated

that offspring of exercised fathers displayed higher levels of memory ability and lower level of brain-derived neurotrophic factor, suggesting an intergenerational effect of physical activity on cognitive benefit, which may be associated with hippocampal epigenetic programming in offspring (Goli and Yazdi, 2021).

Some studies use animal models showing that maternal exercise during gestation has beneficial effects in developing neural circuits and the offspring's well-being. In an animal model of prenatal stress, which has been associated with increased vulnerability to psychiatric disturbances such as autism, the authors show that voluntary exercise appeared to have a neuroprotective effect, with an improvement in motor control and a decreased dopamine cell loss in substantia nigra (Mabandla et al., 2009).

The role of voluntary exercise in modulating behavioral and synaptic abnormalities in neurodevelopmental disorders was studied in a mouse model of a neurodevelopmental disorder induced by maternal immune activation (Andoh et al., 2019). It was observed that voluntary wheel running ameliorates the abnormalities in sociability, repetitiveness, and anxiety. Exercise activates a portion of dentate granule cells, normalizing the density synapses, excessive in the MIA-affected offspring. Deficits induce the synaptic surplus in the MIA offspring in synapse engulfment by microglia, normalized by exercise throughmicroglial activation (Andoh et al., 2019).

In a diet-induced obesity model, maternal exercise during gestation can completely mitigate metabolic impairment in adult offspring in mice (Laker et al., 2021). Maternal obesity results in hypermethylation of the Pgc-1α promoter at CpG-260, which can be abolished by maternal exercise (Laker et al., 2021).

However, there is an urgent need for more research exploring transgenerational epigenetic effects in animal models and human populations for ASD development, giving special attention to the role of exercise. These future studies may identify epigenetic mechanisms as potential contributors to the "missing heritability" observed in genome-wide association studies of psychiatric illnesses and other human disorders, facilitating the development of novel strategies to predict, prevent consequences on offspring health andpsychiatric disorders in particular.

4. The authors could better address the role of epigenetics in ASD, which is relevant to both neuroinflammation and exercise. Whilst the primary literature may be too large to address here, there are a number of relevant reviews:

R: We agree with the reviewer's suggestion, and included a new section in the manuscript entitled "Epigenetics in the etiology of ASD".

5. The authors should better address the literature on environmental enrichment (which enhances both cognitive stimulation and physical exercise) on both preclinical and clinical ASD and disorders with autistic features.

R: The reviewer raises a good point. We included a new section in the manuscript about Environmental enrichment.

Reviewer #2: The paper is very interesting as it focuses on a rather novel, but potentiallyimportant aspect for the therapeutic framework of Autism Spectrum Disorder, that is the link between contributive factors for the etiopathogenesis of this condition and possible innovative treatments that could be adapted to the individuals, with

manifold positive outcome expected to occur. However, there are several issues identified in this work, preventing it to be published without a substantial revision.

1. First of all, throughout the manuscript there are several redundancies, making the paperhard to be logically followed. As such, also the quality of English language and grammaris quite low and should be significantly improved. Careful proofread should be performed, checking and correcting the several typos present in the paper.

Also, the logical flow between the factors contributing to ASD and the physical activity as a novel treatment method should be better stated.

R: We agree with the reviewer's comment. We revised all the manuscript

2. In the Introduction, the inclusion of recent epidemiological data (see Maenner et al., 2020 for example) is desirable.

R: These were corrected in the manuscript, and the reference was added.

3. The discussion about the factors somewhat contributing to the burden of autism worldwide should also include a part describing the lack of vitamin D as possible furtherfactor, and the hyper/hypo-sensoriality, since it was mentioned earlier on, but not properlyfaced.

R: We agree with the reviewer's comment. Changes were made as suggested.

We added to the manuscript a brief explanation about the vitamin D deficiency in pregnant women as a risk factor for ASD.

4. Finally, a stronger take-home message should be included. There is no real recommendations about how to

use physical activity as a mean to improve clinical features of subjects with ASD, whether it would be feasible for any ASD individual, independently from their age, IQ and clinical characteristics.

R: We agree with the reviewer's comment and a take-home message was added in the manuscript as suggested.

Minor:- Highlights seem to be incomplete (especially point 2)R: We corrected the highlights.

Highlights:

- Neuroinflammation is involved in the etiology of Autism Spectrum Disorder.
- Microglia activation is detected in postmortem samples of ASD subjects
- Maternal immune activation induces the development of autism spectrum disorder.
- Exercise improves autism symptomatology and associated comorbidities.
- Structured and personalized exercise should be used to managed ASD symptoms.

Abstract:

The worldwide prevalence of ASD is around 1%. Although the pathogenesis of ASD is not entirely understood, it is recognized that a combination of genetic, epigenetics, environmental factors and immune system dysfunction can play an essential role in its development. It has been suggested that autism results from the central nervous system derangements due to low-grade chronic inflammatory reactions associated with the immune system activation. ASD individuals have increased microglial activation, density, and increased proinflammatory cytokines in the several brain regions.

Autism has no available pharmacological treatments, however there are pedagogical and psychotherapeutic therapies, and pharmacological treatment, that help to control behavioral symptoms. Recent data indicate that exercise intervention programs may improve cognitive and behavioral symptoms in children with ASD. Exercise can also modify inflammatory profiles that will ameliorate associated metabolic disorders.

This review highlights the involvement of neuroinflammation in ASD and the beneficial effects of physical exercise on managing ASD symptoms and associated comorbidities.

Keywords: Autism Spectrum Disorders, Neuroinflammation, Microglia, Metabolic Disorders, Exercise, Cytokines

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Neuroinflammation in Autism Spectrum Disorders: Exercise as a"Pharmacological" Tool

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not entirely understood, it is recognized that a combination of genetic, epigenetics, environmental factors and immune system dysfunction can play an essential role in its development. It has been suggested that autism results from the central nervous system derangements due to low-grade chronic inflammatory reactions associated with the immune system activation. ASD individuals have increased microglial activation, density, and increased proinflammatory cytokines in the several brain regions.

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However, the brain in ASD adults may be normal or decreased compared to children with ASD (Ha et al., 2015).

ASD diagnosis is completed according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Clinical signs emerge in childhood and persist into adulthood. ASD is characterized by deficits mainly in social communication and interaction, nonverbal communication, skills in developing, maintaining, and understanding relationships, and the presence of a restricted and/or repetitive pattern ofbehavior (Figure 1) (American Psychiatric Association, 2014). A high prevalence of sensory symptoms has been documented, with reports ranging from 60 to 96% of children with ASD exhibiting some degree of atypical responses to sensory stimuli (Baranek et al., 2013; Dunn et al., 2002). Sensory differences may contribute to many higher-order cognitive and social deficits associated with ASD [see review (Robertson and Baron-Cohen, 2017)]. Patients must have evidence of two of four subdomains of repetitive or restricted behaviors such as insistence on sameness, highly restricted or fixed interests, hypersensitivity or hyposensitivity, and stereotyped behavior (American Psychiatric Association, 2014).

In 2015 was estimated 52 million cases of ASD in the world, equivalent to a prevalence of one in 132 people (Baxter et al., 2015). Autism is more common in males than females, with a 3:1 ratio (Loomes et al., 2017). In the US, the prevalence of ASD is one in 54 children aged 8 years, with a 4.3 times higher prevalence among boys than girls (Maenner et al., 2020). The prevalence of ASD dramatically increased in the last years, caused by how environmental factors interact with the underlying genetics to change brain development in ASD (Loke et al., 2015; Vogel Ciernia and LaSalle, 2016).

Genetic alterations associated with ASD may be a consequence of single-gene mutations or copy number variations (such as duplications, large deletions, inversions, and translocations of chromosomes) (Garcia-Forn et al., 2020). In particular, the perinatal environment has attracted much attention for its implications on brain development and functions. Environmental risk factors that affect ASD neurodevelopment and result in long-term alterations in brain physiology include neonatal hypoxia (Modabbernia et al., 2016), maternal obesity (Windham et al., 2019), valproate use during pregnancy (Christensen et al., 2013), maternal diabetes (Xiang et al., 2015), and advanced maternal and paternal age (Gao et al., 2020). Another important risk factor for ASD is the reduced levels of vitamin D in pregnant women and their infants and toddlers (Magnusson et al., 2016; Vinkhuyzen et al., 2018). Also, obese subjects have vitamin D deficiency (around

35% higher than lean subjects) (Pereira-Santos et al., 2015), suggesting that both associated maternal obesity and vitamin D deficiency can increase the risk of offspring Children with ASD may be suffering from autoimmune disorders (Ormstad et al., 2018). Associated with the immune system activation, it has also been suggested that autism results from the central nervous system derangements due to chronicinflammatory This mini-review focused on neuroinflammatory mechanisms involved in the biogenesis of autism spectrum disorders and a special attention was given to the exercise as a pharmacological tool for managing ASD symptoms.

2. Physiopathology of Autism Spectrum Disorders

Autism results from alterations in the central nervous system's development, which change the brain's physiology and behavior. The physiopathology of autism remains unclear, but it likely involves several systems connectivity, neural, biochemical, neuroanatomical, cellular, and molecular features.

Brain areas involved in emotional control, social interactions, and motor coordination are compromised in subjects with ASD. Postmortem samples from ASD subjects show abnormalities in brain regions, particularly in the cerebellum, hippocampus, cortex, brainstem, and other subcortical areas.

Meta-analyses studies suggest consistent neuroanatomical changes both in grey-matter (such as the amygdala, hippocampus, and precuneus) and in white-matter structures arcuate and uncinate fasciculi) (Bourgeron, 2015; Eissa et al., 2018; Mann et al., 2020; Mei et al., 2020; Varghese et al., 2017). The medial prefrontal cortex, superior temporal sulcus, temporo parietal junction, amygdala, and fusiform gyrus are hypoactive regions in markers of neuronal overgrowth and an increase in the neuronal dendritic spine in young children diagnosed with ASD compared to control brain tissue (Hutsler and Zhang, 2010).

Replicating some of the neuropathological features seen in postmortem studies, a common finding in animal models of ASD is the altered density of dendritic spines (Varghese et al., 2017). The lateral nucleus of amygdala had reduced neuronal cells (Schumann and Amaral, 2006).

It has been documented, by electroencephalography, relevant differences in the timing of the response to auditory, visual, and tactile input in ASD subjects. Increased cortical representation of the visual periphery in ASD subjects was reported, suggesting early

hyper-responsiveness (Frey et al., 2013). The neural responses to tactile stimulation are related to tactile hyper-responsiveness. In contrast, slightly later neural responses are related to tactile hypo-responsiveness and may involve higher-level processes such as attention, allocation, and assignment of emotional valence (Cascio et al., 2015). Using functional magnetic resonance imaging, different spatial activation patterns across cortical areas of the brain were observed as being responsible for these earlier and later sensory processing stages (Schauder and Bennetto, 2016).

Other modifications reported in ASD subjects include a significant increase in the neuropil in the frontal cortex and the anterior cingulate gyrus, reduced dendritic branching

The genetic component is undoubtedly involved in the pathogenesis of ASD, with hundreds of genes linked to this pathology. However, each gene contributes to only a tiny percentage of the affected population. Recent large-scale genetic studies have highlighted hundreds of genes that have a role in synaptic function and development as risk factors for ASD pathogenesis (Bourgeron, 2015; Sanders et al., 2015).

Neuroligins (NLGNs) are well-characterized neuronal, post-synaptic, cellular adhesion molecules that mediate synaptic formation and function. NLGNs are composed of five family members in humans (NLGN1, 2, 3, 4X, and 4Y). Mutations in genes encoding

NLGN3 and NLGN4 are associated with autism (Cast et al., 2021; Jamain et al., 2003; Kopp et al., 2020; Yan et al., 2005). More recently, a nonsense variant of NLGN2 in a human was associated with a neurobehavioral phenotype including anxiety, autism, hyperphagia, and obesity (Parente et al., 2017). Expression levels of NLGN2 influence the balance between excitatory and inhibitory neuronal signals, and dysregulation in this balance is associated with neurobehavioral disturbance, including autism (Bang and Owczarek, 2013; Rubenstein and Merzenich, 2003; Steffen et al., 2021). Alterations in several neurotransmitters are implicated in the development of ASD, including serotonin, dopamine, noradrenaline, GABA, and glutamate (Quaak et al., 2013). For instance, serotonin is involved in sleep, sensory perception, and appetite, which are often disrupted in ASD (Janusonis, 2014).

3. Epigenetics in the etiology of ASD

The difficulty in understanding the etiology and pathophysiological mechanisms of ASD development arises from the complexity and highly interdependent interactions across body systems.

Epigenetics is a mechanism that alters gene activity without changing the DNA sequence, leading to genomic modifications that are heritable during cell division (Waddington, 2011). Epigenetics is critical for the normal development and functioning of the human brain, representing an essential mechanism by which the environment can act on the genome leading to persistent changes in gene expression/ function. However, when epigenetics occurs improperly, they can induce significant adverse health and behavioral effects. Epigenetics modifications can influence and regulate gene activity both at the transcriptional and translational levels. The most characterized epigenetic changes are DNA methylation and post-translational modifications of histones (Vogel Ciernia and LaSalle, 2016). Epigenetic mechanisms generally result in the silencing or expression of genes, and therefore their occurrence during development can have a significant impact on the individual´s behavior. Epigenetic alterations can affect the expression of neuronal proteins, hormonal receptors, and cytokines, and therefore have long-term consequences on the brain.

These factors negatively impact brain development during fetal life and have been found to be associated with ASD development.

Maternal immune activation during prenatal development induces epigenetic alterations in the brain [DNA methylation (Basil et al., 2014), histone methylation (Connor et al., 2012), and miRNA expression (Hollins et al., 2014)], which is correlated with autism.

For instance, microglia from the offspring of dams with allergic asthma displays hypermethylation of pro-inflammatory genes associated with autism (Vogel Ciernia et al., 2018). Maternal overnutrition during pregnancy modulates the epigenome in offspring and increases ASD susceptibility (Banik et al., 2017). Also, obesity as a chronic pro-inflammatory condition is a risk factor for developing neurologic diseases and altered behavior such as ASD (Zheng et al., 2017). It potentially might set a selective epigenetic program in offspring. However, the effect of maternal high-fat diet consumption during gestation and its role on epigenetics modulation of immunity for ASD development has not been completely identified yet.

Genome-wide alterations in DNA methylation in ASD brain samples have shown preliminary evidence to support the role of epigenetics in autism. DNA methylation in neurodevelopmental genes (such as PRRT1, TSPAN32, SPI1, IRF8, TNF-α, ITGB2, NRXN1, SH3, and SHANK3) encode proteins involved in the formation of synapses and the immune system. These are often changed in the dorsolateral prefrontal cortex, temporal cortex, cerebellum, and anterior cingulate gyrus of ASD subjects (Ladd-Acosta et al., 2014; Nardone et al., 2014; Nardone et al., 2017). Also, methylated genes involved in microglial cell specification and synaptic pruning during brain development were the most affected (De Rubeis et al., 2014; Nardone et al., 2017). It was also detected in

al., 2017). The offspring of the autoimmune ASD mice model showed hypermethylated DNA regions in several transcription factor motifs critical for early microglial development and immune activation, and the cytokines IL-6, IL4, IL-8, and Jak-STAT, TNF, and mTOR signaling (Vogel Ciernia et al., 2018).

Altogether, data support the hypothesis that environmental factors occurring in-utero, especially those with stress or inflammatory-related component, can alter epigenetic programming, in term of DNA methylation, contributing to neurodevelopmental and behavioral deficits in the off spring.

4. The role of maternal immune activation in the etiology of ASD

Multiple prenatal exposures, especially infection, have been linked to an increased risk of ASD in offspring. Perinatal brain development, especially during the first weeks of neonatal life, is particularly susceptible to abnormal immune activation consequences with detrimental consequences on brain development and, consequently, being important in the etiology of neuropsychiatric conditions such as ASD (Deverman and Patterson, 2009; Tsafaras et al., 2020) (Figure 2).

Clinical and preclinical studies have suggested a link between maternal immune activation during pregnancy and the development of autism spectrum disorders (Atladóttir et al., 2009; Atladóttir et al., 2010; Brown et al., 2014; Choi et al., 2016; Lee et al., 2015; Rudolph et al., 2018). An emerging human diagnostic marker for ASD is detecting increased IL-6 concentrations in the umbilical cord plasma and elevations in several other cytokines (Madsen-Bouterse et al., 2010).

hat could cross the placenta and alter fetal brain development (Braunschweig et al., 2012; Dalton et al., 2003; Zimmerman et al., 2007).

Severe bacterial or viral infections in pregnancy are similarly associated with an increased risk of ASD in children (Atladóttir et al., 2010). Increasing maternal levels of C-reactive protein were significantly associated with autism in offspring, suggesting that maternal inflammation may significantly impact ASD development (Brown et al., 2014). Increase concentrations of IFN-γ, IL-4, and IL-5 in the maternal serum of during midgestation were significantly associated with a 50% increased risk of ASD (Goines et al., 2011). Increasing the plasma level of cytokines in the mother can cross the placental and blood-

brain barrier to trigger cytokine expression in the brain.

In preclinical studies with rodent models, it has been observed that prenatal lipopolysaccharide (LPS) injections induce activation of the immune system with alterations in synaptic plasticity, white matter development, and in social, cognitive, and motor behaviors of offspring (Choi et al., 2016; Fernández de Cossío et al., 2017; Foley et al., 2015; Urakubo et al., 2001). In detail, LPS injection in E16 pregnancy rats induces an increase in IL-1β, IL-6, and TNF-α in the placenta and amniotic fluid. An increase in TNF-α was also observed in the fetal brain (Urakubo et al., 2001). Maternal immune activation induced by LPS injection in E17 mice induces an increased number of pyramidal and granular cells in the hippocampus, as well as the shrinkage of pyramidal cells, impaired distinct forms of learning and memory, but not motor function or exploration in the adult offspring (Golan et al., 2005). Specifically, IL-17 seems to have

Maternal nutrition directly affects offspring's health. There is a significant vitamin D deficiency in pregnant women and their infants and toddlers, and it is associated with the current increase in ASD. Vitamin D has anti-inflammatory effects, reducing the risk and severity of inflammatory cytokines in the brain. The lowest vitamin D levels during the first trimester of pregnancy were associated with a fourfold risk of ASD in the offspring (Chen et al., 2016; Vinkhuyzen et al., 2018). Calcitriol (Vitamin D) has anti-autoimmune effects, increases T-regulatory cells, protects neural mitochondria, and can be used to alleviate neuroinflammation (Huang et al., 2015). Obesity is also a risk factor for vitamin D deficiency.

Obesity is considered a low-grade chronic inflammatory disease. Rates of maternal obesity have risen due to more significant numbers of obese pregnant women and excess weight gain during pregnancy. Maternal obesity influences the health of both the mother and child. A clear association between maternal obesity and cognitive function, mental health, and increased risk of autism spectrum disorder in the offspring may be influenced by additional biological or social factors (Contu and Hawkes, 2017; Hatanaka et al., 2017). Large cohort studies from Canada, the US, and the UK observed that children born to obese mothers were more likely to be diagnosed with autism (Bilder et al., 2013; Dodds et al., 2011; Krakowiak et al., 2012). High-fat diet-fed dams generate offspring with increased anxiety- and depression-related behaviors and decreased cognitive functions,

correlated with microglial activation and increased expression of proinflammatory cytokines in the amygdala and hippocampus (Bilbo and Tsang, 2010). Maternal high-fat inflammatory cytokines, leptin, and insulin in obese mothers severely influence fetal brain development (Estes and McAllister, 2015; Rivera et al., 2015). The effect of gestational programming by high-fat diet consumption sets a pro-inflammatory profile is partially dependent on an epigenetic program of immunity, promoting brain structural abnormalities in the offspring.

In conclusion, acute or chronic maternal immune activation alters proinflammatory cytokine levels in the fetal environment, impacting neuroinflammation mechanisms, developing brain, and consequently ASD development.

5. Neuroinflammation in Neurodevelopment Disorders

The complexity and high interactions across body systems during the course and development of ASD make it difficult to clarify this disorder's etiology. However, several studies hypothesized that chronic inflammation and neuroinflammation during early brain development could cause behavioral and cognitive impairments, impacting the etiological pathway of ASD (Ashwood et al., 2011; Liao et al., 2020; Sciara et al., 2020) (Figure 2). Inflammation and dysregulation of the immune system are clinical features of ASD (Ashwood et al., 2011; Ashwood and Wakefield, 2006). Elevated plasma levels of pro-inflammatory cytokines such as IL-1β, IL-6, IL-17, IL-12p40, and TNF-α were found in 3 to 9-year-old children with ASD (Inga Jácome et al., 2016b). The plasma cytokine levels were also correlated with the severity of ASD symptoms, meaning that increasing cytokine levels were associated with more impaired communication and aberrant behaviors (Ashwood et al., 2011; Inga Jácome et al., 2016a). Peripheral inflammatory

markers can also predict comorbidities in autism, as well as reinforce and aid informed decision-making in ASD (Inga Jácome et al., 2016a), highlighting the role of chronic inflammation in ASD etiology.

