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CURSO DE DOUTORADO

INTERVENÇÃO COMBINANDO EXERCÍCIO FÍSICO E EDUCAÇÃO
ALIMENTAR E NUTRICIONAL MODULA O MICROBIOMA INTESTINAL E
MELHORA A INFLAMAÇÃO E A FUNÇÃO AUTONÔMICA CARDÍACA NA
OBESIDADE INFANTIL: UMA ABORDAGEM TRANSLACIONAL

Discente: Paulo César Trindade da Costa

Orientador: Prof. Dr. José Luiz de Brito Alves

João Pessoa

2025

PAULO CÉSAR TRINDADE DA COSTA

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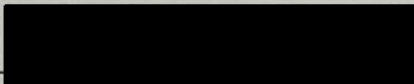
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E a todos que se julgam por direito,

*A importância de uma coisa há que ser medida pelo encantamento que a coisa produza em
nós.*

Manoel de Barros

RESUMO

Crianças com obesidade apresentam maior probabilidade de desenvolver alterações cardiovasculares e metabólicas na vida adulta. A realização de exercício físico, assim como ações de educação alimentar e nutricional, são estratégias importantes para a prevenção e/ou tratamento da obesidade e de desordens associadas. No entanto, sua efetividade de intervenções de estilo de vida em relação à perda de peso, à modulação do microbioma intestinal e à melhora de parâmetros metabólicos e cardiovasculares em crianças com obesidade ainda não está completamente elucidada. Dessa forma, este trabalho teve como objetivo avaliar se uma intervenção composta por um circuito com exercício físico e educação alimentar e nutricional contribui para a melhora da composição corporal, de parâmetros metabólicos, inflamatórios, cardiovasculares e da microbiota intestinal em escolares com obesidade. No estudo, crianças de 7 a 11 anos foram recrutadas em escolas de João Pessoa - PB. Antes da intervenção, alunos com peso normal e com obesidade foram avaliados na linha de base para análises transversais. Após a intervenção, os escolares com obesidade foram reavaliados quanto à composição corporal, perfil inflamatório, pressão arterial, variabilidade da frequência cardíaca (VFC), metabólica e microbiota intestinal. Adicionalmente, foi desenvolvido um protocolo experimental com exercício físico em ratos, visando melhor compreender os mecanismos biomoleculares envolvidos nos efeitos fisiológicos observados. Na análise transversal, foi possível observar que crianças com obesidade apresentam disfunção autonômica cardíaca e perfil pró inflamatório, em comparação com crianças com peso normal. A frequência cardíaca se mostrou um preditor válido para disfunção autonômica cardíaca em escolares. Verificou-se ainda que o supercrescimento bacteriano do intestino delgado estava relacionado ao consumo elevado de alimentos ultraprocessados. Na revisão sistemática realizada, observou-se ainda que não é possível determinar qual o tipo de exercício físico mais eficaz para melhorar a VFC em crianças com obesidade, embora todos os tipos analisados tenham demonstrado efeitos benéficos sobre alguns índices. No estudo de intervenção, foi constatado redução no escore z do índice de massa corporal para idade, no percentual de gordura corporal, na pressão arterial sistólica e diastólica, e melhora na VFC, no perfil inflamatório e metabólico, bem como na composição do microbioma intestinal de crianças com obesidade. Em ratos, os resultados mostraram que o exercício físico promoveu melhora da microbiota intestinal, da função autonômica cardíaca e da expressão de genes relacionados à função autonômica e à inflamação no cólon e tronco encefálico de ratos alimentados com dieta hiperlipídica. Os resultados indicam que essa estratégia se configura

como fundamental no enfrentamento da obesidade infantil e de suas complicações associadas, elucidando os mecanismos fisiológicos e biomoleculares envolvidos nesse processo. As evidências obtidas nesse estudo poderão embasar a implementação de políticas públicas que garantam a prática regular de exercício físico aliada à educação alimentar e nutricional no ambiente escolar.

Palavras-chave: Obesidade infantil; exercício físico; educação alimentar e nutricional; variabilidade da frequência cardíaca; SUS; análise biomolecular; metabolômica; microbioma intestinal.

ABSTRACT

Children with obesity are more likely to develop cardiovascular and metabolic alterations in adulthood. Physical exercise and food and nutrition education are important strategies for preventing and/or treating obesity and associated disorders. However, the effectiveness of these strategies in promoting weight loss, modulating the gut microbiome, and improving metabolic and cardiovascular parameters in children with obesity is not fully understood. This study aimed to evaluate the effectiveness of an intervention consisting of physical exercise and food and nutrition education in improving body composition, metabolic and inflammatory parameters, and cardiovascular health in schoolchildren with obesity. Children aged 7 to 10 were recruited from schools in João Pessoa, Paraíba, for the study. Before the intervention, students with normal-weight and obesity were assessed for cross-sectional analyses. After the intervention, the students with obesity were reassessed for body composition, inflammatory profile, blood pressure, heart rate variability (HRV), metabolomics, and gut microbiota. Additionally, an experimental protocol involving physical exercise in rats was developed to better understand the biomolecular mechanisms involved in the observed physiological effects. Cross-sectional analyses revealed that obese children exhibit cardiac autonomic dysfunction and a proinflammatory profile compared to normal-weight children. Heart rate was found to be a reliable indicator of cardiac autonomic dysfunction in schoolchildren. Small intestinal bacterial overgrowth was also found to be related to the consumption of large quantities of ultra-processed foods. However, the systematic review found that the most effective type of physical exercise for improving HRV in children with obesity remains unclear, though all analyzed types demonstrated beneficial effects on some indices. The intervention study found a reduction in the body mass index z-score for age, body fat percentage, systolic and diastolic blood pressure, and improvements in HRV, inflammatory and metabolic profiles, as well as the composition of the gut microbiome in children with obesity. In rats, the results showed that physical exercise improved gut microbiota, cardiac autonomic function, and the expression of genes related to autonomic function and inflammation in the colon and brainstem of rats fed a high-fat diet. The results indicate that this strategy is fundamental in combating childhood obesity and its associated complications, elucidating the physiological and biomolecular mechanisms involved in this process. The evidence obtained in this study could support the implementation of public policies that ensure regular physical exercise combined with food and nutrition education in schools.