The inflammatory process associated with ASD is extended to the central nervous system as neuroinflammation. Neuroinflammation is a well-orchestrated inflammatory response within the brain or spinal cord, mediated by various groups of glial cells, particularly astrocytes and microglial cells. The inflammatory mediators, namely cytokines, chemokines, reactive oxygen species, and secondary messengers, are mainly produced by microglia, astrocytes, and peripherally derived immune cells (DiSabato et al., 2016). Astrocytes and microglia act in concert as a surveillance system in the central nervous system, ensuring efficiency of the inflammatory processes against pathogens without disrupting homeostasis under physiological conditions. Astrocytes are the most abundant cells in the central nervous system, with essential roles in support neuronal function, transport of substances across the blood-brain barrier, energy storage, regulation of neurotransmission, and the immune regulation of the brain. Astrocytes can maintain brain homeostasis in response to metabolic alterations by sensing nutrients, hormones, and other metabolites (Meldolesi, 2020). Microglial cells contribute to immune surveillance in the central nervous system. Microglia can acquire various phenotypes that determine the consequences of the inflammation, controlling the balance between promotion and suppression in neuroinflammation. Microglia are responsible for regulating the majority of cytokine levels in extracellular areas of neighboring neurons and astrocytes (Eroglu and Barres, 2010). Both microglia and astrocytes modify their morphology to rapidly adapt to brain changes, influencing each other with a series of stimulatory signals, such as cytokines, and ATP, to initiate an immune response (DiSabato et al., 2016). As a disturbing process occurring at early brain development stages, chronic neuroinflammation can be involved in the biogenesis of behavioral and cognitive impairments. Neuroinflammation plays a role in developing and maintaining the dendritic spines in glutamatergic and GABAergic neurotransmission (Alabdali et al., 2014; El-Ansary and Al-Ayadhi, 2014). Cytokines can impact the length, location, and organization of dendritic spines on excitatory and inhibitory neurons and recruit and impact glial cell function around the neurons (Eroglu and Barres, 2010), with

consequences on the development that may ultimately contribute to the ASD behavioral and cognitive symptoms.

ASD children had increased numbers of circulating monocytes, essential precursors for macrophages, dendritic, and microglial cell activation (Rodriguez and Kern, 2011; Sweeten et al., 2003). Additionally, evidence of immune system activation, including an abnormal CD4:CD8 T cell ratio, a high number of DR+ (activated) T cells, high urinary neopterin levels, and increased cytokine production, has been reported in autistic children (Sweeten et al., 2003). Immunoreactivity of astrocytes was detected in brain regions of ASD subjects. A significant increase in GFAP was observed in the postmortem superior

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frontal cortex, parietal cortex, prefrontal cortex, and cerebellum (Edmonson et al., 2014; Laurence and Fatemi, 2005). GFAP levels are also increased in fresh-frozen brain tissue of the cerebellum, middle frontal gyrus, and anterior cingulate gyrus from ASD subjects (Vargas et al., 2005).

Samples from cerebrospinal fluid, blood, and postmortem brain tissue from ASD patients have shown an imbalance in the immune cell with changes in response patterns, the presence of autoantibodies, increased levels of proinflammatory cytokines (Businaro et al., 2016; Masi et al., 2015; Nadeem et al., 2019), decreased levels of anti-inflammatory brain sections of the dorsolateral prefrontal cortex, with markedly morphological alterations of microglia including enlargement of soma, retraction, and thickening of processes, and extension of filopodia (Morgan et al., 2010). This study also shows an increase in microglial cell density in the gray-matter and an increased somal volume of microglia in the white-matter of ASD subjects (Morgan et al., 2010). Amygdala also shows strong signs of excessive microglia activation; however, an evident heterogeneity activation profile within the ASD cohort was detected (Morgan et al., 2014).

Changes in microglia density can be accompanied by changes in microglia phenotype and morphology, which correlate with specific functions and indicate the brain's pathophysiological state. Six structurally and functionally microglial phenotypes were quantified using an unbiased stereological approach in the postmortem human temporal cortex (immuno-stained with Iba1) of ASD subjects, showing a decreased density of ramified microglia. However, an increased density of primed microglia in ASD subjects compared with controls, whereas no group difference in the total density of all microglial phenotypes was found (Lee et al., 2017). Positron emission tomography and a radiotracer for microglia were used to identify brain regions associated with excessively activated

microglia and distribution patterns in the whole brain of men diagnosed with ASD (Suzuki et al., 2013). The authors identify increased microglia activity in the cerebellum, fusiform gyri, and the anterior cingulate and orbitofrontal cortices (Suzuki et al., 2013).

Microglial-specific markers TREM2, DAP12, and CX3CR1, were detected with a higher expression in the prefrontal cortex of autistic people compared to matched controls. The expression of TREM2 was the highest of all microglial markers in brain tissue from ASD patients (Edmonson et al., 2014). However, in the postmortem cerebellum, the expression of microglial markers TREM2, DAP12, CX3CR1, and AIF1 was lower in autism tissue than in control tissue (Edmonson et al., 2014). Corroborating the neuroinflammatory profile of microglia activation in postmortem brain of ASD subjects is the higher levels of proinflammatory cytokines (such as IL-1β, IL-6, IL-8, INF-γ, and TNF-α) detected in both brain specimens and blood of autistic patients compared with controls (Li et al., 2009; Vargas et al., 2005; Wei et al., 2011). The middle frontal gyrus of ASD patients showed an increase TGF- β1, increased MCP-1, IL-6, and IL-10 in the anterior cingulate gyrus, and only MCP-1 was increased in CSF (Vargas et al., 2005). However, in the dorsolateral prefrontal cortex of ASD subjects, there are no changes in characteristic markers of microglial activation, such as IL-6, IL-1 β, and TNF-α (Chana et al., 2015). Elevated levels of TNF-α are associated with disrupted pineal melatonin release and sleep dysfunction in ASD (da Silveira Cruz-Machado et al., 2021). It is proposed that circadian dysregulation in ASD is intimately linked to amplified activation of the Th1 pathway rather than activation of the Th2 pathway (Li et al., 2009).

In a mouse model of autism, the increased levels of IL-6 were associated with neuro-anatomical abnormalities (Wei et al., 2012a; Wei et al., 2012b). Both excitatory and inhibitory synapse formation and transmission are changed by increased levels of IL-6, as well as the shape, length, and distribution pattern of dendritic spines (Wei et al., 2012a).

These observations suggest that innate neuroimmune reactions may play a pathogenic role in ASD.

Altogether, these studies demonstrate abnormal microglial-specific gene expression in autistic brains, indicating that microglial activation patterns may play a pathogenic role in ASD development. Future therapies might involve drugs that modify neuroglial responses in the brain. While the precise influence of peripheral cytokines on the central

nervous system immune environment in ASD has yet to be elucidated, neuroimmune alterations can be responsible for the phenotypic heterogeneity and severity observed in patients with autism. Indeed, many of the ASD features seem to have different severity levels also depending on changes in immune responses, confirming the intimate bond between neurodevelopment and immune processes

5. Benefits of Exercise in ASD

Physical exercise is a non-pharmacological intervention with well-documented beneficial effects both in healthy people and in people with metabolic and neuropsychiatry disorders, such as depression, anxiety, schizophrenia, dementia, and other neurodegenerative diseases (Chen et al., 2020; Zhao and Jiang, 2020). Currently, no drugs are available to cure autism. However, ASD treatment requires integrated approaches, such as pedagogical and psychotherapeutic interventions and pharmacological treatment, to control specific behavioral symptoms, such as self- and hetero-aggression, hyperactivity, stereotyped behavior, and insomnia.

From the 1970s, the first studies related to the positive effects of the intervention with physical exercise, as an adjunct treatment, for the population with ASD (Figure 3) (Best and Jones, 1974). Systematic reviews and meta-analysis studies have shown the existence of a positive relationship between physical exercise and the reduction of stereotypical behavior (Petrus et al., 2008; Toscano et al., 2018a), as well as, reduction deficits in social interaction (Bremer et al., 2016) in ASD subjects. However, in some studies the description of the exercise interventions models for the ASD population are still not clear in specific details such intensity, volume and frequency of exercise (Bremer et al., 2016; Lang et al., 2010; Sowa and Meulenbroek, 2012), as well as in the diagnostic category of ASD (Fragala-Pinkham et al., 2008; Fragala-Pinkham et al., 2011; Hinckson et al., 2013). Besides the beneficial effect of exercise in ASD, there are no specific recommendations for the characteristics of the exercise intervention programs as well as procedures to motivate the adherence and participation of ASD subjects in physical exercise programs.

Studies have proposed that limited levels of physical activity, motor skills and fitness, particularly in children and adolescents with ASD, may accentuate social and emotional

deficits and the associated comorbidities (Bandini et al., 2013; Batey et al., 2014; Bremer et al., 2016; Curtin et al., 2014; Tyler and MacDonald, 2014). More specifically, children with poor motor coordination spent significantly less time doing moderate to vigorous physical activity (measured over seven days using accelerometry), and physical exercise reduces physical motor deficits in children with developmental coordination disorder (Batey et al., 2014). Exercise improves psychopathological profile and cognitive function, and also decrease of behavioral stereotypy (by recording the frequency of predetermined child-specific stereotypic behaviors from baseline recordings) and aggressive behaviors (Bremer et al., 2016; Oriel et al., 2011; Tan et al., 2016).

Intervention studies with aerobic physical exercises, aquatic and terrestrial, have shown a reduction in stereotyped behaviors (Bahrami et al., 2016; Celiberti et al., 1997; Kern et al., 1984; Kern et al., 1982; Levinson and Reid, 1993; Liu et al., 2016; Rosenthal-Malek and Mitchell, 1997; Watters and Watters, 1980). In a controlled trial to assess the impact of exercise on stereotypic behaviors, Bahrami et al. (2012) found significant reductions in stereotypic behavior (using the Gilliam Autism Rating Scale – 2nd ed.) following martial arts intervention (60 min session, four days/week during 14 weeks) (Bahrami et al., 2016). Similarly, a significant reduction in stereotypic behavior (using Aberrant Behaviour Checklist – Community) was observed with horseback riding intervention (60min session/week/ 10 consecutive weeks) (Gabriels et al., 2012). Both studies reported an effect size of 0.9. Intervention studies using jogging (1–5 sessions/week, 20 min/session for a total of 10 sessions) significantly reduces stereotypic behaviors (measured by the frequency of child-specific behaviors, such as body rocking, biting self, hand flapping, etc.), with an effect size of 3.0 (Rosenthal-Malek and Mitchell, 1997).

Physical exercise intervention programs based on coordination and strength exercises (40 min/session, two sessions/week for 40 weeks) showed a decrease in the accumulated number of item for social interaction, attention deficit, reactivity, verbal stereotypes

motor stereotypes, and sleep disturbances in children and adolescents with ASD (accessed by the Autistic Traits Assessment Scale) (unpublished results).

It appears to exist an association between the reduction of primary behavioral symptoms and improvements in the academic responses, observed by the increase in the frequency of correct academic answers given and a significant increase in the number of work tasks performed (Kern et al., 1984; Rosenthal-Malek and Mitchell, 1997). Interventions with 15-min jogging increased about 7.5% the time spent engaged in academic tasks (Nicholson et al., 2011; Oriel et al., 2011). These results can be explained by the improvements in attention (especially in the dorsolateral prefrontal cortex) and changes in neurotransmitters' concentration (Zhang et al., 2020).

activity (horseback riding) significantly improves social responsiveness and social interactions, measured by parent-reports (Bass et al., 2009; Ward et al., 2013). Also, horseback riding intervention significantly improves adaptive behavior (evaluated by Vineland Adaptive Behaviour Scales – Interview Edition, Survey Form), including communication, social, and daily living skills (Gabriels et al., 2012). Long-term Kata techniques training (1 session/day, four days/week for 14 weeks) also has beneficial effects in social dysfunction of children with ASD, accessed by the Gilliam Autism Rating Scale, with a large effect size of 1.4 (Movahedi et al., 2013). Similar reductions in behavioral symptoms were observed using yoga and dance interventions (8 sessions 45 min/session), accessed by Behavioural Assessment System for Children (Rosenblatt et al., 2011). Recently, it has been shown that a mini-basketball training program (40min/session, five sessions/ week) for 12 consecutive weeks had an improvement in social communication (lower scores, assessed by Childhood Autism Rating Scale) in children with ASD (Cai and Yu, 2020). This study shows that white matter integrity of the exercise group showed higher fractional anisotropy in the body of corpus callosum,

corona radiate and left superior fronto-occipital fasciculus (Cai and Yu, 2020).

The impact of exercise intensity is not well understood in the literature (Bremer et al., 2016). In a systematic review with meta-analysis, identified that an average frequency of three times per week, with the duration per session that could vary from 15 to 90 min per session and exercise programs ranging from 8 to 48 weeks had positive effects in ASD symptomatology (Ferreira et al., 2019). An intervention study with a small group of children with ASD showed that interventions with short 10-minute sessions, light to moderate intensity, induce better responses in reducing stereotyped behaviors than more prolonged and intense sessions (Schmitz Olin et al., 2017).

Sleep disorders occur in 44–83 % of these children with ASD (Shui et al., 2018). Studies have shown that ASD children with better physical activity levels have less difficulty falling asleep and fewer sleep disorder patterns, improving the overall sleep quality, reported by parents and by completing the Children's Sleep Habits Questionnaire (Wachob and Lorenzi, 2015).

Obesity has a high prevalence in children and adolescence with ASD, mainly caused by sedentary behavior resulting from the symptomatology profile, developmental coordination disorders, sleep disorders, and the continuous use of psychotropic medications (Bandini et al., 2013; Curtin et al., 2014; Tyler and MacDonald, 2014). Both obesity and neuropsychiatric disorders are characterized by low-grade systemic inflammation and neuroinflammation in several brain regions (Martins et al., 2019). Persistent low-grade inflammation interferes with the regulation and consequent function of neurotransmitters related to emotions (Barha et al., 2017; Hodes et al., 2014). Physical exercise reduces body mass, with improvements in obesity, in children with ASD. capacity and monthly caloric expenditure coupled with a decrease in body mass index (Pitetti et al., 2007). ASD individuals that endorsed in an intervention study playing an active video game for six weeks, 30 minutes on four days a week, have a reduced body mass and body mass index, with minimal changes to waist-to-hip ratios, triceps skinfolds, and stress and anxiety (Strahan and Elder, 2015). Physical activity program based on coordination and strength exercises for 48 weeks, 30-minutes on two days a week with

moderate level showed improvements in the metabolic profile (high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and total cholesterol). However, no changes in body mass in children with ASD were observed (Toscano et al., 2018b). Although the molecular mechanisms involved in the beneficial effects of exercise remains unknown, we could hypothesize that exercise improvements in body mass and in whole-body metabolism could also improve inflammatory profile and neuroinflammation in ASD children leading to the amelioration of the symptomatology.

On the other hand, obese individuals have a higher risk for developing neuropsychiatric disorders such as depression. Depression is four times more prevalent in the ASD population. The impairments associated with depression may be compounded by additional psychiatric comorbidities, such as anxiety, which is also highly prevalent in individuals with ASD (Hudson et al., 2019). Similar to ASD, depression is associated with increased immune system activity, increased leukocyte function, and the release of pro-inflammatory cytokines (IL-1, IL-2, IL-6, and TNF-α) (Colasanto et al., 2020; Liu et al., 2020). It is widely accepted that exercise effectively treats mild to moderate depression comparable to mains antidepressant medication and cognitive behavioral therapy (for review, see (Bueno-Antequera and Munguía-Izquierdo, 2020). Exercise-

As reviewed throughout this manuscript, inflammation and neuroinflammation are relevant biological factors that interact with genetic, external stimuli,and neurophysiological mechanisms and can contribute to ASD development and symptoms. Physical exercise stimulates several organs to secrete cytokines or metabolic hormones that act all body, including on the brain having pro-cognitive functions. The exercise-stimulated cytokine release has essential roles in modulating neuronal metabolism, neuroinflammation, and neuroplasticity underlying brain function changes(Bay

and Pedersen, 2020; Murphy et al., 2020). It is well accepted that physical exercise has beneficial effects for the management of several psychiatric disorders, promoting molecular changes that induce an anti-inflammatory state over a chronic pro-inflammatory condition in the periphery and central nervous system (Ignácio et al., 2019).

We could hypothesize that the beneficial effects of physical exercise over ASD symptomatology and comorbidities could be trigger by a decrease in the neuroinflammatory profile.

In summary, literature recognizes a wide range of benefits of exercise intervention programs in the symptomatology in the ASD population. However, the molecular and cellular mechanisms induced by exercise interventions need to be explored and investigated. The available data are limited by small sample size and large variability in the frequency of intervention sessions (Bremer et al., 2016; Ferreira et al., 2019; Sowa & Meulenbroek, 2012; Tan et al., 2016). Additional research is necessary to better understand which specific physical exercise interventions are more appropriate for the population with ASD, considering the variability in the intensity of disorder's symptomatology. It is recommended that specific exercise intervention protocols should

5.1 Environmental Enrichment

Physical exercise can have similar beneficial effects as enriched environments on brain and behavior in humans and other animals (for review, see (Hillman et al., 2008)). Environmental enrichment is a combination of complex inanimate and social stimulation to reduce stereotypical behavior by counteracting boredom and engaging specific actions. Environmental enrichment involves increasing novelty and complexity in environmental

conditions to enhance sensory, cognitive, and motor stimulation. One way to attempt one of the components of environmental enrichment has been to provide animals with ad libitum access to running wheels or treadmills to assess the effects of increased physical activity alone. For humans, sensory integration is a preferred term for "environmental enrichment",[21] involves different types of sensory and motor exercises on a daily basis. In humans, enriching the children's environments is an attempt to increase social interactions and cognition, promoting learning in a guided participation context (Ball et al., 2019).

In children, enriched physical education (specifically tailored physical activity games) provide a unique form of enrichment that impacts children's cognitive development through motor coordination improvement, improving children's physical activity habits later in life (Pesce et al., 2016). Using animals, a comparison of enrichment, running, and a combination[48] of enrichment and running revealed that only mice with access to running had increases in neurogenesis, neural number and survival, and neurotrophin levels (Kobilo et al., 2011).

In neurodevelopment disorders, environmental enrichment and increased voluntary physical exercise have generally been associated with sensory integration therapy and enrichment can compensate for the deprivation of sensory/social/motor inputs caused by dysfunctional sensory systems (Ball et al., 2019). Rett syndrome is an autistic spectrum developmental disorder associated with mutations in the X-linked Mecp2 gene and severe behavioral and neuropathological deficits. In a genetic animal model of Rett syndrome

(MeCP2^{tm1Tam} mice), environmental enrichment and running wheel exercise reverses the effect of MeCP2 deficit affective phenotype, normalized the hedonic response, and ameliorates motor coordination, by partial normalization of HPA axis function (by rescued basal serum corticosterone), and by an increase in the hippocampal BDNF protein levels (Kondo et al., 2008; Kondo et al., 2016). Environmental enrichment also reduced ventricular volumes, which correlated with improved locomotor activity (Nag et al., 2009).

In an animal model of ASD (valproic acid exposed rats), physical exercise, multisensory stimulation, and enriched housing were associated with improved anxiety-like behavior, social and cognitive deficits as well as reduced repetitive/stereotypic behavior. In another animal model of ASD, the BTBR T + Itpr3tf/J (BTBR) mice, environmental enrichment improves systemic metabolism, learning/memory, anxious behavior, increased social affiliation, and locomotor activity (Queen et al., 2020). In a genetic mouse model of ASD (NL3R451C), environmental enrichment reduces body weight and increased social

techniques are commonly used to treat symptoms of ASD, and other developmental disorders, to improve dysfunctional sensory processing (Aronoff et al., 2016; Woo et al., 2015). Sensory integration using fine and gross motor activity is especially effective in reducing hyperactivity and attentional deficit in school-age children when combined with executive functioning therapy (Salami et al., 2017). Benefits of increased activity have been shown without the other aspects of enriched environments in children with neurodevelopment disorders. Still, at least one study (Salami et al., 2017) suggests that

combination therapy, environmental enrichment, plus physical exercise is most beneficial.

In summary, the etiology of ASD is associated with increased immune system activity, astrogliosis, microglial activation, and the release of proinflammatory cytokines (IL-1, IL-2, IL-6, and TNF-α) in the brain. The detailed effects of cytokines on neural immune environment in ASD has yet to be elucidated, however these changes can be responsible for the phenotypic heterogeneity and severity observed in patients with autism. Indeed, many of the ASD features seem to have different severity levels also depending on changes in immune responses, confirming the intimate bond between neurodevelopment and immune processes profile and neuroinflammation in ASD subjects leading to the amelioration of the symptomatology.