Keywords: Childhood obesity; physical exercise; food and nutrition education; heart rate variability; SUS; biomolecular analysis; metabolomics; intestinal microbiome.

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LISTA DE ABREVIATURAS E SIGLAS

SUS	Sistema Único de Saúde
SNA	Sistema nervoso autônomo
SNS	Sistema nervoso simpático
SNP	Sistema nervoso parassimpático
VFC	Variabilidade da frequência cardíaca
IMC	Índice de massa corporal
FTO	Do inglês, Fat mass and obesity associated
NHANES	Do inglês, National Health and Nutrition Examination Surveys
TLR4	Do inglês, Toll-like receptor 4
TNFR1	Do inglês, Tumor necrosis factor receptor 1
HIF1 α	Do inglês, Hypoxia Inducible Factor 1 Subunit Alpha
NF- κ B	Do inglês, nuclear factor kappa B
MCP-1	Do inglês, Monocyte chemoattractant protein-1
MAPKK	Do inglês, Mitogen-activated protein kinase kinase
PKC	Do inglês, Protein Kinase C
MAPK	Do inglês, Mitogen-Activated Protein Kinase
IRS 1/2	Do inglês, Insulin Receptor Substrate proteins 1 and 2
PI3K-AKT	Do inglês, Phosphoinositide 3-kinase - Protein Kinase B
BAT	Do inglês, Brown adipose tissue
UCP-1	Do inglês, Uncoupling Protein 1
JNK	Do inglês, c-Jun N-terminal kinase
cAMP	Do inglês, Cyclic adenosine monophosphate
ROS	Do inglês, reactive oxygen species
IKK-beta	Do inglês, Inhibitor of nuclear factor kappa B kinase subunit beta
SLC6A2	Do inglês, Solute carrier family 6 member 2
MAOa	monoamina oxidase A
NS	nódulo sinoatrial
AV	nódulo atrioventricular
FC	frequência cardíaca
NTS	núcleo do trato solitário
GLP-1	Do inglês, Glucagon-like peptide-1

RMSSD	Do inglês, Square root of the mean of the square of the differences between consecutive NN intervals
SDNN	Do inglês, Standard deviation between NN intervals
PNN50	Do inglês, Percentage of adjacent NN with differences in duration greater than 50
ULF	Do inglês, Ultra-low-frequency
VLF	Do inglês, Very-low-frequency
LF	Do inglês, Low frequency
HF	Do inglês, High frequency
SD1	Do inglês, Standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph
SD2	Do inglês, Standard deviation of long-term continuous NN intervals
LPS	Lipopolissacarídeo
DM2	Diabetes mellitus tipo 2
AGCC	Ácidos graxos de cadeia curta
TMA	Trimetilamina
GABA	Ácido gama-aminobutírico
SNC	Sistema nervoso central
OMS	Organização Mundial de Saúde
PPSUS	Programa de Pesquisa para o SUS
EAN	Educação alimentar e nutricional
CBA	Do inglês, Cytometric bead array
IL	Interleucina
IFN- γ	Interferon-gama
TNF- α	Do inglês, tumor necrosis factor alpha
SIBO	Do inglês, Small Intestinal Bacterial Overgrowth
REBEC	Registros Brasileiros de Ensaios Clínicos
ALT	Alanina transaminase
AST	Aspartato aminotransferase
GGT	Gama-glutamil transferase
CT	Colesterol Total
LDL-c	Do inglês, Low-density lipoprotein cholesterol
HDL-c	Do inglês, High-density lipoprotein cholesterol
TG	Triglicerídeos

HbA1c	Hemoglobina glicada
Hs-CRP	Proteína c-reativa de alta sensibilidade
HOMA-IR	Homeostase da resistência à insulina
HFD	High-fat diet
PAP	Pressão arterial pulsátil
PAM	Pressão arterial média
PAS	Pressão arterial sistólica
PAD	Pressão arterial diastólica
TBP	Do, inglês, TATA-box binding protein
Cldn1	Do inglês, Claudin-1
Ffar3	Do inglês, Free fatty acid receptor 3
Gpx2	Do inglês, glutathione peroxidase 2
Muc2	Do inglês, Mucin 2
NLRP3	Do inglês, NLR family pyrin domain containing 3
NTRK2	Do inglês, Neurotrophic Receptor Tyrosine Kinase 2
Ocln	Do inglês, Occludin
Sod2	Do inglês, Superoxide dismutase 2
Tjp1	Do inglês, Tight junction protein 1
TLR2	Do inglês, Toll like receptor 2
RGS6	Do inglês, Regulator of G protein signaling 6
Calca	Do inglês, Calcitonin related polypeptide alpha
Fos	Do inglês, Fos proto-oncogene
Nos1	Do inglês, Nitric oxide synthase 1
VIP	Do inglês, Vasoactive intestinal peptide
RAMP1	Do inglês, Receptor Activity Modifying Protein 1
ASVs	Variantes de sequência de amplicon

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1. INTRODUÇÃO

A obesidade é definida como uma condição de doença que, assim como outras doenças crônicas, resulta diretamente do efeito do excesso de adiposidade sobre a função de órgãos e tecidos (Rubino *et al.*, 2025). Trata-se de uma condição multifatorial, influenciada por diversos fatores, como os socioeconômicos, genéticos e ambientais (Lingvay *et al.*, 2024). O aumento da ingestão de alimentos ultraprocessados, hipercalóricos, com alto teor de gordura, aliado à crescente inatividade física, constitui um dos principais fatores de risco dessa doença crônica não transmissível (Koskinas *et al.*, 2024; Lingvay *et al.*, 2024). A obesidade contribui para a ocorrência de diversas condições debilitantes à saúde, como o risco aumentado de complicações cardiovasculares, diabetes mellitus tipo 2, alterações metabólicas e comprometimentos psicossociais (Zhang *et al.*, 2024; Di Cesare *et al.*, 2019). Além disso, crianças com excesso de peso apresentam maior probabilidade de obesidade na vida adulta (Bradwisch *et al.*, 2020).

O aumento da prevalência de obesidade infantil no mundo configura um sério problema de saúde pública (Zhang *et al.*, 2024). No Brasil, estudos apontam uma prevalência de obesidade infantil, nas últimas três décadas, entre 8 e 9%, com aumento proporcional à idade e maior concentração nas regiões mais desenvolvidas (Zhang *et al.*, 2024; Ferreira *et al.*, 2021). O excesso de peso, associado a outras desordens metabólicas, resulta em custos bilionários para o Sistema Único de Saúde (SUS). Estimativas indicam que os custos diretos atribuíveis à obesidade somam cerca de 269,6 milhões de dólares, considerando apenas despesas com assistência médica de média e alta complexidade. Esses custos podem atingir US\$ 10,1 bilhões até 2050. Ademais, os impactos da obesidade infantil e de suas comorbidades psicológicas, cardiovasculares e metabólicas, tanto em curto quanto em longo prazo, ainda são pouco compreendidos e podem gerar consequências ainda mais graves para o SUS (Blüher, 2019; O'donnell *et al.*, 2000; Rtveladze *et al.*, 2013). No cenário global, os custos anuais relacionados à obesidade giram em torno de aproximadamente 1.878 bilhões de dólares (Okunogbe *et al.*, 2022).

Na obesidade, a inflamação desempenha um papel importante no desenvolvimento de disfunções metabólicas e cardiovasculares (Saltiel; Olefsky, 2017). Sabe-se que, com a expansão do tecido adiposo na obesidade crônica, ocorre ativação de processos inflamatórios que induzem inflamação crônica, afetando negativamente o funcionamento de diversos órgãos

(Kawai *et al.*, 2021). Essa inflamação é sustentada por adipócitos estruturalmente alterados e com função comprometida, que passam a secretar citocinas pró-inflamatórias. Além disso, há infiltração de células imunológicas da medula óssea, também produtoras dessas citocinas, como o TNF- α (Tumor Necrosis Factor-alpha) e a IL-1 β (Interleukin-1 β) (Costa *et al.*, 2024; Kawai *et al.*, 2021).

A inflamação sistêmica de baixo grau tem sido reconhecidamente uma das causas da neuroinflamação (Costa *et al.*, 2024; Semmler *et al.*, 2008), uma alteração que acarreta diminuição na sensibilidade do controle barorreflexo (Sinski *et al.*, 2009), maior quimiossensibilidade respiratória (Del Rio *et al.*, 2012) e hiperatividade simpática (Chan; Chan, 2012). Esses fatores, em conjunto, favorecem o desenvolvimento de complicações cardiovasculares e metabólicas. Embora existam evidências de comprometimento autonômico cardíaco em crianças com obesidade, os resultados na literatura ainda são divergentes (Campos *et al.*, 2022; Papadopoulos *et al.*, 2024). Em longo prazo, a disfunção autonômica (disautonomia) é reconhecida como fator de risco para desenvolvimento de doenças cardiovasculares e maior risco de mortalidade precoce (Carvalho *et al.*, 2019; Brar *et al.*, 2025). Diante disso, nosso primeiro objetivo deste estudo foi investigar a ocorrência de inflamação crônica de baixo grau e a função autonômica cardíaca em crianças com obesidade, comparando-as com crianças com peso normal.

O funcionamento adequado do sistema nervoso autônomo (SNA) permite a comunicação entre o cérebro, especialmente o tronco encefálico e o hipotálamo, e órgãos periféricos, como o coração e o trato gastrointestinal (Scott-Solomon; Boehm; Kuruvilla, 2021). A interação entre os dois componentes do SNA, o sistema nervoso simpático (SNS) e o parassimpático (SNP) é complexa: o aumento da atividade de um pode reduzir, aumentar ou não afetar a atividade do outro. Ou seja, o SNS pode suprimir a atividade do SNP ou, em alguns casos, induzir uma resposta compensatória aumentada (Billman, 2013).

A variabilidade da frequência cardíaca (VFC) é uma medida que expressa a função neurocardíaca e representa a variação natural nos intervalos entre batimentos cardíacos consecutivos. A VFC tem sido considerada um indicador excelente para avaliação da função autonômica cardíaca (Tiwari *et al.*, 2021). A VFC compreende índices nos domínios do tempo, da frequência e não lineares, que indicam predominantemente a atuação do SNS, do SNP ou da variabilidade geral da FC (Shaffer; Ginsberg, 2017). Assim, compreender o funcionamento autonômico em crianças pode auxiliar na predição de riscos cardiovasculares.

Nesse sentido, o segundo objetivo do presente estudo foi investigar a FC como preditor de risco cardiovascular, com base nos parâmetros de VFC em crianças com obesidade e peso normal.

A conexão entre microbiota intestinal, obesidade, inflamação e disfunção autonômica tem se mostrado interligada, desempenhando papel essencial no equilíbrio entre saúde e doença (Brar *et al.*, 2025). Estudos demonstram que disbiose intestinal se associa à obesidade não apenas pelo consumo elevado de alimentos ultraprocessados e pelos efeitos locais do estresse oxidativo e inflamação, mas também pela interferência da disbiose em centros reguladores do apetite, estimulando maior ingestão calórica (Liu *et al.*, 2021). Assim, investigar a ocorrência de supercrescimento bacteriano do intestino delgado (SIBO) e sua relação com fatores como obesidade, consumo de ultraprocessados e inflamação é de suma relevância para população infantil, e esse foi o terceiro objetivo deste trabalho.

Na disbiose, a barreira intestinal é alterada, tornando-se mais permeável e facilitando a translocação de moléculas nocivas para corrente sanguínea, como lipopolissacarídeos (LPS) e citocinas pró-inflamatórias, que aumentam as chances de ocorrência de inflamação sistêmica (Vincenzo *et al.*, 2024). Além disso, a disbiose intestinal tem sido destacada como um dos principais fatores que levam à disfunção do SNA, por meio da produção de substâncias que interagem com células neurais localizadas nos centros de controle autonômico (Costa *et al.*, 2024). Ainda, uma má comunicação vagal bidirecional entre o intestino e o cérebro pode influenciar centros cerebrais de controle do apetite, estimulando o consumo calórico excessivo e o ganho de peso (Fox, 2013). Diante desses aspectos, torna-se extrema relevante encontrar estratégias para conter o avanço da obesidade infantil, especialmente aquelas com foco em exercício físico e nutrição (Koskinas *et al.*, 2024).