6. Conclusion

In conclusion, the essential benefits of exercise for ASD symptoms highlight exercise programs as a powerful complementary therapy to minimize symptoms among ASD subjects. Using exercise activities improves motor performance and may indirectly affect the core social communication impairments of individuals with ASDs by providing greater opportunities for socialization with peers and better attentional focus. We strongly recommend that structured and personalized exercise programs (combining components of aerobic, resistance, flexibility, and neuromuscular training) should be included within

the care plan for individuals with ASD, given the multisystem and systemic effects in the ASD of exercise interventions.

Authors´ Contributions

All authors revised and edited the manuscript, approved the final version to be submitted, and take responsibility for the manuscript's integrity and accuracy.

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Figure Caption

Figure 1: Etiology and symptoms of autism spectrum disorders. The etiology and pathogenesis of ASD have not been completely identified. However, the combination of

genetic and environmental factors and immune dysfunction can be underlying ASD development.

Figure 2: Neuroinflammation associated with autism spectrum disorders. Maternal **immune system activation is a risk factor that increases the chances of a child develops ASD. The increased inflammatory cytokines and autoantibodies that react to fetal brain tissue may change proper synaptic development in the offspring and that are linked to behavioral abnormalities see in ASD, including repetitive behaviors, stereotypes, anxiety and impaired behaviors.**

Figure 3: Benefits of exercise in autism spectrum disorders. Physical Exercise intervention has a positive effect both in primary symptoms of ASD as well as in comorbidities.

References:

Alabdali, A., Al-Ayadhi, L., El-Ansary, A., 2014. Association of social and cognitive impairment and biomarkers in autism spectrum disorders. Journal of neuroinflammation

American Psychiatric Association, 2014. Diagnostic and statistical manual of mental disorders, 5th ed.

Aronoff, E., Hillyer, R., Leon, M., 2016. Environmental Enrichment Therapy for Autism: Outcomes with Increased Access. Neural Plast 2016, 2734915-2734915.

Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I., Van de Water, J.,2011. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain, behavior, and immunity 25, 40-45.

Ashwood, P., Wakefield, A.J., 2006. Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms. Journal of neuroimmunology 173, 126-134. autism spectrum

disorders. Pediatrics 124, 687-694.

Bahrami, F., Movahedi, A., Marandi, S.M., Sorensen, C., 2016. The Effect of Karate Techniques Training on Communication Deficit of Children with Autism Spectrum Disorders. Journal of autism and developmental disorders 46, 978-986.

Ball, N.J., Mercado, E., 3rd, Orduña, I., 2019. Enriched Environments as a Potential Treatment for Developmental Disorders: A Critical Assessment. Frontiers in psychology

Bandini, L.G., Gleason, J., Curtin, C., Lividini, K., Anderson, S.E., Cermak, S.A., Maslin, M., Must, A., 2013. Comparison of physical activity between children with autism spectrum disorders and typically developing children. Autism : the international journal of research and practice 17, 44-54. Bang, M.L., Owczarek, S., 2013. A matter of balance: role of neurexin and neuroligin at the synapse. Neurochemical research 38, 1174-1189.

Banik, A., Kandilya, D., Ramya, S., Stünkel, W., Chong, Y.S., Dheen, S.T., 2017. Maternal Factors that Induce Epigenetic Changes Contribute to Neurological Disorders in Offspring. Genes 8, 150.

Baranek, G.T., Watson, L.R., Boyd, B.A., Poe, M.D., David, F.J., McGuire, L., 2013.

Bass, M.M., Duchowny, C.A., Llabre, M.M., 2009. The effect of therapeutic horseback riding on social functioning in children with autism. Journal of autism and developmental disorders 39, 1261-1267.

Batey, C.A., Missiuna, C.A., Timmons, B.W., Hay, J.A., Faught, B.E., Cairney, J., 2014.

Baxter, A.J., Brugha, T.S., Erskine, H.E., Scheurer, R.W., Vos, T., Scott, J.G., 2015. The epidemiology and global burden of autism spectrum disorders. Psychological Medicine, 601-613.

Bay, M.L., Pedersen, B.K., 2020. Muscle-Organ Crosstalk: Focus on Immunometabolism. Frontiers in physiology 11, 567881.

Bechard, A.R., Cacodcar, N., King, M.A., Lewis, M.H., 2016. How does

environmental enrichment reduce repetitive motor behaviors? Neuronal activation and dendritic morphology in the indirect basal ganglia pathway of a mouse model. Behavioural brainr esearch 299, 122-131.

Bjorklund, G., Saad, K., Chirumbolo, S., Kern, J.K., Geier, D.A., Geier, M.R., Urbina, M.A., 2016. Immune dysfunction and neuroinflammation in autism spectrum disorder. Acta neurobiologiae experimentalis 76, 257-268.

Bourgeron, T., 2015. From the genetic architecture to synaptic plasticity in autism spectrum disorder. Nature reviews. Neuroscience 16, 551-563.

Braunschweig, D., Duncanson, P., Boyce, R., Hansen, R., Ashwood, P., Pessah, I.N., Hertz-Picciotto, I., Van de Water, J., 2012. Behavioral correlates of maternal antibody status among children with autism. Journal of autism and developmental disorders 42, 1435-1445.

Bremer, E., Crozier, M., Lloyd, M., 2016. A systematic review of the behavioural outcomes following exercise interventions for children and youth with autism spectrum disorder. Autism : the international journal of research and practice 20, 899-915.

Brown, A.S., Sourander, A., Hinkka-Yli-Salomäki, S., McKeague, I.W., Sundvall, J., Surcel, H.M., 2014. Elevated maternal C-reactive protein and autism in a national birth cohort. Molecular psychiatry 19, 259-264.

Businaro, R., Corsi, M., Azzara, G., Di Raimo, T., Laviola, G., Romano, E., Ricci, L., Maccarrone, M., Aronica, E., Fuso, A., Ricci, S., 2016. Interleukin-18 modulation in autism spectrum disorders. Journal of neuroinflammation 13, 2.

Cai, K., Yu, Q., 2020. Mini-Basketball Training Program Improves Social Communication and White Matter Integrity in Children with Autism. 10.

Cascio, C.J., Gu, C., Schauder, K.B., Key, A.P., Yoder, P., 2015. Somatosensory Event- Related Potentials and Association with Tactile Behavioral Responsiveness Patterns in Children with ASD. Brain topography 28, 895-903.

Cast, T.P., Boesch, D.J., Smyth, K., Shaw, A.E., Ghebrial, M., Chanda, S., 2021. An Autism-Associated Mutation Impairs Neuroligin-4 Glycosylation and

Enhances Excitatory Synaptic Transmission in Human Neurons. 41, 392-407.

Celiberti, D.A., Bobo, H.E., Kelly, K.S., Harris, S.L., Handleman, J.S., 1997. The differential and temporal effects of antecedent exercise on the self-stimulatory behavior of a child with autism. Research in developmental disabilities 18, 139-150.

Chana, G., Laskaris, L., Pantelis, C., Gillett, P., Testa, R., Zantomio, D., Burrows, E.L., Hannan, A.J., Everall, I.P., Skafidas, E., 2015. Decreased expression of mGluR5 within the dorsolateral prefrontal cortex in autism and increased microglial number in mGluR5

Chen, Z., Lan, W., Yang, G., Li, Y., Ji, X., Chen, L., Zhou, Y., Li, S., 2020. Exercise Intervention in Treatment of Neuropsychological Diseases: A Review. Frontiers in psychology 11, 569206.

Chiarotti, F., Venerosi, A., 2020. Epidemiology of Autism Spectrum Disorders: A Review of Worldwide Prevalence Estimates Since 2014. Brain sciences 10, 274.

Choi, G.B., Yim, Y.S., Wong, H., Kim, S., Kim, H., Kim, S.V., Hoeffer, C.A., Littman, D.R., Huh, J.R., 2016. The maternal interleukin-17a pathway in mice promotes autism- like phenotypes in offspring. Science 351, 933-939.

Christensen, J., Grønborg, T.K., Sørensen, M.J., Schendel, D., Parner, E.T., Pedersen, L.H., Vestergaard, M., 2013. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. Jama 309, 1696-1703.

Colasanto, M., Madigan, S., Korczak, D.J., 2020. Depression and inflammation among children and adolescents: A meta-analysis. Journal of affective disorders 277, 940-948.

Connor, C.M., Dincer, A., Straubhaar, J., Galler, J.R., Houston, I.B., Akbarian, S., 2012. Maternal immune activation alters behavior in adult offspring, with subtle changes in the cortical transcriptome and epigenome. Schizophrenia research 140, 175-184.

Contu, L., Hawkes, C.A., 2017. A Review of the Impact of Maternal Obesity on
58
the Cognitive Function and Mental Health of the Offspring. International journal of molecular sciences 18. Markus, R.P., 2021. Disrupted nocturnal melatonin in autism:
16
Association with tumor necrosis factor and sleep disturbances. Journal of pineal research, e12715.

Dalton, P., Deacon, R., Blamire, A., Pike, M., McKinlay, I., Stein, J., Styles, P., Vincent, A., 2003. Maternal neuronal antibodies associated with autism and a language disorder. Annals of neurology 53, 533-537.

De Rubeis, S., He, X., Goldberg, A.P., Poultney, C.S., Samocha, K., Ercument Cicek, A., Kou, Y., Liu, L., Fromer, M., Walker, S., Singh, T., Klei, L., Kosmicki, J., Fu, S.-C., Aleksic, B., Biscaldi, M., Bolton, P.F., Brownfeld, J.M., Cai, J., Campbell, N.G., Carracedo, A., Chahrour, M.H., Chiocchetti, A.G., Coon, H., Crawford, E.L., Crooks, L., Curran, S.R., Dawson, G., Duketis, E., Fernandez, B.A., Gallagher, L., Geller, E., Guter, S.J., Sean Hill, R., Ionita-Laza, I., Jimenez Gonzalez, P., Kilpinen, H., Klauck, S.M., Kolevzon, A., Lee, I., Lei, J., Lehtimäki, T., Lin, C.-F., Ma'ayan, A., Marshall, C.R., McInnes, A.L., Neale, B., Owen, M.J., Ozaki, N., Parellada, M., Parr, J.R., Purcell, S., Puura, K., Rajagopalan, D., Rehnström, K., Reichenberg, A., Sabo, A., Sachse, M., Sanders, S.J., Schafer, C., Schulte-Rüther, M., Skuse, D., Stevens, C., Szatmari, P., Tammimies, K., Valladares, O., Voran, A., Wang, L.-S., Weiss, L.A., Jeremy Willsey, A., Yu, T.W., Yuen, R.K.C., Cook, E.H., Freitag, C.M., Gill, M., Hultman, C.M., Lehner, T., Palotie, A., Schellenberg, G.D., Sklar, P., State, M.W., Sutcliffe, J.S., Walsh, C.A., Scherer, S.W., Zwick, M.E., Barrett, J.C., Cutler, D.J., Roeder, K., Devlin, B., Daly, M.J., details. Journal of neurochemistry 139 Suppl 2, 136-153.

Dodds, L., Fell, D.B., Shea, S., Armson, B.A., Allen, A.C., Bryson, S., 2011. The role of prenatal, obstetric and neonatal factors in the development of autism. Journal of autism and developmental disorders 41, 891-902.

Dunn, W., Myles, B.S., Orr, S., 2002. Sensory processing issues associated with Asperger syndrome: a preliminary investigation. The American journal of occupational therapy : official publication of the American Occupational Therapy Association 56, 97-102.

Edmonson, C., Ziats, M.N., Rennert, O.M., 2014. Altered glial marker expression in autistic post-mortem prefrontal cortex and cerebellum. Molecular autism 5, 3.

Eissa, N., Al-Houqani, M., Sadeq, A., Ojha, S.K., Sasse, A., Sadek, B., 2018. Current Enlightenment About Etiology and Pharmacological Treatment of Autism Spectrum Disorder. Frontiers in neuroscience 12, 304.

El-Ansary, A., Al-Ayadhi, L., 2014. GABAergic/glutamatergic imbalance relative to excessive neuroinflammation in autism spectrum disorders. Journal of neuroinflammation 11, 189.

Eroglu, C., Barres, B.A., 2010. Regulation of synaptic connectivity by glia. Nature 468, 223-231.

Fernández de Cossío, L., Guzmán, A., van der Veldt, S., Luheshi, G.N., 2017. Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring. Brain, behavior, and immunity 63, 88-98.

Ferreira, J.P., Ghiarone, T., Júnior, C.R.C., Furtado, G.E., Carvalho, H.M., Rodrigues, A.M., Toscano, C.V.A., 2019. Effects of Physical Exercise on the Stereotyped Behavior of Children with Autism Spectrum Disorders. Medicina (Kaunas, Lithuania) 55, 685.

Foley, K.A., MacFabe, D.F., Kavaliers, M., Ossenkopp, K.P., 2015. Sexually dimorphic effects of prenatal exposure to lipopolysaccharide, and prenatal and postnatal exposure to propionic acid, on acoustic startle response and prepulse inhibition in adolescent rats: relevance to autism spectrum disorders. Behavioural brain research 278, 244-256.

Fragala-Pinkham, M., Haley, S.M., O'Neil, M.E., 2008. Group aquatic aerobic exercise for children with disabilities. Developmental medicine and child neurology 50, 822-827.

Fragala-Pinkham, M.A., Haley, S.M., O'Neil, M.E., 2011. Group swimming and aquatic exercise programme for children with autism spectrum disorders: a pilot study. Developmental neurorehabilitation 14, 230-241.

Frey, H.P., Molholm, S., Lalor, E.C., Russo, N.N., Foxe, J.J., 2013. Atypical cortical representation of peripheral visual space in children with an autism spectrum disorder. The European journal of neuroscience 38, 2125-2138.

Gabriels, R.L., Agnew, J.A., Holt, K.D., Shoffner, A., Zhaoxing, P., Ruzzano, S., Clayton, G.H., Mesibov, G., 2012. Pilot study measuring the effects of therapeutic horseback riding on school-age children and adolescents with autism spectrum disorders.

Research in Autism Spectrum Disorders 6, 578-588.

Genes to Disruption of Cortical Development. 9.

Goines, P.E., Croen, L.A., Braunschweig, D., Yoshida, C.K., Grether, J., Hansen, R., Kharrazi, M., Ashwood, P., Van de Water, J., 2011. Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A case-control study. Molecular autism 2, 13.

Golan, H.M., Lev, V., Hallak, M., Sorokin, Y., Huleihel, M., 2005. Specific
neurodevelopmental damage in mice offspring following maternal inflammation during pregnancy. Neuropharmacology 48, 903-917.

Ha, S., Sohn, I.J., Kim, N., Sim, H.J., Cheon, K.A., 2015. Characteristics of Brains in Autism Spectrum Disorder: Structure, Function and Connectivity across the Lifespan. Experimental neurobiology 24, 273-284.

Hatanaka, Y., Kabuta, T., Wada, K., 2017. Disturbance in Maternal Environment Leads to Abnormal Synaptic Instability during Neuronal Circuitry Development. Frontiers in neuroscience 11, 35.

Hillman, C.H., Erickson, K.I., Kramer, A.F., 2008. Be smart, exercise your heart: exercise effects on brain and cognition. Nature reviews. Neuroscience 9, 58-65.

Hinckson, E.A., Dickinson, A., Water, T., Sands, M., Penman, L., 2013. Physical activity, dietary habits and overall health in overweight and obese children and youth with intellectual disability or autism. Research in developmental disabilities 34, 1170-1178.

Hollins, S.L., Zavitsanou, K., Walker, F.R., Cairns, M.J., 2014. Alteration of imprinted

Huang, Y.N., Ho, Y.J., Lai, C.C., Chiu, C.T., Wang, J.Y., 2015. 1,25-Dihydroxy vitamin attenuates endotoxin-induced production of inflammatory mediators by inhibiting MAPK activation in primary cortical neuron-glia cultures. Journal of neuroinflammation 12, 147.

Hudson, C.C., Hall, L., Harkness, K.L., 2019. Prevalence of Depressive Disorders in Individuals with Autism Spectrum Disorder: a Meta-Analysis. Journal of Abnormal Child

Psychology 47, 165-175.

Hutsler, J.J., Zhang, H., 2010. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. Brain research 1309, 83-94.

Ignácio, Z.M., da Silva, R.S., Plissari, M.E., Quevedo, J., Réus, G.Z., 2019. Physical Exercise and Neuroinflammation in Major Depressive Disorder. Molecular neurobiology Inga Jácome, M.C., Morales Chacòn, L.M., Vera Cuesta, H., Maragoto Rizo, C., Whilby Santiesteban, M., Ramos Hernandez, L., Noris García, E., González Fraguela, M.E., Agramonte, M.d.L.A., 2016b. Peripheral Inflammatory Markers Contributing to Comorbidities in Autism, Behav Sci (Basel).

Jamain, S., Quach, H., Betancur, C., Rastam, M., Colineaux, C., Gillberg, I.C., Soderstrom, H., Giros, B., Leboyer, M., Gillberg, C., Bourgeron, T., Paris Autism Research International Sibpair, S., 2003. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nature genetics 34, 27-29.

Janusonis, S., 2014. Serotonin dynamics in and around the central nervous system: is autism solvable without fundamental insights? International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 39, 9-15.

Kern, L., Koegel, R.L., Dunlap, G., 1984. The influence of vigorous versus mild exercise on autistic stereotyped behaviors. Journal of autism and developmental disorders 14,

Kern, L., Koegel, R.L., Dyer, K., Blew, P.A., Fenton, L.R., 1982. The effects of physical exercise on self-stimulation and appropriate responding in autistic children. Journal of autism and developmental disorders 12, 399-419.

Kondo, M.A., Gray, L.J., Pelka, G.J., Leang, S.K., Christodoulou, J., Tam, P.P.,

Hannan, A.J., 2016. Affective dysfunction in a mouse model of Rett syndrome: Therapeutic effects of environmental stimulation and physical activity. Developmental neurobiology 76, 209-224.

Kopp, N., Amarillo, I., Martinez-Agosto, J., 2020. Pathogenic paternally inherited NLGN4X deletion in a female with autism spectrum disorder: Clinical, cytogenetic,

54

and molecular characterization. e62025.

Krakowiak, P., Walker, C.K., Bremer, A.A., Baker, A.S., Ozonoff, S., Hansen, R.L., Hertz-Picciotto, I., 2012. Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. Pediatrics 129, e1121-1128.

Ladd-Acosta, C., Hansen, K.D., Briem, E., Fallin, M.D., Kaufmann, W.E., Feinberg, A.P., 2014. Common DNA methylation alterations in multiple brain regions in autism. Molecular psychiatry 19, 862-871.

Lai, M.C., Lombardo, M.V., Baron-Cohen, S., 2014. Autism. Lancet 383, 896-910.

Lang, R., Koegel, L.K., Ashbaugh, K., Regester, A., Ence, W., Smith, W., 2010. Physical exercise and individuals with autism spectrum disorders: A systematic review. Research in Autism Spectrum Disorders 4, 565-576.

Lee, B.K., Magnusson, C., Gardner, R.M., Blomström, Å., Newschaffer, C.J., Burstyn, I., Karlsson, H., Dalman, C., 2015. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. Brain, behavior, and immunity 44, 100- 105.

Levinson, L.J., Reid, G., 1993. The Effects of Exercise Intensity on the Stereotypic Behaviors of Individuals with Autism. Adapted Physical Activity Quarterly 10, 255-268.

Li, X., Chauhan, A., Sheikh, A.M., Patil, S., Chauhan, V., Li, X.M., Ji, L., Brown, T., Malik, M., 2009. Elevated immune response in the brain of autistic patients. Journal of neuroimmunology 207, 111-116.

Liao, X., Liu, Y., Fu, X., Li, Y., 2020. Postmortem Studies of Neuroinflammation in Autism Spectrum Disorder: a Systematic Review. Molecular neurobiology 57, 3424-423438.

Liu, J.J., Wei, Y.B., Strawbridge, R., Bao, Y., 2020. Peripheral cytokine levels and response to antidepressant treatment in depression: a systematic review and meta-analysis. 25, 339-350.

Liu, T., Fedak, A.T., Hamilton, M., 2016. Effect of Physical Activity on the Stereotypic Behaviors of Children With Autism Spectrum Disorder. International Journal of

School Health 3, 17-22.

Loomes, R., Hull, L., Mandy, W.P.L., 2017. What Is the Male-to-Female Ratio in Autism Spectrum Disorder? A Systematic Review and Meta-Analysis. Journal of the American Academy of Child and Adolescent Psychiatry 56, 466-474.