A atividade física regular desempenha um papel fundamental na prevenção de diversos tipos de doenças, e o ambiente escolar configura-se como um espaço importante para aplicação de protocolos de exercício físico em crianças e adolescentes, principalmente por fornecer acesso independente do histórico ou status socioeconômico (Love; Adams; Sluijs, 2019). Incluir um componente pliométrico em programas de exercício físico tem se mostrado relevante na investigação de estratégias para a prevenção e o tratamento de doenças crônicas não transmissíveis, como a obesidade (Almeida *et al.*, 2021; Gómez-Bruton *et al.*, 2017). Esse tipo de exercício é caracterizado pela combinação de força e potência, tendo como base o ciclo do alongamento-encurtamento, que corresponde à rápida transição da fase excêntrica

para a fase concêntrica (Gómez-Bruton *et al.*, 2017). Considerando os efeitos positivos do exercício sobre parâmetros cardiovasculares em crianças, o quarto objetivo deste estudo foi realizar uma revisão sistemática com o intuito de identificar qual o melhor tipo de exercício para melhorar a VFC em crianças com obesidade.

A educação alimentar e nutricional (EAN) também é considerada um componente essencial de intervenções planejadas para crianças com obesidade. Tem sido demonstrado que a EAN, quando associada à prática de exercício físico no ambiente escolar - principalmente com o envolvimento dos pais -, pode contribuir significativamente para a prevenção e redução da obesidade (Guerra; Silveira; Salvador, 2016). Neste sentido, a utilização da EAN em conjunto com a prática de exercícios físicos com componente pliométrico pode se configurar-se como uma estratégia eficiente para a modificação da composição corporal de escolares com obesidade (Bibeau *et al.*, 2008; Nobre *et al.*, 2017), bem como para a atenuação de alterações cardiometabólicas e da microbiota intestinal relacionadas a essa condição.

Os fatores discutidos acima, somados ao ineditismo do protocolo de intervenção utilizado, reforçam a importância deste trabalho no sentido fornecer evidências que possam melhorar a compreensão e o enfrentamento da obesidade infantil, especialmente diante dos resultados controversos disponíveis na literatura (Mead *et al.*, 2017). Desta forma, o quinto objetivo deste trabalho foi avaliar se uma intervenção composta por exercício físico e EAN contribui para melhoria da composição corporal, dos parâmetros bioquímicos, das variáveis cardiovasculares e do microbioma intestinal em escolares com obesidade. Finalmente, com o intuito de aprofundar melhor compreensão dos mecanismos biomoleculares que sustentam os resultados fisiológicos encontrados nas crianças, foi executado um protocolo experimental com animais, estabelecido como o sexto objetivo deste trabalho.

2. REFERENCIAL TEÓRICO

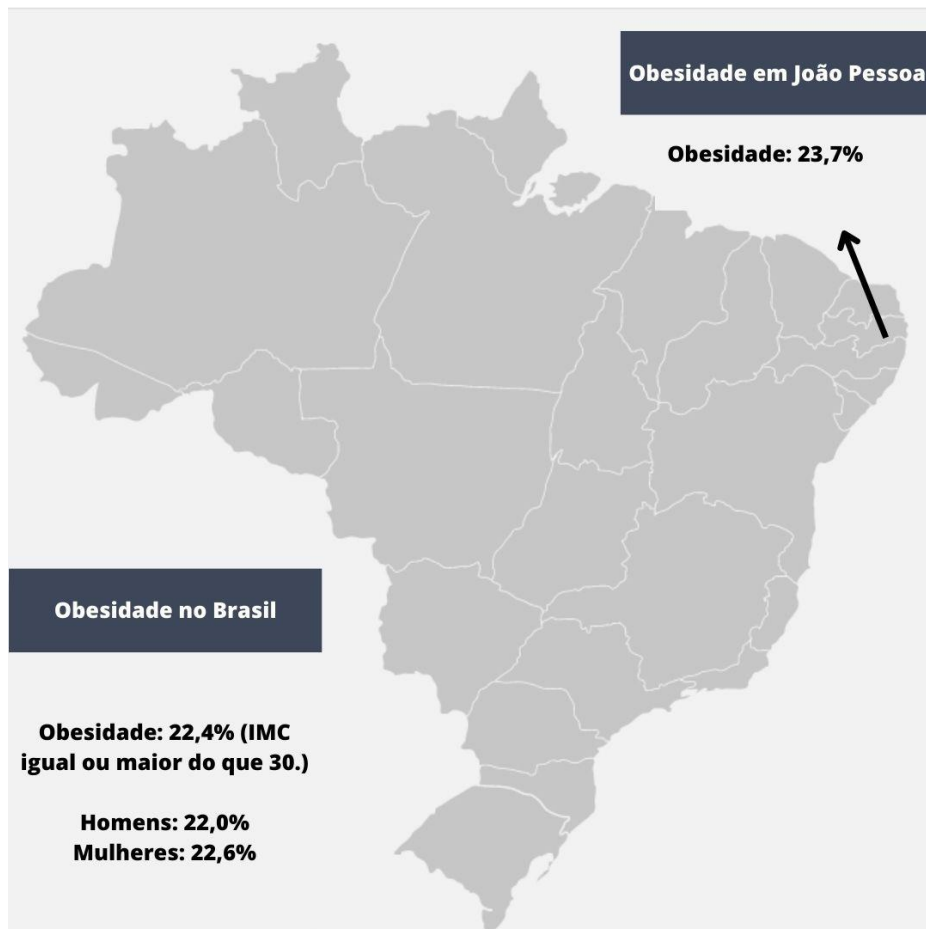
2.1. ASPECTOS RELACIONADOS À OBESIDADE, SUA RELAÇÃO COM OUTRAS COMORBIDADES E OBESIDADE INFANTIL

2.1.1. Aspectos relacionados à obesidade e sua relação com outras comorbidades

A obesidade é definida como uma condição de doença que, de maneira semelhante a outras doenças crônicas, resulta diretamente dos efeitos do excesso de adiposidade sobre a função de órgãos e tecidos (Rubino *et al.*, 2025). Ela é classificada principalmente pelo índice de massa corporal (IMC), um critério limitado, mas ainda adotado amplamente como ferramenta clínica simples e rápida para a classificação inicial e o monitoramento de mudanças no peso corporal, tanto para indivíduos como para populações. Além disso, embora o debate ainda seja preliminar, a obesidade pode ser classificada com base em biomarcadores específicos. Nesse contexto, propõe-se uma subdivisão entre indivíduos com obesidade metabolicamente saudáveis, indivíduos com obesidade e distúrbios metabólicos, pessoas com peso normal com obesidade metabólica e aqueles com obesidade sarcopênica (Mayoral *et al.*, 2020).

A prevalência da obesidade segue em crescimento contínuo, afetando mais de 2 bilhões de pessoas no mundo, o que representa 30% da população global (Lingvay *et al.*, 2024). No Brasil, a prevalência atinge 22,4%, e na Paraíba, aproximadamente 23,7% dos indivíduos maiores de 18 anos têm IMC superior a 30 kg/m², conforme ilustrado na Figura 1 (VIGITEL, 2019). De acordo com especialistas, não há perspectivas animadoras de redução desses índices nos próximos anos (Caballero, 2019; OMS, 2013).

Figura 1 - Mapa da obesidade em pessoas maiores de 18 anos no Brasil e em João Pessoa no ano de 2021.



Fonte: Autor, adaptado de VIGITEL, 2022.

Historicamente, o IMC da população humana tem aumentado nos últimos 300 anos, impulsionado pela transição de contextos de escassez alimentar para um cenário de produção e consumo em massa de alimentos. Sob essa ótica, é possível compreender com mais clareza os fatores sociais e econômicos que contribuem para o aumento global da obesidade (Fogel; Grotte, 2011). Entre os fatores sociais, destacam-se o estado civil (ser casado), idade avançada, sexo feminino, êxodo rural e substituição do agronegócio local por redes varejistas de alimentos, que influenciam positivamente a prevalência de obesidade (Endalifer; Diress, 2020). No aspecto econômico, a concentração de renda exerce impacto direto sobre a qualidade de vida, sendo responsável por uma redução média de 2,1 anos da expectativa de vida de pessoas em estratos socioeconômicos mais baixos (Stringhini *et al.*, 2017).

Além dos fatores socioeconômicos, aspectos comportamentais como má alimentação e sedentarismo são determinantes importantes da obesidade. O consumo de alimentos hipercalóricos, açucarados, e com concentração elevada de lipídios, é reconhecidamente associado ao desenvolvimento de obesidade e de doenças crônicas não transmissíveis (Hruby *et al.*, 2016). A prática regular de exercício físico, por sua vez, atua como moduladora do surgimento dessas doenças, principalmente as cardiovasculares. Um dos mecanismos de atuação do exercício físico é o seu efeito sobre a expressão gênica. Por exemplo, Kilpelainen *et al.* (2011) demonstraram que a atividade física reduziu em cerca de 30% os efeitos obesogênicos mediados pelo gene FTO (*fat mass and obesity associated*), por meio da diminuição da sua expressão.

Os efeitos negativos da obesidade são amplamente documentados, sobretudo pela sua associação com o surgimento de outras doenças, como diabetes mellitus tipo 2 (DM2), esteatose hepática, doenças cardiovasculares, dislipidemias e alguns tipos de câncer - todas as quais podem levar a um aumento do risco de mortalidade (Purnamassari *et al.*, 2011; Mayoral *et al.*, 2020).

A relação entre obesidade e DM2 é reconhecida há muito tempo e ajuda a explicar a elevada prevalência da doença em diversos países. O principal mecanismo dessa associação é a resistência à insulina, que impede a cascata de eventos que culminaria na expressão de receptores de glicose nas membranas celulares, bloqueando a entrada de glicose nas células e contribuindo para a hiperglicemia. Há evidências de que a presença concomitante de obesidade e DM2 representa um fenótipo de alto risco para desfechos cardiovasculares, a exemplo da insuficiência cardíaca (Piché; Tchernof; Després, 2020).

A obesidade, quando ocorre concomitantemente à hipertensão arterial, está associada ao aumento da morbidade e elevação progressiva dos custos médicos. Os mecanismos envolvidos são complexos e por vezes interdependentes, com destaque para a atuação do SNS, além de fatores genéticos e ambientais. Indivíduos portador de obesidade têm maior risco de desenvolver hipertensão arterial, sendo que aproximadamente 60% dos casos de hipertensão tem como fator causal principal o aumento da adiposidade corporal. Dados do *National Health and Nutrition Examination Surveys* (NHANES) indicam que a prevalência de hipertensão arterial é de 42,5% entre indivíduos com IMC >30 kg/m², em comparação com 15,3% entre aqueles com peso normal (Seravalle; Grassi, 2017; Lewis *et al.*, 2012).

Diante de todos os desafios relacionados à obesidade apresentados nesta seção, torna-se essencial compreender sua manifestação ainda na infância, a fim de reduzir o risco de complicações futuras e promover uma melhor qualidade de vida ao longo da vida adulta.

2.1.2. Obesidade infantil

A obesidade infantil emerge como um dos desafios mais críticos da saúde pública contemporânea, demandando investigações sólidas sobre seus determinantes biológicos, comportamentais e sociais, bem como seus impactos em curto e longo prazo (Zhang et al., 2024). A prevalência global de excesso de peso em crianças menores de cinco anos tem aumentado de forma modesta, inclusive em países de baixa e média renda. Já a prevalência de obesidade entre crianças de 2 a 4 anos aumentou moderadamente (Zhang et al., 2024). Nos anos de 1980, crianças de 5 a 19 anos com obesidade eram casos relativamente raros, realidade que se transformou significativamente nas últimas décadas (Zhang *et al.*, 2024; Assari; Bazargan, 2019).

Apesar dos custos sociais, financeiros e de saúde relacionados ao excesso de peso, os impactos da obesidade infantil, especialmente em termos psicológicos, cardiovasculares e metabólicas, ainda são pouco compreendidos e podem ser ainda mais graves do que se imagina (Assari; Bazargan, 2019). O manejo dessa condição envolve desafios consideráveis, exigindo programas estruturados e individualizados de redução de peso, aliados a hábitos alimentares saudáveis (Assari; Bazargan, 2019). Os medicamentos para tratamento da obesidade têm um papel limitado na infância e não são recomendados para crianças e adolescentes mais jovens. Além disso, a cirurgia bariátrica é recomendada para um grupo mais específico, composto por adolescentes com idade mais avançada e com obesidade grave, embora os dados sobre sua segurança em longo prazo nessa faixa etária ainda sejam limitados (Assari; Bazargan, 2019).