Madsen-Bouterse, S.A., Romero, R., Tarca, A.L., Kusanovic, J.P., Espinoza, J., Kim, C.J., Kim, J.-S., Edwin, S.S., Gomez, R., Draghici, S., 2010. The transcriptome of the fetal inflammatory response syndrome. Am J Reprod Immunol 63, 73-92.

Maenner, M.J., Shaw, K.A., Baio, J., Washington, A., Patrick, M., DiRienzo, M., Christensen, D.L., Wiggins, L.D., Pettygrove, S., Andrews, J.G., Lopez, M., Hudson, A., Baroud, T., Schwenk, Y., White, T., Rosenberg, C.R., Lee, L.C., Harrington, R.A., Huston, M., Hewitt, A., Esler, A., Hall-Lande, J., Poynter, J.N., Hallas-Muchow, L., Constantino, J.N., Fitzgerald, R.T., Zahorodny, W., Shenouda, J., Daniels, J.L., Warren, Z., Vehorn, A., Salinas, A., Durkin, M.S., Dietz, P.M. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016.

Magnusson, C., Lundberg, M., Lee, B.K., Rai, D., Karlsson, H., Gardner, R., Kosidou, K., Arver, S., Dalman, C., 2016. Maternal vitamin D deficiency and the risk of autism spectrum disorders: population-based study. BJPsych open 2, 170-172.

Mann, C., Schäfer, T., Bletsch, A., 2020. Examining volumetric gradients based on the frustum surface ratio in the brain in autism spectrum disorder. Molecular psychiatry 20, 440-446.

Mei, T., Llera, A., Floris, D.L., Forde, N.J., Tillmann, J., Durston, S., Moessnang, C., Banaschewski, T., Holt, R.J., Baron-Cohen, S., Rausch, A., Loth, E., Dell'Acqua, F., Charman, T., Murphy, D.G.M., Ecker, C., Beckmann, C.F., Buitelaar, J.K., 2020. Gray matter covariations and core symptoms of autism: the EU-AIMS Longitudinal European Autism Project. Molecular autism 11, 86.

Meldolesi, J., 2020. Astrocytes: News about Brain Health and Diseases. Biomedicines Modabbernia, A., Mollon, J., Boffetta, P., Reichenberg, A., 2016. Impaired Gas Exchange at Birth and Risk of Intellectual Disability and Autism: A Meta-analysis.

Journal of autism and developmental disorders 46, 1847-1859.

Morgan, J.T., Barger, N., Amaral, D.G., Schumann, C.M., 2014. Stereological study of amygdala glial populations in adolescents and adults with autism spectrum disorder. PloS one 9, e110356.

Morgan, J.T., Chana, G., Pardo, C.A., Achim, C., Semendeferi, K., Buckwalter, J., Courchesne, E., Everall, I.P., 2010. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biological psychiatry 68, 368-376.2

Murphy, R.M., Watt, M.J., Febbraio, M.A., 2020. Metabolic communication during exercise. Nature metabolism 2, 805-816.

Nadeem, A., Ahmad, S.F., Attia, S.M., Al-Ayadhi, L.Y., Bakheet, S.A., Al-Harbi, N.O., 2019. Oxidative and inflammatory mediators are upregulated in neutrophils of autistic
children: Role of IL-17A receptor signaling. Progress in neuro-psychopharmacology & biological psychiatry 90, 204-211.

Nag, N., Moriuchi, J.M., Peitzman, C.G.K., Ward, B.C., Kolodny, N.H., Berger- Sweeney, J.E., 2009. Environmental enrichment alters locomotor behaviour and ventricular volume in Mecp21lox mice. Behavioural brain research 196, 44-48.

Nardone, S., Sams, D.S., Reuveni, E., Getselter, D., Oron, O., Karpuj, M., Elliott, E., 2014. DNA methylation analysis of the autistic brain reveals multiple dysregulated biological pathways. Translational psychiatry 4, e433.

Nardone, S., Sams, D.S., Zito, A., Reuveni, E., Elliott, E., 2017. Dysregulation of Cortical Neuron DNA Methylation Profile in Autism Spectrum Disorder. Cerebral cortex (New York, N.Y. : 1991) 27, 5739-5754.

Nicholson, H., Kehle, T.J., Bray, M.A., Heest, J.V., 2011. The effects of antecedent physical activity on the academic engagement of children with autism spectrum disorder. Psychology in the Schools 48, 198-213.

Ormstad, H., Bryn, V., Saugstad, O.D., Skjeldal, O., Maes, M., 2018. Role of the Immune System in Autism Spectrum Disorders (ASD). CNS & neurological disorders drug

targets 17, 489-495.

Parente, D.J., Garriga, C., Baskin, B., Douglas, G., Cho, M.T., Araujo, G.C., Shinawi, M., 2017. Neuroligin 2 nonsense variant associated with anxiety, autism, intellectual disability, hyperphagia, and obesity. American journal of medical genetics. Part A 173, 213-216.

Pereira-Santos, M., Costa, P.R., Assis, A.M., Santos, C.A., Santos, D.B., 2015. Obesity and vitamin D deficiency: a systematic review and meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity 16, 341-349.

Pesce, C., Masci, I., Marchetti, R., Vazou, S., Sääkslahti, A., Tomporowski, P.D., 2016. Deliberate Play and Preparation Jointly Benefit Motor and Cognitive Development: Mediated and Moderated Effects. Frontiers in psychology 7, 349-349.

Petrus, C., Adamson, S.R., Block, L., Einarson, S.J., Sharifnejad, M., Harris, S.R., 2008. Effects of exercise interventions on stereotypic behaviours in children with autism spectrum disorder. Physiother Can 60, 134-145.

Pitetti, K.H., Rendoff, A.D., Grover, T., Beets, M.W., 2007. The Efficacy of a 9-Month Treadmill Walking Program on the Exercise Capacity and Weight Reduction for Queen, N.J., Boardman, A.A., Patel, R.S., Siu, J.J., Mo, X., Cao, L., 2020. Environmental enrichment improves metabolic and behavioral health in the BTBR mouse model of autism. Psychoneuroendocrinology 111, 104476.

Rivera, H.M., Christiansen, K.J., Sullivan, E.L., 2015. The role of maternal obesity in the risk of neuropsychiatric disorders. Frontiers in neuroscience 9, 194.

Robertson, C.E., Baron-Cohen, S., 2017. Sensory perception in autism. Nature reviews. Neuroscience 18, 671-684.

Rodriguez, J.I., Kern, J.K., 2011. Evidence of microglial activation in autism and its possible role in brain underconnectivity. Neuron glia biology 7, 205-213.

Rosenblatt, L.E., Gorantla, S., Torres, J.A., Yarmush, R.S., Rao, S., Park, E.R.,Denninger, J.W., Benson, H., Fricchione, G.L., Bernstein, B., Levine, J.B., 2011.

Rosenthal-Malek, A., Mitchell, S., 1997. Brief Report: The Effects of Exercise on the Self-Stimulatory Behaviors and Positive Responding of Adolescents with Autism.

29

Journal of autism and developmental disorders 27, 193-202.

Rubenstein, J.L., Merzenich, M.M., 2003. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes, brain, and behavior 2, 255-267.

Schauder, K.B., Bennetto, L., 2016. Toward an Interdisciplinary Understanding of Sensory Dysfunction in Autism Spectrum Disorder: An Integration of the Neural and Symptom Literatures. Frontiers in neuroscience 10, 268-268.

Schmitz Olin, S., McFadden, B.A., Golem, D.L., Pellegrino, J.K., Walker, A.J., Sanders, D.J., Arent, S.M., 2017. The Effects of Exercise Dose on Stereotypical Behavior in Children with Autism. Medicine and science in sports and exercise 49, 983-990.

Schumann, C.M., Bloss, C.S., Barnes, C.C., Wideman, G.M., Carper, R.A., Akshoomoff, N., Pierce, K., Hagler, D., Schork, N., Lord, C., Courchesne, E., 2010. Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. The Journal of neuroscience : the official journal of the Society for Neuroscience 30, 4419-4427.

Sciara, A.N., Beasley, B., Crawford, J.D., Anderson, E.P., Carrasco, T., Zheng, S., Ordway, G.A., Chandley, M.J., 2020. Neuroinflammatory Gene Expression Alterations in Anterior Cingulate Cortical White and Gray Matter of Males With Autism Spectrum Disorder. 13, 870-884.

Shui, A.M., Katz, T., Malow, B.A., Mazurek, M.O., 2018. Predicting sleep problems in children with autism spectrum disorders. Research in developmental disabilities 83, 270- 279.

Sowa, M., Meulenbroek, R., 2012. Effects of physical exercise on Autism Spectrum Disorders: A meta-analysis. Research in Autism Spectrum Disorders 6, 46-57.

Steffen, D.M., Ferri, S.L., Marcucci, C.G., Blocklinger, K.L., Molumby, M.J., Abel,.,Weiner, J.A., 2021. The γ-Protocadherins Interact Physically and Functionally with Yoshihara, Y., Omata, K., Matsumoto, K., Tsuchiya, K.J., Iwata, Y., Tsujii, M. Sugiyama, T., Mori, N., 2013. Microglial activation in young adults with autism spectrum disorder. JAMA psychiatry 70, 49-58.

Sweeten, T.L., Posey, D.J., McDougle, C.J., 2003. High blood monocyte counts

and neopterin levels in children with autistic disorder. The American journal of psychiatry 160, 1691-1693.

Tan, B.W., Pooley, J.A., Speelman, C.P., 2016. A Meta-Analytic Review of the Efficacy of Physical Exercise Interventions on Cognition in Individuals with Autism Spectrum Disorder and ADHD. Journal of autism and developmental disorders 46, 3126-3143.

Tetreault, N.A., Hakeem, A.Y., Jiang, S., Williams, B.A., Allman, E., Wold, B.J., Allman, J.M., 2012. Microglia in the cerebral cortex in autism. Journal of autism and developmental disorders 42, 2569-2584.

Toscano, C.V.A., Carvalho, H.M., Ferreira, J.P., 2018a. Exercise Effects for Children With Autism Spectrum Disorder: Metabolic Health, Autistic Traits, and Quality of Life. Perceptual and motor skills 125, 126-146.

Toscano, C.V.A., Ferreira, J.P., Gaspar, J.M., Carvalho, H.M., 2018b. Growth and weight status of Brazilian children with autism spectrum disorders: A mixed longitudinal study. Jornal de pediatria.

Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W., Pardo, C.A., 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. Annals of neurology 57, 67-81.

Varghese, M., Keshav, N., Jacot-Descombes, S., Warda, T., Wicinski, B., Dickstein, D.L., Harony-Nicolas, H., De Rubeis, S., Drapeau, E., Buxbaum, J.D., Hof, P.R., 2017. Autism spectrum disorder: neuropathology and animal models. Acta neuropathologica.

Vinkhuyzen, A.A.E., Eyles, D.W., Burne, T.H.J., Blanken, L.M.E., Kruithof, C.J., Verhulst, F., Jaddoe, V.W., Tiemeier, H., McGrath, J.J., 2018. Gestational vitamin D deficiency and autism-related traits: the Generation R Study. Molecular psychiatry 23, 240-246.

Vogel Ciernia, A., Careaga, M., LaSalle, J.M., Ashwood, P., 2018. Microglia from offspring of dams with allergic asthma exhibit epigenomic alterations in genes dysregulated in autism. Glia 66, 505-521.

Vogel Ciernia, A., LaSalle, J., 2016. The landscape of DNA methylation amid a

perfect storm of autism aetiologies. Nature reviews. Neuroscience 17, 411-423.

Ward, S.C., Whalon, K., Rusnak, K., Wendell, K., Paschall, N., 2013. The association between therapeutic horseback riding and the social communication and sensory reactions of children with autism. Journal of autism and developmental disorders 43, 2190-2198.

Watters, R.G., Watters, W.E., 1980. Decreasing self-stimulatory behavior with physical exercise in a group of autistic boys. Journal of autism and developmental disorders 379-387.

Wei, H., Chadman, K.K., McCloskey, D.P., Sheikh, A.M., Malik, M., Brown, W.T., Li, X., 2012a. Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. Biochimica et biophysica acta 1822, 831-842.

Wei, H., Mori, S., Hua, K., Li, X., 2012b. Alteration of brain volume in IL-6 overexpressing mice related to autism. International journal of developmental neuroscience: the official journal of the International Society for Developmental Neuroscience 30, 554-559.

Wei, H., Zou, H., Sheikh, A.M., Malik, M., Dobkin, C., Brown, W.T., Li, X., 2011. IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. Journal of neuroinflammation 8, 52.

Windham, G.C., Anderson, M., Lyall, K., Daniels, J.L., Kral, T.V.E., Croen, L.A., 2019. Maternal Pre-pregnancy Body Mass Index and Gestational Weight Gain in Relation to enrichment as a therapy for autism: A clinical trial replication and extension. Behavioral neuroscience 129, 412-422.

Xiang, A.H., Wang, X., Martinez, M.P., Walthall, J.C., Curry, E.S., Page, K., Buchanan, T.A., Coleman, K.J., Getahun, D., 2015. Association of maternal diabetes with autism in offspring. Jama 313, 1425-1434.

Yamaguchi, H., Hara, Y., Ago, Y., Takano, E., Hasebe, S., Nakazawa, T., Hashimoto, H., Matsuda, T., Takuma, K., 2017. Environmental enrichment attenuates behavioral abnormalities in valproic acid-exposed autism model mice. Behavioural brain research

Yan, J., Oliveira, G., Coutinho, A., Yang, C., Feng, J., Katz, C., Sram, J., Bockholt,

A., Jones, I.R., Craddock, N., Cook, E.H., Jr., Vicente, A., Sommer, S.S., 2005. Analysis of the neuroligin 3 and 4 genes in autism and other neuropsychiatric patients. Molecular psychiatry 10, 329-332.

Zhang, M., Liu, Z., Ma, H., Smith, D.M., 2020. Chronic Physical Activity for Attention Deficit Hyperactivity Disorder and/or Autism Spectrum Disorder in Children: A Meta- Analysis of Randomized Controlled Trials. Frontiers in Behavioral Neuroscience 14.

Zhao, J.L., Jiang, W.T., 2020. Exercise, brain plasticity, and depression. CNS Neurosci-Ther. 26, 885-895.

Zheng, Z., Zhang, L., Li, S., Zhao, F., Wang, Y., Huang, L., Huang, J., Zou, R., Qu, Y., Mu, D., 2017. Association among obesity, overweight and autism spectrum disorder: a systematic review and meta-analysis. Scientific reports 7, 11697.

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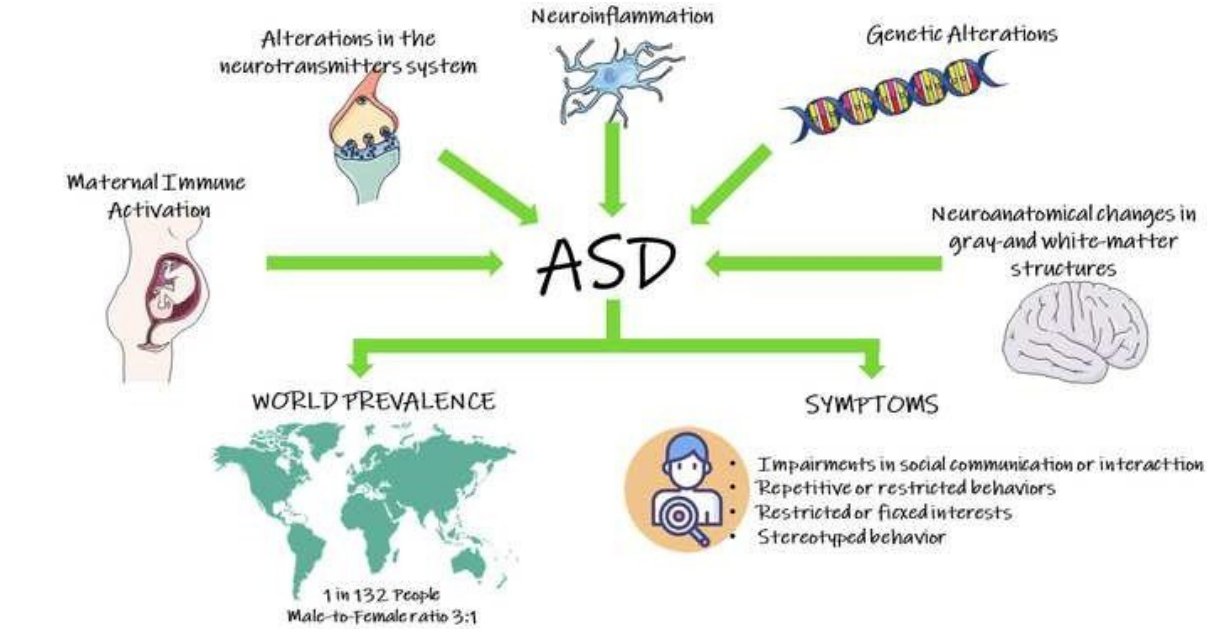
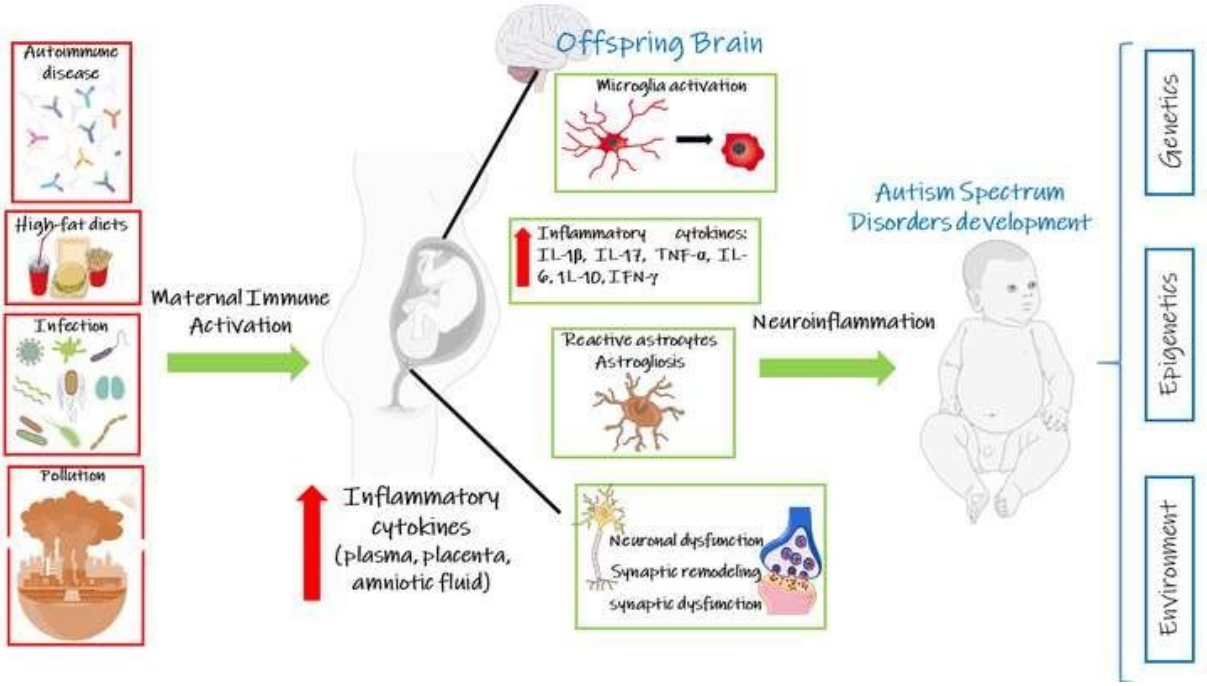


Figure 1: Etiology and symptoms of Autism spectrum disorders



Obesity associated with autism spectrum disorders: mitochondrial dysfunction and neuroinflammation as a links

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Highlights:

Abstract: Autism and obesity are highly prevalent for mitochondrial dysfunction. More specifically, obesity and autism are strongly associated with mitochondrial dysfunctions in the CNS. There are some characteristics in common between autism and obesity, suchas the activation of the immune system, the release of pro-inflammatory cytokines, the presence of oxidative stress, neuroinflammation, morphophysiological alterations in the CNS, deficit in areas such as memory, learning and cognition, among others.

Due this link between obesity, autism and mitochondrial dysfunction, this article set out to review the underlying biochemical mechanisms of all these events, suggesting that the triggering of some these biochemical mechanisms may occur through common routes, and may be good targets in the treatment of the etiology and/or symptoms of these comorbidities.

The metabolic profiles of patients with ASD suggest a possible implication of mitochondrial pathways.

In this review, we summarize the mechanism of mitochondria and its role in neurons, and the consequence of mitochondrial dysfunction in ASDs. Deeper appreciation of the role of mitochondria in ASDs pathology is required for developing new therapeutic approaches.

Keywords: Autism; Obesity; Mitochondrial Dysfunction; High-fat diet; Neuroinflammation

INTRODUCTION

Autism spectrum disease (ASD) are a group of neurodevelopmental disorders characterized by deficits in communication and social interaction, restricted interests and repetitive behaviors. ASD symptoms start to manifest at the beginning of childhood, being present between 2 and 6 first months of age (Diagnostic Stat. Man. Ment. Disord. 5th Ed., 2013). The symptoms of autism spectrum disease range from mild to severe (MAYES et al., 2012).

The world prevalence of ASD is estimated in about 1.5% (LYALL et al., 2017a), with a higher incidence in males in relation to females (MAENNER et al., 2020). The prevalenceof ASD in Brazil is 1 in 367 in children aged between 7 to 12 years old (PAULA et al., 2011). In 2020, the United States prevalence of ASD was 1 in 54 children aged 8 (MAENNER et al., 2020), showing a significantly increase in the last decade (about 2.5 times). In Europe the ASD prevalence changes across different countries. In Italy, the prevalence is 1 in 87 in children aged 7 to 9 years old, diagnosed according to DSM-IV and CIE10 (Classification of International Illness, 10ª Edition, WHO) criteria (NARZISIet al., 2018). In Germany, the prevalence is 1 in 66 in children aged 6 to 11 years old (BACHMANN; GERSTE; HOFFMANN, 2018). The prevalence in Norway is 1 in 125,and in Spain the prevalence is 1 in 64 in children aged 3 to 4 years old, and 1 in 100 in children aged 10 to 11 years old (MORALES-HIDALGO et al., 2018).

ASD symptoms include motor impairments (AMENT et al., 2015), sleep disorders (TZISCHINSKY et al., 2018) and food selectivity (CHISTOL et al., 2018). Moreover, ASD can be associated with several comorbidities and associated disorders such as epilepsy (20%) (TUCHMAN; RAPIN, 2002; EWEN et al., 2019) and gastrointestinal problems (9-91%) (COURY et al., 2012). ASD is also associated a high prevalence of obesity and overweight (TOSCANO et al., 2019) being estimated a prevalence of 42% for overweight and 21% for obesity compared to the incidence in healthy children that have a prevalence of 26% for overweight and 12% for obesity (CRIADO et al., 2018a).

The etiology of ASD is still unclear, but several studies suggest an interaction between genetic and environmental components (GHIRARDI et al., 2019; HEGARTY et al., 2020; SHARON et al., 2019).

Obesity is a disease characterized by a high and abnormal fat accumulation and is a risk factor for metabolic disorders. Increase consumption of hypercaloric diets associated with

sedentary behaviors are the most causes of obesity (ARLUK et al., 2003; WORLD, 2016). Hypercaloric diet leads to an incomplete fat acids oxidation triggering a mitochondrial dysfunction in different regions of the Central Nervous System (CNS) (CAVALIERE etal., 2019; DIAZ et al., 2015; KOWALTOWSKI et al., 2019; LANGLEY et al., 2020;

TONIAZZO et al., 2019). We hypothesized mitochondrial dysfunction can be the link factor in explaining the development of ASD, symptoms and associated comorbidities, in which obesity is a potential aggravating factor in the severity of symptoms.

In this review we will highlight pathological mechanisms of obesity and mitochondrial dysfunction and their association with the symptoms of autism, also trying to understand which are the causes for the development of comorbidities associated with ASD.

1. AUTISM SPECTRUM DISORDERS

Autism spectrum disorders, also knew as autism, are characterized mainly by repetitive and stereotyped behaviors, restricted interests, repetitive movements, deficits in communication and social interaction, non-verbal communication behaviors and hyper or hiporeactivity to sensory stimuli (Diagnostic Stat. Man. Ment. Disord. 5th Ed., 2013). ASD is a complex disorder with high phenotypic heterogeneity (TOAL et al., 2010).

Autism can usually be associated concurrently to others neurological and psychiatric dysfunctions like epilepsy (EWEN et al., 2019), sleep dysfunction (FLETCHER et al., 2020), cognitive deficit, attention deficit and hyperactivity (GODOY et al., 2020), ZABLOTSKY; BRAMLETT; BLUMBERG, 2020), and anxiety (POSTORINO et al.,

2017). ASD is also associated with food selectivity (specially refusing vegetables) (CHISTOL et al., 2018; LUCARELLI et al., 2017), gastrointestinal dysfunctions (COURY et al., 2012), dyslipidemia (LUO et al., 2020) and obesity (TOSCANO et al., 2019). ASD is a disease with a multiple debilitating condition, involving multiple physiological systems. The comprehension of mechanisms involved in the ASD and associated comorbidity is of great relevance for setting targets and therapeutic treatments. Although the etiology of ASD is still a matter of debate in the scientific community, it isalready acceptable that results from the complex interaction between genetic, environmental factors, and immune system activation (GHIRARDI et al., 2019; HEGARTY et al., 2020; SHARON et al., 2019; LE BELLE et al., 2014).

Among the environmental factors that have association with ASD development are maternal nutrition and obesity (SANCHEZ et al., 2018; LYALL et al., 2013), maternal metabolic disorders (CORDERO et al., 2019; CURRAN et al., 2018; WAN et al., 2018; KONG et al., 2018), maternal exposure to environmental pollution and pesticides (LYALL et al., 2017b) and maternal immune system activation (GUMUSOGLU et al., 2017).

Several mechanisms are proposed to be involved in the pathophysiology of ASD, including changes in synaptic proteins (neuroligins, neurexins, post synaptic scaffold proteins) (CAST et al., 2020; ZHANG et al., 2018; HALI et al., 2020; AMAL et al., 2020), neurotrophins (BARBOSA et al., 2020), neurotransmitors and their transporters (DICARLO et al., 2019; GARBARINO et al., 2019), neurodevelopment disturbances through activation of maternal immune system (ZERBO et al., 2015), and mitochondrialdysfunction in CNS (CORRIGAN et al., 2012)**.**

Neuroinflammation is a potential key factor for ASD symptoms and is a hallmark event in ASD subjects. Furthermore, neuroinflammation is a process that can be triggered by mitochondrial dysfunction events (ISHIHARA et al., 2015). Therefore, these two mechanisms can be closely associated and have a direct connection with obesity.

Astrogliosis and microglia activation were observed in postmortem brain samples of ASD subjects, being identified in cerebellum, cingulate gyrus, cortex, white matter and hippocampus (LUCCHINA; DEPINO, 2014; VARGAS et al., 2005). Higher citokines expression, MCP-1, TGF- β1, IL-1 β, IL-6 and TNF-α, was also observed in brain samples of ASD subjects and in animal models of autism (HU et al., 2018). These data suggest the presence of an inflammatory state in autistic individuals asa recurrent factor in the disease, also suggesting that the activation of the peripheral and central immune systems may be involved in the onset of autism.

Autistic individuals commonly present neuroanatomical, neurophysiological and molecular changes in the brain regions involved in social behavior, memory, cognition and emotions (CIEŚLIK et al., 2020; KOJIMA et al., 2019; POSTEMA et al., 2019; STERNBERG, 2006; STOODLEY et al., 2017; WEGIEL et al., 2010). Changes in brain connectivity (cerebellum, parietal lobo, cortex, cingulate cortex, temporal areas, orbitofrontal cortex) (STOODLEY et al., 2017; POSTEMA et al., 2019), increased volumes (thalamus, striated, ventricular area and pale globe) (SCHOEN et al., 2019; OWEN et al., 2018) and decreased in gray matter, posterior cingulate cortex, precuneus and white matter (KOJIMA et al., 2019; OWEN et al., 2018) are demonstrated in ASD individuals. Another important feature

is the increased brain size of autistic individuals compared to age-matched controls (SACCO; GABRIELE; PERSICO, 2015; AMARAL et al., 2017). Lower thickness of neurons from the amygdala, hippocampus and cortex of

post-mortem brains of ASD individuals has also been reported (AZMITIA et al., 2011). It was also demonstrated a reduction in the density of apical dendrites in pyramidal neuronin the neocortex (WILLIAMS et al., 1980), as well as a lower density of myelinated axons in the orbitofrontal cortex (LIU et al., 2020), and in corpus callosum (OWEN et al., 2018). Autists also presented in postmortem brains dysplasia with thickening ofsubependymal cells and subependymal nodules (indicating active neurogenesis), hippocampal, subcortical, cerebellar and periventricular heterotopia (indicating abnormal neuronal migration), cerebral multifocal dysplasia (result of distortion of the cytoarchitecture of neocortex, cortex, Ammon's horn and dentate gyrus (WEGIEL et al., 2010). Cerebellar and vermis flocculonodular dysplasias and cerebellar hypoplasia were also found (WEGIEL et al., 2010).

Magnetic resonance images show that ASD subjects has lower activation of frontal and parietal cortex, and striatum that were associated to lower cognitive capacity (SHAFRITZ et al., 2008). Furthermore, the severity in repetitive behaviors were negatively associated to the activation of the anterior cingulate gyrus and posterior parietal cortex, indicating that executivedysfunctions may be associated with dysfunctions in these circuits (SHAFRITZ et al., 2008).

Several molecular mechanisms have been implicated in ASD, such as changes in neurotransmission, in neurotrophic factors and in synaptic proteins. Specifically, it was demonstrated an increase in serotonin and serotonin transporters in blood samples from ASD children, that was positively associated with the severity of ASD comorbidity (ABDULAMIR; ABDUL-RASHEED; ABDULGHANI, 2018). The brain-derived

neurotrophic factor (BDNF) transcription was found higher in the peripheral blood of ASD children compared to healthy control children (GHAFOURI-FARD et al., 2020). However, in ASD newborns the levels of BDNF expression was found to be decreased (SKOGSTRAND et al., 2019). These results suggest that the period in which theexpression of BDNF is changed can be relevant to the pathophysiology of autism.

ASD pathophysiology is associated with mutations in synaptic proteins, such as neurexins (NRXN2 rs12273892 and NRXN3 rs12879016 variants associated with the autistic phenotype) (WANG et al., 2018), and neuroligins (BURROWS et al., 2017). Mutations in neuroligin-3 decrease at synaptic transmission and induces deficits in socialbehavior both in

humans and in animals models (BURROWS et al., 2017) (ZHANG et al., 2017).

Deficits in the Shank3, a postsynaptic protein, is well-characterized in ASD subjects. These deficits in the Shank3 leads to changes in mobilization of synaptic vesicles andconsequently causing deficits in synaptic function, that are related with impairments in social interactions and communication (AMAL et al., 2020; BOZDAGI et al., 2010). ASD animal models, Shank2$^{-/-}$ and Shank3 αβ$^{-/-}$, have changes in the glutamatergic and GABAergic neurotransmission in striatum, thalamus, cortex and hippocampus (HEISE et al., 2018). In animal models of ASD, it has been demonstrated a severe reduction in synaptic excitation and inhibition in pyramidal neuron of medial prefrontal cortex (LAZARO etal., 2019), increase in the connectivity between the somatosensory cortex and the cingulate cortex, and decrease in the connectivity between the somatosensory cortex and the prefrontal cortex, that indicates dysfunctions in synaptic pruning (KIM et al., 2017). Mutations in CNTNAP2 and atg7 genes (with adhesion functions of molecules and receptors in the central nervous system and autophagic function, respectively), involved in ASD phenotypes, induces changesin the synaptic connection and microglial autophagy, and are associated with decreasedneural spines and synapses, increased activity on inhibitory neurons in the prefrontalcortex region, increased dendritic spines in the cortex and dysregulation in synaptic pruning activity, resulting in changes in connectivity between brain structures (KIM et al., 2017).

2. OBESITY

Obesity is defined as an abnormal and excessive accumulation of adipose tissue, that results from positive energetic balance (WORLD, 2016). Obesity is a risk factor for several diseases, including not only metabolic (HIGGINS et al., 2020; SHAH et al.,2016), but also cardio-cerebrovascular diseases (CHEN et al., 2019), psychiatric diseases (WORLD, 2016), and liver diseases (LI et al., 2020). Obesity is classified according to the body mass index (BMI), a ratio of body weight (kilograms) per square of height (meter). The BMI classifies the body weight in five categories: underweight ($\leq$ 18.5 kg/m^2); normal weight (18.5 to 24.9kg/m^2); overweight (25 to 29.9kg/m^2); and obesity ($\geq$ 30 kg/m^2). Obesity is frequently divided into categories: class 1 (30 to 34.9kg/m^2); class 2 (35 to 39.9kg/m^2); and class 3 or morbid obesity ($\geq$ 40 kg/m^2).

Since 1975, obesity rates has tripled worldwide, being estimated that in 2016more than 1.9 billion people over 18 years old were overweight. Of these, 650 million were obese (WORLD, 2016). It was estimated that in 2019, 38 million children under 5 years of agewere overweight or obese (WORLD, 2016).

Obesity is considered a low grade inflammatory disease. The increase in the adipose tissue occurs in cell size and number, and leads to changes in their secretory function, with alterations in hormone and cytokines release (JO et al., 2009). The increase in adipose tissue induces the release of pro-inflammatory cytokines, generating a chronic inflammatory state responsible for metabolic alterations (MCARDLE et al., 2013; STRASSER, 2017).

Obesity and overweight are more prevalent in ASD individuals compared with individuals without ASD (CRIADO et al., 2018b; TOSCANO et al., 2019; BRODER-FINGERT et al.,2014; HILL; ZUCKERMAN; FOMBONNE, 2015). The increase in obesity prevalence in ASD can be caused by many factors like genetic mutations, where greater sensibility to bitter taste and food refusals (generally refusing fruits and vegetables) is associated with the TASR38 genotype, commonly found in autistic individuals (RICCIO et al., 2018), sedentary behaviors (MCCOY; MORGAN, 2020), sleep disorders and circadian rhythm alterations (YAVUZ-KODAT et al., 2020).

The deposition of body fat occurs mainly in two places, in the subcutaneous adipose tissue(SAT) and in the visceral adipose tissue (VAT), and the increased size of these adipose cells is related to different metabolic dysfunctions (HOFFSTEDT et al., 2010). The increase in visceral adipocytes is strongly associated with impaired glucose metabolism,

hyperinsulinemia and insulin resistance (HOFFSTEDT et al., 2010).

Simultaneously with adipose tissue expansion, occurs the recruitment and infiltration of macrophages (WEISBERG et al., 2003a). Recruited macrophages are activated and release proinflammatory cytokines, that induces a low degree chronic inflammation (HAN et al., 2020).

Once recruited to adipose tissue, the macrophages are activated to perform their classic functions. The macrophage activation occurs through two polarization states: M1 and M2,that depend mainly on cytokines, proteins expressed in the membrane and chemokines (VOGEL et al., 2014). Maturation in the M1 state occurs through the exposure of macrophages to Th1 cytokines such as IFN-γ, IL-2 and LPS (GOERDT et al., 1999). Such M1 macrophages secrete proinflammatory cytokines, the main being IL-6, TNF-αe IL-1β (GOERDT et al., 1999). The polarization of macrophages in the M2 state occursmainly through their exposure to IL-6, IL-4, GM-CSF and IL-13 (BRAUNE et al., 2017).M2 macrophages have a tissue repair function and secrete anti-inflammatory cytokines such as IL-10 e IL-1RA, in addition to also secreting the transforming growth factor (TGF)-β (BOURLIER et al., 2012). The recruitment of macrophages and the polarization phenotype play a strong role in the inflammatory state, in addition to regulating insulin sensitivity (JIA; MORGAN-BATHKE; JENSEN, 2020), angiogenesis (PANG et al., 2008), adipogenesis (LEE; PETKOVA; GRANNEMAN, 2013) and consequently in the glycemic homeostasis and metabolic profile.

T lymphocytes also have an important role at metabolic inflammation. In an animal modelof diet-induced obesity, adipose tissue is infiltrated with lymphocyte $TCD8^{+}$, that are important for the macrophages recruitment, and for M1 polarization, also being associated with insulin resistance (NISHIMURA et al., 2009). The interaction of B lymphocytes with fatty acids, activates the secretion of proinflammatory cytokines and IgG antibodies,inducing the polarization of M1 macrophages, and the activation of T lymphocytes, establishing the importance of B cells and adaptive immunity in insulin resistance with high-fat diets consumption (WINER et al., 2011). Saturated fatty acids activate inflammatory pathways through activation of Toll-like receptors (TLRs) in macrophage cells (SINDHU et al., 2020). TLRs activation initiates a signaling cascade that activates NFκB and release of pro-inflammatory cytokines and chemokines (BARTON; MEDZHITOV, 2003).

2.1 NEUROINFLAMMATORY PROCESS THROUGH OBESITY STATE AND/OR HIGH-FAT DIETS

Besides peripheral inflammation, high-fat diet consumption and obesity are also associated with a central nervous system inflammation, also called, neuroinflammation (THALER et al., 2012). The neuroinflammation is an important risk factor for neurodegenerative disorders which are characterized by cognitive impairment and are generally associated with altered neuronal connectivity and atypical synaptic plasticity (KUMAR, 2018). When neuroinflammation is triggered by high-fat diet and obesity, is associated with astrocytes and microglia activation, a weakened psychosocial profile, with decreased cognition, deficit in memory, learning, communication, beyond impairments in social behaviors, anxiety behaviors and mood disorders (DALVI et al., 2017; DOUGLASS et al., 2017; WAISE et al., 2015).

Neuroinflammation is a common mechanism both in obesity/high-fat diet consumption and in ASD (KIM et al., 2018; LUCCHINA; DEPINO, 2014). This neuroinflammation can be associated in obese animal models and ASD animal models with low cognitive performance (SAMARA et al., 2020; ALABDALI; AL-AYADHI; EL-ANSARY, 2014), as well as with impairments in memory and learning processes (MARKO et al., 2015; WILLIAMS; GOLDSTEIN; MINSHEW, 2005; ALABDALI; ARNORIAGA RODRÍGUEZ et al., 2019; DE LUCA et al., 2016).

The activation of microglia is a hallmark during the neuroinflammatory process. Activated microglia secretes proinflammatory cytokines, make phagocytosis, and present antigens to protect brain under toxic stimulus (JHA; LEE; SUK, 2016). Although it is not yet clear how the peripheral immune response communicates with central immune response, the systemic inflammation (such as caused by obesity) induces damage in the blood brain barrier (RANSOHOFF et al., 2015) which are associated with lymphocytes penetration, cytokine release and the activation of microglia in the central nervous system,causing neuroinflammation and neuronal dysfunction (VARATHARAJ; GALEA, 2017).Moreover, the interaction of B-lymphocytes with fatty acids induces immune dysfunction, with the secretion of IgG proinflammatory antibodies (WINER et al., 2011)that adhere and activate microglial cells (YI et al., 2012).

Many animal models have been used to better understand the mechanisms by which the diet modulates the inflammatory response and what impacts are generated on the CNS of

these animals.

In mice, obesity induced-neuroinflammation is associated with activation of microglia, neuronal stress in mediobasal hypothalamus (MBH), and altering the impact of saturatedfat on leptin responsiveness through microglia (VALDEARCOS et al., 2014).

Saturated fatty acids bind in Toll-like receptor 4 (TLR4) and trigger the secretion of inflammatory cytokines through the IKK- NFκB pathways and leading to the hypothalamic inflammation (neuroinflammatory process), beyond, insulin and leptin resistance (MARIC; WOODSIDE; LUHESHI, 2014; VALDEARCOS et al., 2014). In the hypothalamus, the neuroinflammatory process is associated with the long-term weight gain, lipotoxicity and associated comorbidities (VALDEARCOS et al., 2014). The short- term (1-3 days) consumption of high-fat diet is enough to induce hypothalamicneuroinflammation (WAISE et al., 2015; THALER et al., 2012; DALVI et al., 2017; YI et al., 2012). This neuroinflammation associated with obesity was also observed inprefrontal cortex leading impairments in motor and exploratory behavior in addition to a pattern of depression in behavioral tests (VENIAMINOVA et al., 2020). This phenotypeis also found in individuals with ASD (LICARI et al., 2020; HOLLOCKS et al., 2019). Hippocampal neuroinflammation, with an increase in the number and size of reactiveastrocytes, was demonstrated in rats fed on a high-fat and high-fructose diet for 7 days.These animals also had a reduction in dendritic arborization and in the number ofdendritic spines in hippocampal CA1 region (CALVO-OCHOA et al., 2014). Hippocampal neuroinflammation is also associated with deficits in long term potentiation(LTP) and a decrease in synaptic density (VALCARCEL-ARES et al., 2019).