Estratégias focadas no estilo de vida têm se mostrado fundamentais no tratamento da obesidade. Uma revisão sistemática demonstrou a eficácia de intervenções baseadas em estilo de vida, combinando alimentação saudável e atividade física, na redução do IMC e da circunferência abdominal (diminuição média de IMC: $-1,77 \text{ kg/m}^2$ e redução de IMC-z: $-0,30$) em crianças com excesso de peso (Deng *et al.*, 2024).

Evidências robustas e bem estabelecidas demonstram os riscos metabólicos associados à obesidade infantil, como a tolerância à glicose prejudicada e resistência à insulina em

crianças e adolescentes com obesidade (Sinha *et al.*, 2002). Nesse sentido, a obesidade infantil não apenas sinaliza riscos metabólicos imediatos à saúde, como hipertensão arterial, dislipidemias e resistência à insulina, mas também se configura como um marcador precoce da trajetória de risco cardiovascular ao longo da vida (Freedman *et al.*, 2001). O emblemático Bogalusa Heart Study, iniciado nos anos 1970, foi pioneiro ao revelar que fatores de risco para doenças cardiovasculares, incluindo obesidade, níveis elevados de colesterol e pressão arterial, podem ser identificados já na infância e tendem a persistir até a vida adulta, reforçando a importância de intervenções precoces e multiprofissionais desde os primeiros anos de vida (Freedman *et al.*, 2001).

A obesidade infantil envolve uma complexa disfunção neuroendócrina e metabólica, que abrange desde os mecanismos de apetite e saciedade até processos inflamatórios e resistência à insulina. No nível neuroendócrino, desequilíbrios em adipocinas como leptina e adiponectina alteram a sinalização de saciedade, enquanto a inflamação crônica de baixo grau, com elevação de TNF- α , IL-6 e IL-1 β , prejudica a sinalização da insulina via vias como PKC (Protein Kinase C) e MAPK (Mitogen-Activated Protein Kinase), reduzindo a eficácia do IRS-1/2 (Insulin Receptor Substrate proteins 1 and 2) e interrompendo o caminho PI3K-AKT (Phosphoinositide 3-kinase - Protein Kinase B), o que compromete a captação de glicose nos tecidos periféricos (Luo *et al.*, 2024). Além disso, o acúmulo de ácidos graxos livres e metabólitos lipídicos está associado à disfunção mitocondrial e ao estresse do retículo endoplasmático, contribuindo para a resistência à insulina (Luo *et al.*, 2024). O metabolismo alterado manifesta-se também na diminuição da utilização oxidativa de glicose, com predomínio de vias glicolíticas, sobretudo em estados de hiperglicemia e inflamação persistente. Por fim, a redução da atividade do tecido adiposo marrom (BAT), responsável pela termogênese, compromete o gasto energético, favorece a retenção de gordura e piora o perfil metabólico na obesidade e infantil (Cree-Green *et al.*, 2019).

É importante destacar que as obesidades infantil e adulta compartilham alguns dos mecanismos fisiológicos mencionados anteriormente, como inflamação de baixo grau, a resistência à insulina e a disfunção do tecido adiposo. No entanto, na infância, esses processos apresentam maior plasticidade: há maior capacidade de formação de novos adipócitos (hiperplasia), maior atividade do tecido adiposo marrom e respostas imunometabólicas mais reversíveis (Landgraf *et al.*, 2015). Já na obesidade adulta, predominam alterações mais estabelecidas e menos reversíveis, como hipertrofia dos adipócitos, inflamação crônica

intensa, resistência à insulina persistente e menor termogênese (Al-Mansoori et al., 2022). A programação metabólica é outro fator que diferencia a obesidade na infância e na vida adulta (Martinho, 2022). Essas diferenças tornam as intervenções na infância mais eficazes na prevenção de complicações metabólicas futuras.

Diante da gravidade da obesidade, especialmente quando se manifesta na infância, e das comorbidades associadas à essa condição, é fundamental compreender em maior profundidade os mecanismos subjacentes, a fim de se alcançar uma visão mais ampla de seus impactos. Neste sentido, a inflamação crônica de baixo grau aparece como um alvo importante nas vias relacionadas às disfunções metabólicas da obesidade, como doenças cardiovasculares, e será discutida mais detalhadamente na seção seguinte (Saltiel; Olefsky, 2017).

2.2. ASPECTOS FISIOPATOLÓGICOS DA OBESIDADE RELACIONADOS COM A INFLAMAÇÃO

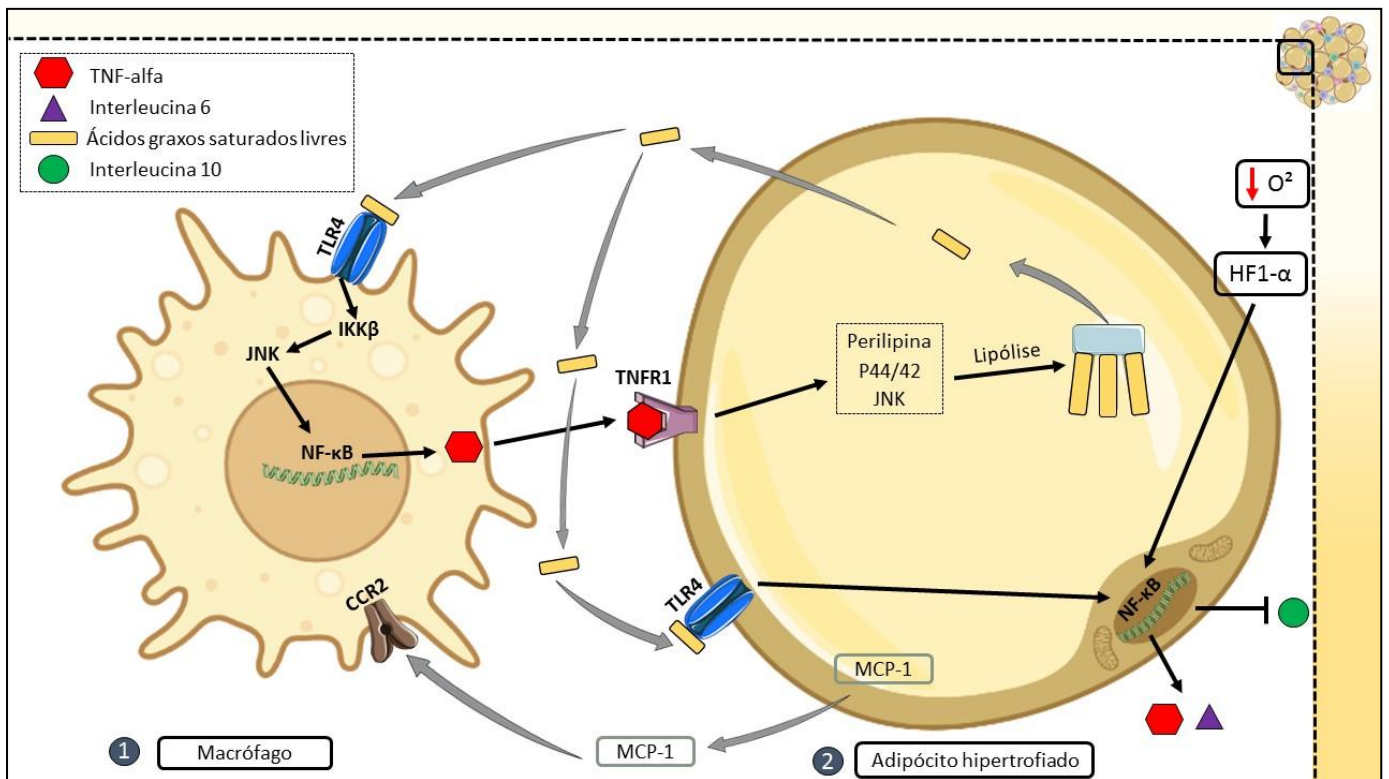
Sabe-se que a expansão do tecido adiposo está associada à ativação do processo inflamatório, contribuindo para um estado inflamatório sistêmico (Saltiel; Olefsky, 2017), o qual pode afetar o funcionamento de diversos órgãos e potencializar as consequências da obesidade (Kawai *et al.*, 2021). Essa inflamação é sustentada ao longo do tempo por adipócitos disfuncionais, que passam a secretar adipocinas inflamatórias, e pela infiltração de células imunológicas derivadas da medula óssea, as quais medeiam a sinalização inflamatória por meio da secreção de citocinas pró-inflamatórias, como TNF- α e IL-1 β (Costa *et al.*, 2024; Kawai *et al.*, 2021).

O tecido adiposo branco participa da síntese da adipocina leptina, um hormônio implicado na regulação da homeostase energética por meio da interação com centros de controle do apetite localizados em núcleos hipotalâmicos (Nappo *et al.*, 2017). Outras adipocinas, como a adiponectina, IL-6 e TNF- α , também são reconhecidas por desempenharem papéis importantes na regulação da inflamação e da resistência à insulina (Nappo *et al.*, 2017). Além disso, o tecido adiposo marrom é responsável principalmente pela termogênese, processo no qual há atuação destacada do complexo desacoplador de proteínas 1 (UCP-1) (Scheele; Wolfrum, 2020).

A disfunção metabólica dos adipócitos é reconhecidamente um dos fatores principais que contribuem para o surgimento da inflamação crônica de baixo grau, através da síntese de citocinas e quimiocinas. Em sequência, os macrófagos ativados no tecido adiposo perpetuam o processo inflamatório. Esse processo regula positivamente as vias do receptor *toll-like 4* (TLR4)/NF- κ B, devido à interação desses receptores com substâncias ligantes, tais como os ácidos graxos saturados e endotoxinas, a exemplo dos LPS, resultando na produção de citocinas como a IL2, IL-6, IL-1 β e TNF- α . Estas, por sua vez, também podem ativar a via do NF- κ B por meio da ligação a receptores específicos, como, no caso do TNF- α com o receptor do fator de necrose tumoral 1 (TNFR1). Outro fator que contribui para a manutenção da inflamação é o fator induzível por hipóxia 1 α (HIF1 α), produzido em resposta à hipóxia resultante da redução da perfusão sanguínea causada pela expansão do tecido adiposo. Além disso, a obesidade favorece um mecanismo inflamatório caracterizado pelo aumento da liberação da proteína quimioatraente de monócitos 1 (MCP-1), a qual participa do recrutamento de macrófagos que são ativados por ácidos graxos livres via TLR4 (Stoll; Denning; Weintraub, 2006; Chavakis; Alexaki; Ferrante, 2023; Gordon; Shaw; Kirshenbaum, 2011) (Figura 2).

Na figura 2, também está ilustrada a atuação do TNF- α na potencialização da inflamação. Essa citocina pró-inflamatória pode ser produzida tanto por adipócitos disfuncionais quanto por macrófagos ativados, aumentando a lipólise em adipócitos por meio da interação com o receptor TNFR1. Além disso, proteínas envolvidas na regulação da lipólise, como a perilipina, a p44/42 e a JNK (c-Jun N-terminal kinase), também são moduladas por TNF- α . Os ácidos graxos saturados interagem com os macrófagos, conferindo continuidade ao ciclo inflamatório (Chavakis; Alexaki; Ferrante, 2023; Gordon; Shaw; Kirshenbaum, 2011).

Figura 2 – Interação entre macrófagos e adipócitos hipertrofiados induzindo inflamação crônica de baixo grau



Fonte: Autor, 2025

Além disso, a elevada quantidade de tecido adiposo corporal desencadeia disfunção mitocondrial e, consequentemente, a geração de espécies reativas de oxigênio (ROS), induzindo estresse e inflamação do retículo endoplasmático e potencializando ainda mais a inflamação sistêmica. O retículo endoplasmático atua como um estimulador da inflamação, inclusive por meio da ativação do NF-κB, considerado um dos principais reguladores da inflamação em nível transcricional. Em condições de homeostase, o NF-κB permanece inativo devido à sua ligação ao complexo inibidor do fator nuclear kappa-B quinase (IKK) quinase. No entanto, após vários estímulos inflamatórios, a fosforilação da subunidade IKKβ do complexo IKK promove a degradação desse inibidor, resultando na ativação do NF-κB, que se transloca para o núcleo celular e ativa a transcrição de múltiplos genes inflamatórios. Dessa maneira, acredita-se que o estresse do retículo endoplasmático induza a ativação de NF-κB e, consequentemente, de outras vias pró-inflamatórias (Ajooolabady *et al.*, 2023).