In experiments with rodents fed for 1 month with high-fat diet and high sucrose diet, wasobserved hippocampal inflammation with high levels of TNF-α, IL-1β and oxidative stress, and animals presented worsening memory in hippocampal-dependent recognitiontasks (BEILHARZ; MANIAM; MORRIS, 2014). Compulsive and anxious behavior was

also detected in high-fat diet animals, and this was associated microglia activation, increased TNF, IL-6 levels, and increase in nitrite levels in the prefrontal cortex and hippocampus (GOMES et al., 2018).

In another study, was used a mice model with obesity induced by high-fat diet to demonstrate that 4-1BB receptors (present in T cells, adipocytes and endothelial cells) and their 4-1BBL ligands (highly expressed in macrophages and antigen presenting cells) are involved in activation of s and microglia (KIM et al., 2018). After being activated, astrocytes

and microglia produce inflammatory mediators (TNF-α, MCP-1, e IL-6) causing neuroinflammation in the hypothalamus. It was found that astrocytes and microglia exposed to factors such as LPS, FFA, high glucose and ATCM, increased the levels of 4-1BB transcripts in both glial cells. Levels of TNF-α, MCP-1, and IL-6 were also increased, with increased secretion of MCP-1 and IL-6 in both cells (KIM et al., 2018).

It was found in mice feed for 20 weeks on a high-fat diet that there was a decrease in mitochondrial respiratory function in the hippocampus, an increase in ROS levels, an increase in apoptosis in the hippocampus and insulin resistance (PARK; CHO; KIM, 2018). This suggests that obesity induced by a high-fat diet causes damage to mitochondrial function in the brain concomitantly with damage to memory function (PARK; CHO; KIM, 2018).

A study showed that mice fed with western diet presented decreased expression of serotonin transporter (SERT) and neuroinflammation with a high number of activated microglia in the prefrontal cortex (VENIAMINOVA et al., 2020). These animals also showed impairments in motor and exploratory behavior, in addition to a pattern of despair in behavioral tests (VENIAMINOVA et al., 2020). The deficiency in the motor pattern and the despair behavioral (typical behavior found in more anxious individuals) are also found in ASD animals (LICARI et al., 2020; HOLLOCKS et al., 2019).

In a study where rodents received a highly palatable diet (high levels of sucrose), they showed high levels of protein damage in the frontal cortex (indicated by decreased amounts of tryptophan and tyrosine) and a higher grade of anxiety in behavioral tests compared with control diets (SOUZA et al., 2007). Another study also shows that rodentswith diet induced obesity showed cognitive deficits concomitantly with neuroinflammation and ROS in the hippocampus (TUCSEK et al., 2014a).

Analysis in the hippocampus of rats fed 7 days on a high-fat/high-frutose showed an increase in the number and size of reactive astrocytes, a decrease in the hippocampal weight, a reduction in dendritic afforestation and in the number of dendritic spines in the CA1 region of the hippocampus(CALVO-OCHOA et al., 2014). In an experiment with rodents fed for 1 month on a high-fat diet and high sucrose content, they presented worsening memory of hippocampal-dependent recognition, presenting hippocampal inflammation with high levels of TNF-αand IL-1β mRNA and oxidative stress in the hippocampus (BEILHARZ; MANIAM; MORRIS, 2014). Another study with young and old groups of mice fed with high-fat dietalso showed a deficit in the memory of hippocampal-dependent recognition (VALCARCEL-ARES et al.,

2019). Moreover, the mice showed a deficit in long term potentiation (LTP) and a decrease in synaptic density in the radiated stratum of the hippocampus (VALCARCEL-ARES et al., 2019).

Mice fed with a high-fat diet for 5 months showed a reactive astrogliosis in the striatum and in the substantia nigra, decreased dopaminergic neurons in the substantia nigra, decreased density of dendritic spines in the substantia nigra and decreased peroxisome proliferator activation receptor (PPAR) (KAO et al., 2020).

Studies with chronic exposure of mice to highly refined carbohydrates showed that theseanimals presented compulsive and anxious behavior in behavior tests, associated with neuroinflammation and activation of microglia and an increase in nitrite levels in the prefrontal cortex and hippocampus (GOMES et al., 2018). The expression of iNOS was increased in the prefrontal cortex and there was an increase in the levels of TNF, IL-6 and leptin in the hippocampus (GOMES et al., 2018).

3. HIGH-FAT DIET INDUCED OBESITY AND MITOCHONDRIAL DYSFUNCTION IN THE CNS OF ANIMALS MODELS

Mitochondria are the organelles that provide energy to cells through ATP synthesis and the oxidative phosphorylation (OXPHOS), and for this reason they have a fundamental role in the CNS that requires high energy demand for their normal functioning. They are the primary sources and also targets of ROS, and the accumulationof ROS causes oxidative stress and dysfunction in the mitochondrial structures due to thehigh potential for damage that ROS presents (HARMAN, 1972).

The obesity and high consumption of hyperlipidic diets leads to a significant increase of ROS (TUCSEK et al., 2014b). High levels of ROS cause oxidative stress and are toxic to mitochondria, being a common event in individuals with ASD (ROSE et al., 2012). ROS cause aggressions in the structure of mitochondria, such as mutations and deletionsin mtDNA that lead to morphophysiological dysfunctions in the mitochondria (NAPOLI;WONG; GIULIVI, 2013). Mutations in mtDNA and the insufficient presence of repair mechanisms (antioxidant enzymes) also generate high levels of ROS (FLEMING et al., 1982; LINNANE et al.,1989), and the high levels of these oxy-molecules generate mutations in mtDNA, creating a vicious

cycle of ROS development and mitochondrial dysfunction (MANDAVILLI; SANTOS; VAN HOUTEN, 2002).

Several studies have demonstrated mitochondrial dysfunction as one of the cellular mechanisms that occurs in neurologic and neurodegenerative diseases associated with the high consumption of hyperlipidic diets and obesity, being also associated with increased oxidative stress (ADAV; PARK; SZE, 2019; LEE et al., 2018; VARGA et al., 2018; GUERRA et al., 2019; CARRARO et al., 2018; CUNARRO et al., 2018).

The energy demand in the brain is very high compared to other tissues, and small changes in energy synthesis can lead a large impact on the brain (JOSHI; MOCHLY-ROSEN, 2018). Mitochondria produce large amounts of energy at the presynaptic level, which is largely used for exocytosis and reuptake of neurotransmitters into synaptic vesicles, while at the post-synaptic level a large content of energy produced is used for membrane excitability and functioning of the ion channels (LORES-ARNAIZ et al., 2016). Mitochondrial dysfunction in cells of the nervous system induces reduction in the quality of synaptic transmission and consequently failures in cognitive process due to low energy supply (REDDY; BEAL, 2008). Therefore, mitochondrial malfunction in CNS cells presents a high risk for the development of the pathophysiological state, typical of neurologic and neurodegenerative disorders.

Several studies in rodents show processes of mitochondrial dysfunction, such as changes in biogenesis, dynamics, signaling, respiration and morphology in the CNS induced by hyperlipidic diets (*figure 1*) (CARRARO et al., 2018; CAVALIERE et al., 2019; LANGLEY et al., 2020; DESMOULINS et al., 2019; DIAZ et al., 2015; TONIAZZO etal., 2019; CARVALHO et al., 2012; GAO et al., 2010; SRIPETCHWANDEE; CHATTIPAKORN; CHATTIPAKORN, 2018). Moreover, high-fat diets induce significant effects on ATP production and gene expression levels of relevant antioxidant enzymes (*figure 1*) (REUTZEL et al., 2021).

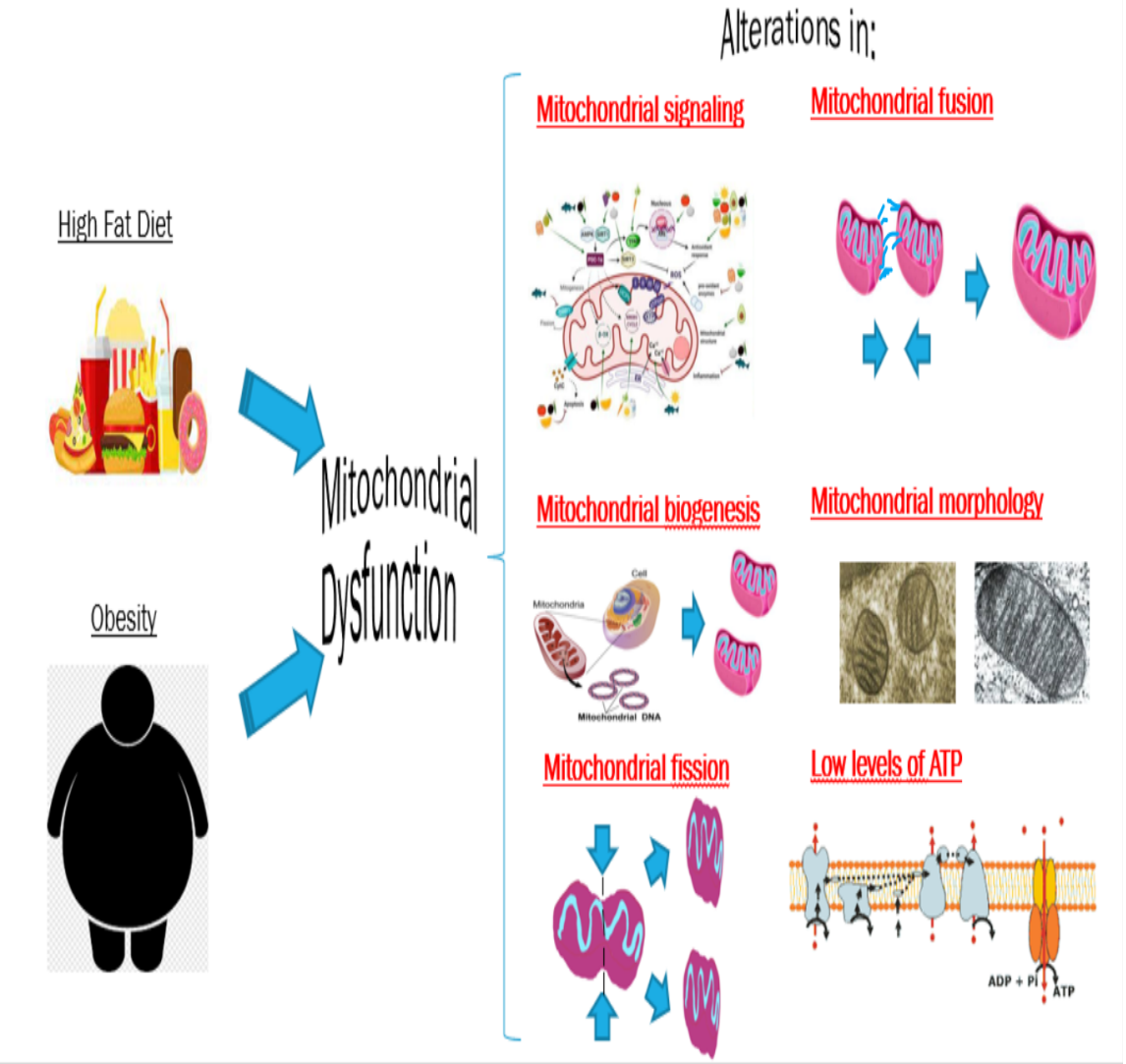

Figure 1. Obesity and high fat diets induce to mitochondrial dysfunction in CNS.

Mice with a high-fat diet consumption for 18 weeks showed increase inflammation and oxidative stress and a decrease in mitochondrial oxidative capacity both in all the brain cortex and their synaptosomal fraction (CAVALIERE et al., 2019). In the cerebral cortex, using the substrates succinate or pyruvate, mitochondrial state 3 respiration decreased in the HFD mice with no changes in state 4. The maximal FCCP-stimulated respiration was reduced in the HFD mice (CAVALIERE et al., 2019). In spinal cord cells of mice fed a high-fat diet for 12 weeks, it was observed a reduction in mitochondrial respiration (LANGLEY et al., 2020). Using a cell culture of mouse oligodendrocytes treated with a saturated fatty acid, palmitate (PA, 100µM por 24h), was observed a decrease in the baseline consumption of oxygen linked to ATP, and no effect on the leakage of protons in cells treated with PA (LANGLEY et al., 2020), reinforcing the hypothesis that saturated fatty acid has a negative effect on cellular respiration. Saturated fatty acids also induce morphological changes in

mitochondria (smaller mitochondria, increased mitochondrial fragmentation) (LANGLEY et al., 2020). Mice fed a high-fat diet for 20 weeks showed deficits in hippocampus dependent memory and insulin resistance, associated with a decrease in mitochondrial respiratory function, an increase in ROS levels, and an increase in neuronal apoptosis in the hippocampus (PARK; CHO; KIM, 2018). In rodents fed a high-calorie diet for 5 weeks, Toniazzo and collaborators analyzed the activity of the electron transport chain in the hypothalamus (TONIAZZO et al., 2019); female mice hada decreased activity in complexes I and II and showed an increase in complex IV activityand male mice had a reduction in complex IV activity. This study showed a gender specific heterogeneous mitochondrial dysfunction influenced by HFD feeding (TONIAZZO et al., 2019). These authors and also showed that females fed a high-fat diet had a lower mitochondrial mass (TONIAZZO et al., 2019).

In rats fed for 3 weeks with high-fat and high-sucrose diet was observed in hypothalamic neurons a decrease in oxygen consumption in states 2 (in the presence of succinate, only) and 3 (in the presence of ADP) of mitochondrial respirationand a decrease in the e respiratory chain coupled to the ADP phosphorylation (DESMOULINS et al., 2019). No changes were found in the protein quantification for mitochondrial complexes which suggests that the decrease in oxygen consumption was related to the decrease in mitochondrial activity (DESMOULINS et al., 2019).

Mitochondrial dynamics, fusion and fission are also changed in animal models of diet- induced obesity (CARRARO et al., 2018; LANGLEY et al., 2020). It was observed a

decrease in the expression of the mitochondrial fusion protein, mitofusin-2 (MFN2) in the mice hypothalamus after 24h of high-fat diet consumption (CARRARO et al., 2018).In cultured hypothalamic neurons treated with palmitic acid (250µM/12h) and palmitoleicacid (250µM/24h), it was also observed a decrease in MFN2 expression levels (DIAZ etal., 2015).

Hypothalamic ER stress is a proposed mechanism for the development of leptin resistance, and a decreased in mitochondria-ER contacts in anorexigenic pro-opiomelanocortin (POMC) neurons was observed in diet-induced obesity mice (SCHNEEBERGER et al., 2013). Ablation of MFN2 in POMC neurons induces loss of mitochondria-ER contacts, defective POMC processing, ER stress-induced leptin resistance, hyperphagia, reduced energy expenditure and obesity (SCHNEEBERGER et al., 2013).

In hippocampus of high-fat diet fed animals, it was observed a decrease in the number of neurons, as well as damaged mitochondria and extended cisterns of the endoplasmic reticulum (ALKANet al., 2021). It was also observed an increased in the oxidative stress

parameters (increase in SOD, CAT, LPO and MPO activity) (ALKANet al., 2021).

The consumption of high-fat diets induces a significant increase in ROS production (BARAZZONI et al., 2012; MORO et al., 2016; RUIZ-RAMÍREZ et al., 2011; SVERDLOV et al., 2015, 2016). Highly palatable diet (high levels of sucrose) increasesthe amount of protein oxidation in frontal cortex and induce anxiety-like behavior in rats(SOUZA et al., 2007). Another study also shows that rodents with diet induced obesity had cognitive deficits associated with neuroinflammation and increased ROS production in the hippocampus (TUCSEK et al., 2014). In a model of high-fat/high-frutose diet, it was demonstrated an increase in oxidative stress in the frontal cortex (BATANDIER et al., 2020).

These results point to a strong association between the high-fat/high-calorie diets induced obesity with the development of mitochondrial dysfunction in the animal CNS. Therefore, suggesting that the obesity and the high intake of this dietary quality can significantly negatively influence individuals who present a pathophysiological condition in the CNS cells, at the risk of aggravating neurophysiological dysfunctions and consequently the severity of the symptoms presented.

5. THE LINK BETWEEN OBESITY INDUCED BY HIGH FAT DIET, MITOCHONDRIAL DYSFUNCTION AND NEUROINFLAMMATORY PROCESSES

Mitochondrial dysfunction is associated with production of many key factors during inflammation and other energy-dependent disturbances, where ROS production can overcome cellular antioxidant capacity and cause cellular oxidative damage (CHAN, 2006). Due to this mitochondrial role in inflammation, many studies have found an association of mitochondrial dysfunction in brain with several neurological and neurodegenerative disorders (CLAY; SILLIVAN; KONRADI, 2011; GIRARD et al., 2012; RAJASEKARAN et al., 2015; SU et al., 2011; YUI; SATO; IMATAKA, 2015).

One of the ways in which HFD-induced obesity promotes alterations in mitochondrial and neuroinflammatory mechanisms is through the mitochondrial uncoupling protein Ucp2, as demonstrated by Kim et al (KIM et al., 2019).

In rodent models of HFD-induced obesity, was shown that the expression of mRNA levels of the Ucp2 was increased in a time-dependent manner in the hypothalamus of these animals, as well as increased expression of proinflammatory cytokines such IL1-β, IL-6, TNF-α and the microglial activation marker Cx3cr1 (KIM et al., 2019). It was also observed in

microglia in the arcuate nucleus of the hypothalamus of animals exposed to HFD, an increase in the number of mitochondria and a decrease in mitochondrial size also in a time-dependent manner (KIM et al., 2019). The mitochondrial fission protein Drp1 was found with increased levels in microglia of animals exposed to HFD in the cortex and hypothalamus, also in a time-dependent manner (KIM et al., 2019). To evaluate the influence of Ucp2 on the alteration of the mitochondrial morphology of the arcuate nucleus (hypothalamus) exposed to HFD, a knockout mice model for the Ucp2 ($Ucp2^{KO}$) protein was used and it was observed that these $Ucp2^{KO}$ animals had greater mitochondrial size and mitochondrial density lower in a time-dependent manner when exposed to HFD compared to wild animals exposed to HFD or SD (KIM et al., 2019). Furthermore, wild mice exposed to HFD showed increased levels of the mitochondrial fission protein Drp1 in the cortex and hypothalamus, while $Ucp2^{KO}$ animals showed no differences in the expression of this protein when exposed to HFD or SD (KIM et al., 2019). Therefore, the Ucp2 protein influences mitochondrial dynamics (fission) through exposure to the HFD, influencing mitochondrial density and size in these mice.

The arcuate nucleus microglia size was observed increased in wild mice exposed to HFD compared to standard diet wild group, and an attenuated effect with respect to microglial size was found in $Ucp2^{KO}$ mice exposed to HFD (KIM et al., 2019). Regarding the expression of inflammatory cytokines IL-6, IL-1β, TNF-α and also the neuronal stress marker Hsp72, these showed higher levels in the wild group exposed to HFD than in the other groups that received standard diet or HFD (wild or knockouts), evidencing a time-dependent role in the inflammatory signaling of the Ucp2 protein triggered by the HFD (KIM et al., 2019).

The $Ucp2^{KO}$ mice also had lower body weight and lower white adipose tissue content when exposed to HFD than wild mice exposed to standard diet or HFD, and also higher glucose tolerance and higher insulin sensitivity than the other groups of animals (KIM et al., 2019), also showing an obesogenic role and in the metabolic profile of the Ucp2 protein. Immunoreactivity in POMC neurons (hypothalamus) was found in $Ucp2^{KO}$ animals when analyzed through reactivity C-Fos (a marker for neuronal activation), and a greater sensitivity to leptin was also detected in $Ucp2^{KO}$ animals in POMC neurons through pSTAT3 when exposed the HFD compared to wild-type HFD group, inducing a decrease in food intake in the $Ucp2^{KO}$ group (KIM et al., 2019).

The mitochondrial protein Drp1 also plays a role in neurodevelopment, as can be seen through the generation of $Drp1^{-/-}$ and $Drp1^{+/-}$ mutant mice, where it was noticed that the $Drp1^{-/-}$ is lethal for embryos, and $Drp1^{+/-}$ mutants showed thinning in the neural tube cell layer and

highly elongated or swollen peroxisomes (ISHIHARA et al., 2009). TUNE positive cells have also been found in neuroepithelium of embryos mouse knockouts ($Drp1^{-/-}$), in addition to reduced forebrain, expanded subdural space, ventricular dilatation, white matter hypoplasia (brain stem and cerebellum) and reduced cortex and of the basal ganglia (ISHIHARA et al., 2009). Knockout mice ($Drp1^{-/-}$) also showed lower expression and arborization of neurites, in addition to an important reduction in the synaptophysin (marker of synapses) and mitochondrial aggregation in the forebrain (ISHIHARA et al., 2009).

Together, these data report possible consequences of mitochondrial dysfunction on neurodevelopment, and the link between mitochondrial dysfunction and neuroinflammatory processes.

6. AUTISM AND MITOCHONDRIAL DYSFUNCTION IN THE CNS

Many individuals with autism report the presence of mitochondrial dysfunction (BENNURI; ROSE; FRYE, 2019; D.A.; R.E., 2012; GIULIVI et al., 2010; GOLDENTHAL et al., 2015; LEGIDO; JETHVA; GOLDENTHAL, 2013; YUI; SATO;IMATAKA, 2015).

Studies involving children with autism report a prevalence of mitochondrial dysfunctionfrom 30 (ROSSIGNOL; FRYE, 2012) to up to 50% (FRYE, 2012). The high prevalenceof mitochondrial dysfunction in autistic individuals has made many recent studies investigating the presence of mitochondrial dysfunction in the CNS of these individuals (N.J. et al., 1993; GU et al., 2013; ANITHA et al., 2013; CHAUHAN; AUDHYA; CHAUHAN, 2012; ROSE et al., 2012; TANG et al., 2013; IPSER et al., 2012; CORRIGAN et al., 2012; INAN et al., 2016).

Was found a reduction in the levels of phoshpocreatine in prefrontal cortex cells of individuals with autism, indicating the high use of this compound for the generation of ATP, suggesting failure in the generation of energy in the mechanisms of mitochondrial respiration (N.J. et al., 1993). The low levels of phosphocreatine found were associated with low language performances in these individuals (N.J. et al., 1993).

In cells of the frontal cortex the result in the activities of mitochondrial complexes of the electron transport chain (ETC) in individuals with autism shows a reduction in all complexes (I, II, III, IV and V) of the chain compared to the healthy control subjects (GUet al.,

2013). Was showed a decrease in the activity of mitochondrial complexes I (in 31%of the autistic group compared to the healthy control group) and V (in 36% of the autisticgroup compared to the healthy control group) in the frontal cortex (GU et al., 2013). Autistic individuals also showed a tendency towards a reduction in the activities of the

other complexes but without significance in relation to the control group. In 43% of the individuals with autism, there was a reduction in activities in complexes I and/or V, and 14% of the autistic individuals showed a reduction in the activities of all mitochondrial complexes in the frontal cortex (GU et al., 2013).

Western blot analyzes showed a reduction in the levels of complex III and V of the respiratory chain in the frontal cortex of autistic children compared to healthy age- matched controls (CHAUHAN et al., 2011). In the temporal cortex there was a reductionin the levels of complex II, III and V in children with autism compared to the control group (CHAUHAN et al., 2011). Was found also an increase in free radicals and oxidativestress measured by the LOOH product levels (resulting from the oxidation of fatty acids) in theparietal, occipital, temporal and frontal cortex, and also in the cerebellum (CHAUHAN et al., 2011).

Another study carried out in postmortem brains showed by qPCR a reduction in 11 complex I genes, 5 complex II and IV genes and 7 complex V genes in autistic individuals compared to control individuals (ANITHA et al., 2013). In total, 11 genes from the electron transport chain in the anterior cingulate gyrus, 12 genes in the motor cortex and19 genes in the thalamus were significantly less expressed (ANITHA et al., 2013).

The activity of the enzyme pyruvate dehydrogenase (PDH) was also reduced in 35% of individuals with autism compared to healthy individuals, where the activity of PDH was determined by the reduction of NAD^+ to NADH (GU et al., 2013). These subjects with ASD also show an increase in mtDNA for the ND1, ND4 and CytB genes (GU et al., 2013).

The levels of the glutathione enzyme and the GSH/GSSG rate were reduced in the cortex,Brodman Area 22 and cerebellum of autists compared to healthy individuals

(CHAUHAN; AUDHYA; CHAUHAN, 2012; ROSE et al., 2012). In another study was showed that the activity of the enzymes superoxide dismutase (SOD) and superoxide dismutase 2 (SOD2) was reduced and there was a high level of oxidative stress in the temporal lobe of autistic individuals compared to healthy control subjects (TANG et al., 2013).

The ND4/ND1 rate showed a significant decrease in 44% in the autistic group

comparedto the control group, and the CytB/ND1 rate showed a significant decrease in 33% in theautistic group, reporting genetic deletions in individuals with ASD (GU et al., 2013).

There was a significant divergence in gene expression between an autistic group compared to healthy control group, with 22 of the divergent genes found in the anterior cingulate cortex (ACG), 15 in the motor cortex (MC), and 12 in the thalamus (THL) (ANITHA et al., 2012). These genes that showed divergent expression have functions inmembrane polarization and potential, mitochondrial transport, small molecule transport,expression of mitochondrial target proteins, outer and inner membrane translocations proteins, mitochondrial fusion and fissions proteins, mitochondrial location and apoptosis. Most of these evaluated genes showed a decrease in their expression in the autistic group compared to the control group (ANITHA et al., 2012).

In a sutdy that used knockout mice for the mitochondrial ETC enzyme Cox10 of parvalbumine neurons in the cortex brain, was found that mice deficient for this enzyme showed significant weight loss (INAN et al., 2016). In a test to evaluate the reobase, it was noted that the neurons of the knockout mice failed to sustain the triggering of the membrane potential in 20mV injections with 500ms of duration compared to the controlmice (INAN et al., 2016). Longer current injections indicated greater fatigue of mutated neurons compared to control neurons as well, where the number of action potentials achieved by knockout neurons was significantly lower than in wild neurons (INAN et al.,2016). Excitability was also shown to be altered between groups, with the mutant group

showing greater excitability than the control group (INAN et al., 2016). These knockout animals also showed less engagement in the social interaction test compared tothe control animals, suggesting that such animals show deficit in social interaction behavior (INAN et al., 2016).

Some studies have shown a decrease in the metabolite N-acetyl-aspartate (NAA), a marker of mitochondrial dysfunction, in the parietal cortex, cerebellum and anterior cingulate gyrus in individuals with autism (IPSER et al., 2012; CHUGANI et al., 1999), and also differences in the levels of this metabolite between autistic children and adults (CORRIGAN et al., 2012).

BIBLIOGRAPHY

ABDULAMIR, Haidar A.; ABDUL-RASHEED, Omar F.; ABDULGHANI, Emad A. Serotonin and serotonin transporter levels in autistic children. **Saudi Medical Journal**, [s. l.], 2018.

ADAV, Sunil S.; PARK, Jung Eun; SZE, Siu Kwan. Quantitative profiling brain proteomes revealed mitochondrial dysfunction in Alzheimer's disease. **Molecular Brain**,[s. l.], 2019.

ALABDALI, Altaf; AL-AYADHI, Laila; EL-ANSARY, Afaf. Association of social and cognitive impairment and biomarkers in autism spectrum disorders. **Journal of Neuroinflammation**, [s. l.], 2014.

ALKAN, Işınsu et al. Hippocampal neural cell loss in high-fat diet-induced obese rats– exploring the protein networks, ultrastructure, biochemical and bioinformatical markers. **Journal of Chemical Neuroanatomy**, [s. l.], 2021.

AMAL, Haitham et al. Shank3 mutation in a mouse model of autism leads to changes in the S-nitroso-proteome and affects key proteins involved in vesicle release and synaptic function. **Molecular Psychiatry**, [s. l.], 2020.

AMARAL, David G. et al. **In pursuit of neurophenotypes: The consequences of having autism and a big brainAutism Research**, 2017.

AMENT, Katarina et al. Evidence for Specificity of Motor Impairments in Catching andBalance in Children with Autism. **Journal of Autism and Developmental Disorders**, [s. l.], 2015.

ANITHA, Ayyappan et al. Brain region-specific altered expression and association of mitochondria-related genes in autism. **Molecular Autism**, [s. l.], 2012.

ANITHA, Ayyappan et al. Downregulation of the expression of mitochondrial electron transport complex genes in autism brains. **Brain Pathology**, [s. l.], 2013.

ARLUK, Shaye L. et al. Childhood obesity's relationship to time spent in sedentary behavior. **Military Medicine**, [s. l.], 2003.

ARNORIAGA RODRÍGUEZ, María et al. Glycated Hemoglobin, but not Insulin Sensitivity, is Associated with Memory in Subjects with Obesity. **Obesity**, [s. l.], 2019.

AZMITIA, Efrain C. et al. Dystrophic serotonin axons in postmortem brains from youngautism patients. **Anatomical Record**, [s. l.], 2011.

BACHMANN, Christian J.; GERSTE, Bettina; HOFFMANN, Falk. Diagnoses of

autism spectrum disorders in Germany: Time trends in administrative prevalence and diagnosticstability. **Autism**, [s. l.], 2018.

BARBOSA, Alexandre Garcia et al. Assessment of BDNF serum levels as a diagnostic marker in children with autism spectrum disorder. **Scientific Reports**, [s. l.], 2020.

BARTON, Gregory M.; MEDZHITOV, Ruslan. **Toll-like receptor signalingpathwaysScience**, 2003.

BATANDIER, C. et al. Acute emotional stress and high fat/high fructose diet modulate brain oxidative damage through NrF2 and uric acid in rats. **Nutrition Research**, [s. l.], 2020.

BEILHARZ, Jessica E.; MANIAM, Jayanthi; MORRIS, Margaret J. Short exposure to adiet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. **Brain, Behavior, and Immunity**, [s. l.], 2014.

BENNURI, Sirish C.; ROSE, Shannon; FRYE, Richard E. Mitochondrial dysfunction isinducible in lymphoblastoid cell lines from children with autism and may involve the TORC1 pathway. **Frontiers in Psychiatry**, [s. l.], 2019.

BENTHAM, James et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. **The Lancet**, [s. l.], 2017.

BHAT, Anjana N.; LANDA, Rebecca J.; GALLOWAY, James C. Current perspectives on motor functioning in infants, children, and adults with autism spectrum disorders. **Physical Therapy**, [s. l.], 2011.

BOURLIER, Virginie et al. TGFbeta family members are key mediators in the induction of myofibroblast phenotype of human adipose tissue progenitor cells by macrophages. **PLoS ONE**, [s. l.], 2012.

BOZDAGI, Ozlem et al. Haploinsufficiency of the autism-associated Shank3 gene leadsto deficits in synaptic function, social interaction, and social communication. **Molecular Autism**, [s. l.], 2010.

BRAUNE, Julia et al. IL-6 Regulates M2 Polarization and Local Proliferation of AdiposeTissue Macrophages in Obesity. **The Journal of Immunology**, [s. l.], 2017.

BRODER-FINGERT, Sarabeth et al. Prevalence of overweight and obesity in a large clinical sample of children with autism. **Academic Pediatrics**, [s. l.], 2014.

BURROWS, E. L. et al. Social isolation alters social and mating behavior in the R451C neuroligin mouse model of autism. **Neural Plasticity**, [s. l.], 2017.

CALVO-OCHOA, Erika et al. Short-term high-fat-and-fructose feeding produces insulinsignaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus. **Journal of Cerebral Blood Flow and Metabolism**, [s. l.], 2014.

CARRARO, Rodrigo S. et al. Hypothalamic mitochondrial abnormalities occur downstream of inflammation in diet-induced obesity. **Molecular and Cellular Endocrinology**, [s. l.], 2018.

CARVALHO, Cristina et al. Metabolic alterations induced by sucrose intake and Alzheimer's disease promote similar brain mitochondrial abnormalities. **Diabetes**, [s. l.],2012.

CAST, Thomas et al. An Autism-Associated Mutation Impairs Neuroligin-4 Glycosylation and Enhances Excitatory Synaptic Transmission in Human Neurons. **The Journal of Neuroscience**, [s. l.], 2020.

CAVALIERE, Gina et al. High-Fat Diet Induces Neuroinflammation and Mitochondrial Impairment in Mice Cerebral Cortex and Synaptic Fraction. **Frontiers in Cellular Neuroscience**, [s. l.], 2019.

CHAUHAN, Abha et al. Brain region-specific deficit in mitochondrial electron transportchain complexes in children with autism. **Journal of Neurochemistry**, [s. l.], 2011.

CHAUHAN, Abha; AUDHYA, Tapan; CHAUHAN, Ved. Brain region-specific glutathione redox imbalance in autism. **Neurochemical Research**, [s. l.], 2012.

CHISTOL, Liem T. et al. Sensory Sensitivity and Food Selectivity in Children with Autism Spectrum Disorder. **Journal of Autism and Developmental Disorders**, [s. l.], 2018.

CHUGANI, Diane C. et al. Evidence of altered energy metabolism in autistic children. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, [s. l.], 1999.

CIEŚLIK, Magdalena et al. Maternal immune activation induces neuroinflammation andcortical synaptic deficits in the adolescent rat offspring. **International Journal of Molecular Sciences**, [s. l.], 2020.

CLARK, Daniel O. et al. Does Body Mass Index Modify Memory, Reasoning, and Speedof Processing Training Effects in Older Adults. **Obesity**, [s. l.], 2016.

CORDERO, Christina et al. Maternal diabetes and hypertensive disorders in associationwith autism spectrum disorder. **Autism Research**, [s. l.], 2019.

CORREIA, C. T. et al. Increased BDNF levels and NTRK2 gene association suggest a disruption of BDNF/TrkB signaling in autism. **Genes, Brain and Behavior**, [s. l.], 2010.

CORRIGAN, Neva M. et al. Proton magnetic resonance spectroscopy and MRI reveal noevidence for brain mitochondrial dysfunction in children with autism spectrum disorder.

Journal of Autism and Developmental Disorders, [s. l.], 2012.

COURY, Daniel L. et al. **Gastrointestinal conditions in children with autism spectrum disorder: Developing a research agendaPediatrics**, 2012.

CRIADO, Kristen K. et al. Overweight and obese status in children with autism spectrumdisorder and disruptive behavior. **Autism**, [s. l.], 2018. a.

CRIADO, Kristen K. et al. Overweight and obese status in children with autism spectrumdisorder and disruptive behavior. **Autism**, [s. l.], 2018. b.

CUNARRO, Juan et al. **Hypothalamic mitochondrial dysfunction as a target in obesity and metabolic diseaseFrontiers in Endocrinology**, 2018.

CURRAN, Eileen A. et al. Exposure to Hypertensive Disorders of Pregnancy Increases the Risk of Autism Spectrum Disorder in Affected Offspring. **Molecular Neurobiology**,[s. l.], 2018.

D.A., Rossignol; R.E., Frye. Mitochondrial dysfunction in autism spectrum disorders: Asystematic review and meta-analysis. **Molecular Psychiatry**, [s. l.], 2012.

DALVI, P. S. et al. High fat induces acute and chronic inflammation in the hypothalamus: Effect of high-fat diet, palmitate and TNF-α on appetite-regulating NPY neurons. **International Journal of Obesity**, [s. l.], 2017.

DE LUCA, Simone N. et al. Early life overfeeding impairs spatial memory performanceby reducing microglial sensitivity to learning. **Journal of Neuroinflammation**, [s. l.], 2016.

DESMOULINS, Lucie et al. Mitochondrial Dynamin-Related Protein 1 (DRP1) translocation in response to cerebral glucose is impaired in a rat model of early alterationin hypothalamic glucose sensing. **Molecular Metabolism**, [s. l.], 2019.

Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. [s.l: s.n.].

DIAZ, Brenda et al. Saturated lipids decrease mitofusin 2 leading to endoplasmicreticulum stress activation and insulin resistance in hypothalamic cells. **Brain Research**,[s. l.], 2015.

DICARLO, Gabriella E. et al. Autism-linked dopamine transporter mutation alters striataldopamine neurotransmission and dopamine-dependent behaviors. **Journal of Clinical Investigation**, [s. l.], 2019.

DOUGLASS, J. D. et al. Astrocyte IKKβ/NF-κB signaling is required for diet-induced obesity and hypothalamic inflammation. **Molecular Metabolism**, [s. l.], 2017.

EWEN, Joshua B. et al. Epilepsy and Autism Severity: A Study of 6,975 Children. **Autism Research**, [s. l.], 2019.

FANTUZZI, Giamila. **Adipose tissue, adipokines, and inflammationJournal of**

Allergy and Clinical Immunology, 2005.

FLEMING, J. E. et al. **Is cell aging caused by respiration-dependent injury to the** mitochondrial genome?Gerontology, 1982.

FLETCHER, Fay E. et al. Atypicalities in sleep and semantic consolidation in autism. **Developmental Science**, [s. l.], 2020.

FRYE, Richard E. Biomarkers of Abnormal Energy Metabolism in Children with AutismSpectrum Disorder. **American Chinese Journal of Medicine and Science**, [s. l.], 2012.GAO, Chun Lin et al. Mitochondrial dysfunction is induced by high levels of glucose andfree fatty acids in 3T3-L1 adipocytes. **Molecular and Cellular Endocrinology**, [s. l.], 2010.

GARBARINO, Valentina R. et al. **Extreme enhancement or depletion of serotonin transporter function and serotonin availability in autism spectrum disorder Pharmacological Research**, 2019.

GHAFOURI-FARD, Soudeh et al. Expression Analysis of BDNF, BACE1, and Their Natural Occurring Antisenses in Autistic Patients. **Journal of Molecular Neuroscience**,[s. l.], 2020.

GHIRARDI, Laura et al. Genetic and environmental contribution to the overlap betweenADHD and ASD trait dimensions in young adults: A twin study. **Psychological Medicine**, [s. l.], 2019.

GINN, S. Islands of Genius: The Bountiful Mind of the Autistic, Acquired, and Sudden Savant. **The British Journal of Psychiatry**, [s. l.], 2011.

GIULIVI, Cecilia et al. Mitochondrial dysfunction in autism. **JAMA - Journal of the American Medical Association**, [s. l.], 2010.

GODOY, Priscilla B. G. et al. Brief Report: Associations Between Cognitive Control Processes and Traits of Autism Spectrum Disorder (ASD), attention- Deficit/Hyperactivity Disorder (ADHD) and Anxiety in Children at Elevated and TypicalFamilial Likelihood for ASD. **Journal of Autism and Developmental Disorders**, [s. l.], 2020.

GOERDT, Sergij et al. Alternative versus classical activation of macrophages. In: PATHOBIOLOGY 1999, **Anais**... [s.l: s.n.]

GOLDENTHAL, Michael J. et al. Mitochondrial enzyme dysfunction in autism spectrumdisorders; A novel biomarker revealed from buccal swab analysis. **Biomarkers in Medicine**, [s. l.], 2015.

GOMES, Júlia Ariana Souza et al. A high-refined carbohydrate diet facilitates compulsive-like behavior in mice through the nitric oxide pathway. **Nitric Oxide - Biology**

and Chemistry, [s. l.], 2018.

GU, F. et al. Alterations in mitochondrial DNA copy number and the activities of electron transport chain complexes and pyruvate dehydrogenase in the frontal cortex from subjectswith autism. **Translational psychiatry**, [s. l.], 2013.

GUERRA, Flora et al. Synergistic Effect of Mitochondrial and Lysosomal Dysfunction in Parkinson's Disease. **Cells**, [s. l.], 2019.

GUMUSOGLU, Serena B. et al. The role of IL-6 in neurodevelopment after prenatal stress. **Brain, Behavior, and Immunity**, [s. l.], 2017.

HALI, Sai et al. Modelling monogenic autism spectrum disorder using mouse cortical organoids. **Biochemical and Biophysical Research Communications**, [s. l.], 2020.

HAN, Myoung Sook et al. Regulation of adipose tissue inflammation by interleukin 6. **Proceedings of the National Academy of Sciences of the United States of America**, [s. l.], 2020.

HARMAN, DENHAM. The Biologic Clock: The Mitochondria? **Journal of the American Geriatrics Society**, [s. l.], 1972.

HARRIS, Rebecca et al. Obesity is the most common risk factor for chronic liver disease: Results from a risk stratification pathway using transient elastography. **American Journal of Gastroenterology**, [s. l.], 2019.

HEGARTY, John P. et al. Genetic and environmental influences on corticostriatal circuitsin twins with autism. **Journal of Psychiatry and Neuroscience**, [s. l.], 2020.

HEISE, Christopher et al. Heterogeneity of cell surface glutamate and GABA receptor expression in shank and CNTN4 autism mouse models. **Frontiers in Molecular Neuroscience**, [s. l.], 2018.

HIGGINS, Victoria et al. Postprandial dyslipidemia, hyperinsulinemia, and impaired gut peptides/bile acids in adolescents with obesity. **Journal of Clinical Endocrinology andMetabolism**, [s. l.], 2020.

HILL, Alison Presmanes; ZUCKERMAN, Katharine E.; FOMBONNE, Eric. Obesity and autism. **Pediatrics**, [s. l.], 2015.

HOFFSTEDT, J. et al. Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. **Diabetologia**, [s. l.], 2010.

HOLLOCKS, Matthew J. et al. **Anxiety and depression in adults with autism spectrum disorder: A systematic review and meta-analysisPsychological Medicine**, 2019.

HU, Chun Chun et al. Alterations in plasma cytokine levels in chinese children with autism spectrum disorder. **Autism Research**, [s. l.], 2018.

INAN, Melis et al. Energy deficit in parvalbumin neurons leads to circuit dysfunction, impaired sensory gating and social disability. **Neurobiology of Disease**, [s. l.], 2016.

IPSER, Jonathan C. et al. 1H-MRS in autism spectrum disorders: A systematic meta-analysis. **Metabolic Brain Disease**, [s. l.], 2012.

JIA, Qingyi; MORGAN-BATHKE, Maria E.; JENSEN, Michael D. Adipose tissue macrophage burden, systemic inflammation, and insulin resistance. **American Journal of Physiology - Endocrinology and Metabolism**, [s. l.], 2020.

JO, Junghyo et al. Hypertrophy and/or hyperplasia: Dynamics of adipose tissue growth. **PLoS Computational Biology**, [s. l.], 2009.

JUGE-AUBRY, Cristiana E. et al. Adipose tissue is a major source of interleukin-1 receptor antagonist: Upregulation in obesity and inflammation. **Diabetes**, [s. l.], 2003.

KAO, Yu Chia et al. High fat diet suppresses peroxisome proliferator-activated receptorsand reduces dopaminergic neurons in the substantia nigra. **International Journal of Molecular Sciences**, [s. l.], 2020.

KIM, H. J. et al. Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects. **Molecular Psychiatry**, [s. l.], 2017.

KIM, Jiye et al. The involvement of 4-1BB/4-1BBL signaling in glial cell-mediated hypothalamic inflammation in obesity. **FEBS Open Bio**, [s. l.], 2018.

KOJIMA, Masaki et al. Neuroanatomical Correlates of Advanced Paternal and MaternalAge at Birth in Autism Spectrum Disorder. **Cerebral Cortex**, [s. l.], 2019.

KONG, Linghua et al. The risk of offspring psychiatric disorders in the setting of maternalobesity and diabetes. **Pediatrics**, [s. l.], 2018.

KOWALTOWSKI, Alicia J. et al. Mitochondrial morphology regulates organellar Ca2+uptake and changes cellular Ca2+ homeostasis. **FASEB journal : official publication of the Federation of American Societies for Experimental Biology**, [s. l.], 2019.

LANGLEY, Monica R. et al. High fat diet consumption results in mitochondrial dysfunction, oxidative stress, and oligodendrocyte loss in the central nervous system. **Biochimica et Biophysica Acta - Molecular Basis of Disease**, [s. l.], 2020.

LARRAIN-VALENZUELA, Josefina et al. Theta and Alpha Oscillation Impairments inAutistic Spectrum Disorder Reflect Working Memory Deficit. **Scientific Reports**, [s. l.], 2017.

LAZARO, Maria T. et al. Reduced Prefrontal Synaptic Connectivity and Disturbed

Oscillatory Population Dynamics in the CNTNAP2 Model of Autism. **Cell Reports**, [s. l.], 2019.

LE BELLE, Janel E. et al. Maternal inflammation contributes to brain overgrowth and autism-associated behaviors through altered redox signaling in stem and progenitor cells. **Stem Cell Reports**, [s. l.], 2014.

LEE, Junghee et al. SIRT3 deregulation is linked to mitochondrial dysfunction in Alzheimer's disease. **Aging Cell**, [s. l.], 2018.

LEE, Yun Hee; PETKOVA, Anelia P.; GRANNEMAN, James G. Identification of an adipogenic niche for adipose tissue remodeling and restoration. **Cell Metabolism**, [s. l.], 2013.

LEGIDO, Agustín; JETHVA, Reena; GOLDENTHAL, Michael J. Mitochondrial dysfunction in autism. **Seminars in Pediatric Neurology**, [s. l.], 2013.

LI, Wenhao et al. Obesity Predicts Liver Function Testing and Abnormal Liver Results. **Obesity**, [s. l.], 2020.

LICARI, Melissa K. et al. Prevalence of Motor Difficulties in Autism Spectrum Disorder:Analysis of a Population-Based Cohort. **Autism Research**, [s. l.], 2020.

LINNANE, Anthony W. et al. MITOCHONDRIAL DNA MUTATIONS AS AN IMPORTANT CONTRIBUTOR TO AGEING AND DEGENERATIVE DISEASES. **The Lancet**, [s. l.], 1989.

LIU, Xuefeng et al. Imbalance of laminar-specific excitatory and inhibitory circuits of theorbitofrontal cortex in autism. **Molecular Autism**, [s. l.], 2020.

LUCARELLI, Jennifer et al. Autism Spectrum Disorder and Avoidant/Restrictive FoodIntake Disorder. **Journal of Developmental and Behavioral Pediatrics**, [s. l.], 2017.

LUCCHINA, Luciana; DEPINO, Amaicha Mara. Altered Peripheral and Central Inflammatory Responses in a Mouse Model of Autism. **Autism Research**, [s. l.], 2014.

LUO, Yuan et al. A multidimensional precision medicine approach identifies an autismsubtype characterized by dyslipidemia. **Nature Medicine**, [s. l.], 2020.

LYALL, Kristen et al. Maternal dietary fat intake in association with autism spectrumdisorders. **American Journal of Epidemiology**, [s. l.], 2013.

LYALL, Kristen et al. The Changing Epidemiology of Autism Spectrum Disorders. **Annual Review of Public Health**, [s. l.], 2017. a.

LYALL, Kristen et al. Polychlorinated biphenyl and organochlorine pesticide concentrations in maternal mid-pregnancy serum samples: Association with Autism spectrum disorder and intellectual disability. **Environmental Health Perspectives**, [s. l.], 2017. b.

LYTLE, Kelli A. et al. Hepatic Fatty Acid Balance and Hepatic Fat Content in Humans with Severe Obesity. **Journal of Clinical Endocrinology and Metabolism**, [s. l.], 2019.

MAENNER, Matthew J. et al. Prevalence of autism spectrum disorder among children aged 8 Years-Autism and developmental disabilities monitoring network, 11 Sites, UnitedStates, 2016. **MMWR Surveillance Summaries**, [s. l.], 2020.

MANDAVILLI, Bhaskar S.; SANTOS, Janine H.; VAN HOUTEN, Bennett. Mitochondrial DNA repair and aging. **Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis**, [s. l.], 2002.

MARKO, Mollie K. et al. Behavioural and neural basis of anomalous motor learning in children with autism. **Brain**, [s. l.], 2015.

MAYES, Susan Dickerson et al. Use of the Childhood Autism Rating Scale (CARS) forchildren with high functioning autism or Asperger syndrome. **Focus on Autism and Other Developmental Disabilities**, [s. l.], 2012.

MCARDLE, Maeve A. et al. **Mechanisms of obesity-induced inflammation and insulin resistance: Insights into the emerging role of nutritional strategiesFrontiers in Endocrinology, 2013.**

MCCOY, Stephanie M.; MORGAN, Kristen. Obesity, physical activity, and sedentary behaviors in adolescents with autism spectrum disorder compared with typically developing peers. **Autism**, [s. l.], 2020.

MORALES-HIDALGO, Paula et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Spanish School-Age Children. **Journal of Autism and DevelopmentalDisorders**, [s. l.], 2018.

N.J., Minshew et al. A preliminary 31P MRS study of autism: Evidence for undersynthesis and increased degradation of brain membranes. **Biological Psychiatry**, [s. l.], 1993.

NAPOLI, Eleonora; WONG, Sarah; GIULIVI, Cecilia. Evidence of reactive oxygen species-mediated damage to mitochondrial DNA in children with typical autism. **Molecular Autism**, [s. l.], 2013.

NARZISI, A. et al. Prevalence of Autism Spectrum Disorder in a large Italian catchmentarea: A school-based population study within the ASDEU project. **Epidemiology and Psychiatric Sciences**, [s. l.], 2018.

NISHIMURA, Satoshi et al. CD8+ effector T cells contribute to macrophage recruitmentand adipose tissue inflammation in obesity. **Nature Medicine**, [s. l.], 2009.

OWEN, Julia P. et al. Brain MR imaging findings and associated outcomes in carriers ofthe reciprocal copy number variation at 16p11.2. **Radiology**, [s. l.], 2018.

PAERATAKUL, Sahasporn et al. Fast-food consumption among US adults and children:Dietary and nutrient intake profile. **Journal of the American Dietetic Association**, [s. l.], 2003.

PANG, Can et al. Macrophage infiltration into adipose tissue may promote angiogenesisfor adipose tissue remodeling in obesity. **American Journal of Physiology-Endocrinology and Metabolism**, [s. l.], 2008.

PANJWANI, Anita A. et al. Maternal Obesity/Diabetes, Plasma Branched-Chain AminoAcids, and Autism Spectrum Disorder Risk in Urban Low-Income Children: Evidence ofSex Difference. **Autism Research**, [s. l.], 2019.

PARK, Hye Sang; CHO, Han Sam; KIM, Tae Woon. Physical exercise promotes memorycapability by enhancing hippocampal mitochondrial functions and inhibiting apoptosis inobesity-induced insulin resistance by high fat diet. **Metabolic Brain Disease**, [s. l.], 2018.P

PAULA, Cristiane S. et al. Brief report: Prevalence of pervasive developmental disorderin Brazil: A pilot study. **Journal of Autism and Developmental Disorders**, [s. l.], 2011.

PELPHREY, Kevin A. et al. **Research review: Constraining heterogeneity: The socialbrain and its development in autism spectrum disorderJournal of Child Psychology and Psychiatry and Allied Disciplines**, 2011.

POSTEMA, Merel C. et al. Altered structural brain asymmetry in autism spectrum disorder in a study of 54 datasets. **Nature Communications**, [s. l.], 2019.

POSTORINO, Valentina et al. **Anxiety Disorders and Obsessive-Compulsive Disorderin Individuals with Autism Spectrum DisorderCurrent Psychiatry Reports**, 2017.

PREHN, Kristin et al. Caloric Restriction in Older Adults-Differential Effects of WeightLoss and Reduced Weight on Brain Structure and Function. **Cerebral cortex (New York,N.Y. : 1991)**, [s. l.], 2017.

PURPURA, Giulia et al. Motor coordination impairment in children with autism spectrum disorder: A pilot study using Movement Assessment Battery for Children-2 Checklist. **Minerva Pediatrica**, [s. l.], 2020.

RAO, Patricia A.; LANDA, Rebecca J. Association between severity of behavioral phenotype and comorbid attention deficit hyperactivity disorder symptoms in children with autism spectrum disorders. **Autism**, [s. l.], 2014.

REDDY, P. Hemachandra; BEAL, M. Flint. Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. **Trends in Molecular Medicine**, [s. l.], 2008.

REUTZEL, Martina et al. Effects of different standard and special diets on cognition andbrain mitochondrial function in mice. **Nutritional Neuroscience**, [s. l.], 2021.

RICCIO, Maria Pia et al. Is food refusal in autistic children related to TAS2R38 genotype? **Autism Research**, [s. l.], 2018.

RINEHART, Nicole J. et al. Gait function in newly diagnosed children with autism: Cerebellar and basal ganglia related motor disorder. **Developmental Medicine and Child Neurology**, [s. l.], 2006.

ROCHA, D. M. et al. **Saturated fatty acids trigger TLR4-mediated inflammatory responseAtherosclerosis**, 2016.

ROSE, S. et al. Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. **Translational Psychiatry**, [s. l.], 2012.

ROSSIGNOL, D. A.; FRYE, R. E. **Mitochondrial dysfunction in autism spectrum disorders: A systematic review and meta-analysisMolecular Psychiatry**, 2012.

SACCO, Roberto; GABRIELE, Stefano; PERSICO, Antonio M. Head circumference andbrain size in autism spectrum disorder: A systematic review and meta-analysis. **Psychiatry Research - Neuroimaging**, [s. l.], 2015.

SAMARA, Amjad et al. Neuroinflammation and White Matter Alterations in Obesity Assessed by Diffusion Basis Spectrum Imaging. **Frontiers in Human Neuroscience**, [s.l.], 2020.

SANCHEZ, C. E. et al. **Maternal pre-pregnancy obesity and child neurodevelopmental outcomes: a meta-analysisObesity Reviews**, 2018.

SCHNEEBERGER, Marc et al. Mitofusin 2 in POMC neurons connects ER stress with leptin resistance and energy imbalance. **Cell**, [s. l.], 2013.

SCHOEN, Michael et al. Shank3 transgenic and prenatal zinc-deficient autism mouse models show convergent and individual alterations of brain structures in MRI. **Frontiersin Neural Circuits**, [s. l.], 2019.

SHAFRITZ, Keith M. et al. The Neural Circuitry Mediating Shifts in Behavioral Response and Cognitive Set in Autism. **Biological Psychiatry**, [s. l.], 2008.

SHAH, Amy S. et al. Superiority of lipoprotein particle number to detect associations with arterial thickness and stiffness in obese youth with and without prediabetes. **Journalof Clinical Lipidology**, [s. l.], 2016.

SHARON, Gil et al. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. **Cell**, [s. l.], 2019.

SINDHU, Sardar et al. MIP-1α Expression Induced by Co-Stimulation of Human Monocytic Cells with Palmitate and TNF-α Involves the TLR4-IRF3 Pathway and Is Amplified by Oxidative Stress. **Cells**, [s. l.], 2020.

SKOGSTRAND, Kristin et al. Reduced neonatal brain-derived neurotrophic factor is associated with autism spectrum disorders. **Translational Psychiatry**, [s. l.], 2019.

SOUBANNIER, Vincent et al. A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. **Current Biology**, [s. l.], 2012.

SOUZA, C. G. et al. Highly palatable diet consumption increases protein oxidation in ratfrontal cortex and anxiety-like behavior. **Life Sciences**, [s. l.], 2007.

SRIPETCHWANDEE, Jirapas; CHATTIPAKORN, Nipon; CHATTIPAKORN, Siriporn Links between obesity-induced brain insulin resistance, brain mitochondrial dysfunction, and dementiaFrontiers in Endocrinology, 2018.

STERNBERG, Esther M. **Neural regulation of innate immunity: A coordinated nonspecific host response to pathogens Nature Reviews Immunology**, 2006.

STOODLEY, Catherine J. et al. Altered cerebellar connectivity in autism and cerebellar-mediated rescue of autism-related behaviors in mice. **Nature Neuroscience**, [s. l.], 2017.STRASSER, Barbara. **Immune-mediated inflammation as a driver of obesity and comorbid conditionsObesity**, 2017.

TANG, Guomei et al. Mitochondrial abnormalities in temporal lobe of autistic brain. **Neurobiology of Disease**, [s. l.], 2013.

TOAL, F. et al. Clinical and anatomical heterogeneity in autistic spectrum disorder: A structural MRI study. **Psychological Medicine**, [s. l.], 2010.

TONIAZZO, Ana Paula et al. Sex-dependent effect on mitochondrial and oxidative stressparameters in the hypothalamus induced by prepubertal stress and access to high fat diet. **Neurochemistry International**, [s. l.], 2019.

TOSCANO, Chrystiane V. A. et al. Growth and weight status of Brazilian children withautism spectrum disorders: A mixed longitudinal study. **Jornal de Pediatria (Versão em Português)**, [s. l.], 2019.

TUCHMAN, Roberto; RAPIN, Isabelle. **Epilepsy in autismLancet Neurology**, 2002. TUCSEK, Zsuzsanna et al. Obesity in aging exacerbates blood-brain barrier disruption, neuroinflammation, and oxidative stress in the mouse hippocampus: Effects onexpression of genes involved in beta-amyloid generation and Alzheimer's disease. **Journals of**

Gerontology - Series A Biological Sciences and Medical Sciences, [s. l.],2014. a.

TUCSEK, Zsuzsanna et al. Obesity in aging exacerbates blood-brain barrier disruption, neuroinflammation, and oxidative stress in the mouse hippocampus: Effects on expression of genes involved in beta-amyloid generation and Alzheimer's disease. **Journals of Gerontology - Series A Biological Sciences and Medical Sciences**, [s. l.], 2014. b.

TZISCHINSKY, Orna et al. Sleep disturbances are associated with specific sensory sensitivities in children with autism. **Molecular Autism**, [s. l.], 2018.

VALCARCEL-ARES, Marta Noa et al. Obesity in aging exacerbates neuroinflammation,dysregulating synaptic function-related genes and altering eicosanoid synthesis in the mouse hippocampus: Potential role in impaired synaptic plasticity and cognitive decline.**Journals of Gerontology - Series A Biological Sciences and Medical Sciences**, [s. l.], 2019.

VARGA, Noémi Ágnes et al. Mitochondrial dysfunction and autism: Comprehensive genetic analyses of children with autism and mtDNA deletion. **Behavioral and Brain Functions**, [s. l.], 2018.

VARGAS, Diana L. et al. Neuroglial activation and neuroinflammation in the brain of patients with autism. **Annals of Neurology**, [s. l.], 2005.

VENIAMINOVA, Ekaterina et al. Prefrontal cortex inflammation and liver pathologies accompany cognitive and motor deficits following Western diet consumption in non- obese female mice. **Life Sciences**, [s. l.], 2020.

VOGEL, Daphne Y. S. et al. Human macrophage polarization in vitro: Maturation and activation methods compared. **Immunobiology**, [s. l.], 2014.

WAISE, T. M. Zave. et al. One-day high-fat diet induces inflammation in the nodose ganglion and hypothalamus of mice. **Biochemical and Biophysical Research Communications**, [s. l.], 2015.

WAN, Hongquan et al. **Association of maternal diabetes with autism spectrum disorders in offspringMedicine (United States)**, 2018. a.

WAN, Hongquan MM et al. Association of maternal diabetes with autism spectrum disorders in offspring: A systemic review and meta-analysis. **Medicine**, [s. l.], 2018. b. WANG, Jia et al. Neurexin gene family variants as risk factors for autism spectrum disorder. **Autism Research**, [s. l.], 2018.

WANG, Min et al. Increased serum levels of brain-derived neurotrophic factor in autismspectrum disorder. **NeuroReport**, [s. l.], 2015.

WANG, Ying et al. Probiotics and fructo-oligosaccharide intervention modulate the

microbiota-gut brain axis to improve autism spectrum reducing also the hyper- serotonergic state and the dopamine metabolism disorder. **Pharmacological Research**, [s. l.], 2020.

WEGIEL, Jerzy et al. The neuropathology of autism: Defects of neurogenesis and neuronal migration, and dysplastic changes. **Acta Neuropathologica**, [s. l.], 2010.

WEISBERG, Stuart P. et al. Obesity is associated with macrophage accumulation in adipose tissue. **Journal of Clinical Investigation**, [s. l.], 2003.

WILLIAMS, Diane L.; GOLDSTEIN, Gerald; MINSHEW, Nancy J. Impaired memory for faces and social scenes in autism: Clinical implications of memory dysfunction. **Archives of Clinical Neuropsychology**, [s. l.], 2005.

WILLIAMS, Roger S. et al. Autism and Mental Retardation: Neuropathologic Studies Performed in Four Retarded Persons with Autistic Behavior. **Archives of Neurology**, [s.l.], 1980.

WINER, Daniel A. et al. B cells promote insulin resistance through modulation of T cellsand production of pathogenic IgG antibodies. **Nature Medicine**, [s. l.], 2011.

WORLD, Organization Health. World Health Organisation Obesity and Overweight FactSheet. **Who**, [s. l.], 2016.

XIAO, Tengfei et al. Butyrate upregulates the TLR4 expression and the phosphorylationof MAPKs and NK-κB in colon cancer cell in vitro. **Oncology Letters**, [s. l.], 2018.

YAVUZ-KODAT, Enise et al. Disturbances of Continuous Sleep and Circadian Rhythms Account for Behavioral Difficulties in Children with Autism Spectrum Disorder. **Journal of Clinical Medicine**, [s. l.], 2020.

YUI, Kunio; SATO, Atsushi; IMATAKA, George. Mitochondrial Dysfunction and Its Relationship with mTOR Signaling and Oxidative Damage in Autism Spectrum Disorders. **Mini-Reviews in Medicinal Chemistry**, [s. l.], 2015.

ZABLOTSKY, Benjamin; BRAMLETT, Matthew D.; BLUMBERG, Stephen J. The Co-Occurrence of Autism Spectrum Disorder in Children With ADHD. **Journal of Attention Disorders**, [s. l.], 2020.

ZERBO, Ousseny et al. Maternal Infection During Pregnancy and Autism Spectrum Disorders. **Journal of Autism and Developmental Disorders**, [s. l.], 2015.

ZHANG, B. et al. Developmental plasticity shapes synaptic phenotypes of autism-associated neuroligin-3 mutations in the calyx of held. **Molecular Psychiatry**, [s. l.], 2017.

ZHANG, Bo et al. Autism-associated neuroligin-4 mutation selectively impairs glycinergic synaptic transmission in mouse brainstem synapses. **Journal of Experimental**

Medicine, [s. l.], 2018.