Além da estrita relação já discutida entre a obesidade e inflamação crônica de baixo grau, outro componente de grande relevância vem sendo cada vez mais estudado em associação com essas condições: o SNA, em especial o SNS. Em síntese, adipócitos estrutural e funcionalmente alterados recrutam macrófagos que promovem inflamação sistêmica por meio da produção de substâncias pró-inflamatórias. Esse processo estimula terminações nervosas simpáticas, que liberam noradrenalina no tecido adiposo com o objetivo de estimular a lipólise. No entanto, uma população de células de defesa caracterizada pela expressão elevada do transportador de noradrenalina (SLC6A2) e da enzima de degradação monoamina oxidase A (MAOa) atenua essa interação entre o SNS e o tecido adiposo ao degradar noradrenalina. Isso reduz a lipólise mediada pela hiperatividade simpática e favorece o acúmulo de gordura, contribuindo para a manutenção da obesidade (Chavakis; Alexaki; Ferrante, 2023). Além disso, a hiperativação do SNS, mantida por esse ciclo disfuncional, está associada ao aumento do risco cardiovascular (Tiwari *et al.*, 2021).

Adicionalmente, muitos dos efeitos prejudiciais observados na obesidade não se originam, diretamente no tecido adiposo, mas resultam do acúmulo de gordura em outros tecidos corporais. Isso ocorre em decorrência da fibrose dos adipócitos e da consequente diminuição da capacidade de armazenamento de gordura, especialmente quando o consumo energético atinge níveis excessivamente elevados. Nesses casos, quando o tecido adiposo não consegue atender à demanda de armazenamento, os triglicerídeos passam a se acumular em tecidos não adiposos - como o fígado, músculo esquelético e coração sob a forma de gordura ectópica, estimulando disfunção metabólica local e ativando processos inflamatórios, os quais contribuem para a inflamação sistêmica (Tanaka, 2019).

O aumento da concentração de citocinas pró-inflamatórias na corrente sanguínea, em decorrência dos mecanismos descritos acima, é um dos fatores que induzem a neuroinflamação. Células do órgão subfornical - região que não é protegida pela barreira hematoencefálica - são capazes de detectar essas citocinas e ativar uma cascata de eventos que culmina na ativação glial e na produção de citocinas pró-inflamatórias, como IL-1 β e TNF- α . A neuroinflamação compromete o controle adequado do SNA sobre os tecidos periféricos, incluindo o coração, levando à disautonomia cardíaca, caracterizada por hiperatividade simpática e diminuição da atividade vagal cardíaca (Costa *et al.*, 2024).

2.3. CONTROLE AUTÔNOMICO CARDÍACO E VARIABILIDADE DA FREQUÊNCIA CARDÍACA

2.3.1. Sistema nervoso autonômico cardíaco

O funcionamento adequado do SNA pode ser considerado um índice de saúde física e emocional. Esse sistema tem a capacidade de exercer a comunicação entre o SNC as regiões periféricas, como o coração. A relação entre os dois sistemas que compõem o SNA, o SNS e o SNP, é complexa e o aumento da atividade de um pode estar associada à diminuição, aumento ou manutenção da atividade do outro, ou seja, embora o SNS possa diminuir a atividade do SNP ele também pode aumentar a reatividade desse sistema (Billman, 2013).

O SNA cardíaco pode ser dividido em duas partes: extrínseca, na qual as fibras e gânglios fazem a conexão entre o coração e o cérebro; e intrínseca, quando as fibras e gânglios estão localizadas no pericárdio. Esse controle acontece por diversos mecanismos, nos quais, na maioria dos casos, receptores detectam estímulos aferentes e induzem a transmissão do sinal via medula para a integração central. Esta, por sua vez, emite e transmite a resposta por vias eferentes, modulando a atividade do coração (Shen, 2021).

O SNA cardíaco extrínseco inclui os gânglios cervicais superiores, cervicotorácicos e torácicos, além dos nervos cardíacos superior, médio e inferior, que conduzem a sinalização simpática ao inervarem o coração. Os neurônios desses gânglios estão sob controle de outros neurônios simpáticos localizados na medula espinal. Já a inervação parassimpática tem sua origem majoritariamente no núcleo ambíguo da medula oblonga, e as fibras estão localizadas dentro do nervo vago, com destino aos nódulos sinoatrial (NS) e atrioventricular (AV) (Shen, 2021; Meng; Shivkumar; Ajijola, 2014).

Quanto ao controle intrínseco do SNA cardíaco, o coração contém células autorritmicas que geram espontaneamente os potenciais marca-passo responsáveis por iniciar as contrações cardíacas. O NS e o AV são os dois marca-passos internos principais, encarregados iniciar os batimentos cardíacos. O NS inicia dá início ao ciclo cardíaco por meio da despolarização espontânea de suas fibras autorritmicas, gerando um impulso elétrico que se propaga até o AV (Shen, 2021; Meng; Shivkumar; Ajijola, 2014).

Em repouso, a atividade do SNP é predominante, resultando em frequência cardíaca (FC) média de 75 bpm, valor inferior à FC intrínseca do NS, que é de 107 bpm. Isso faz

sentido, uma vez que o SNP reduz os mecanismos de despolarização dos nodos NS e AV, por meio da liberação e ligação de acetilcolina aos receptores muscarínicos, especialmente o M2. Nesse contexto, um aumento da FC pode ocorrer devido a um comprometimento no funcionamento do SNP. Além disso, como o SNP induz uma resposta mais rápida, mudanças bruscas na FC são consideradas indicadores de atividade parassimpática. Por outro lado, o aumento da atividade simpática provoca a elevação da FC intrínseca aos nodos NS e AV, que exibe um efeito mais lento e duradouro. O SNS aumenta a FC por meio da interação de norepinefrina e epinefrina com os receptores β -adrenérgicos (β -1), presentes nas fibras musculares cardíacas (Wehrwein; Orer; Barman, 2016; Shaffer; McCraty; Zerr, 2014).

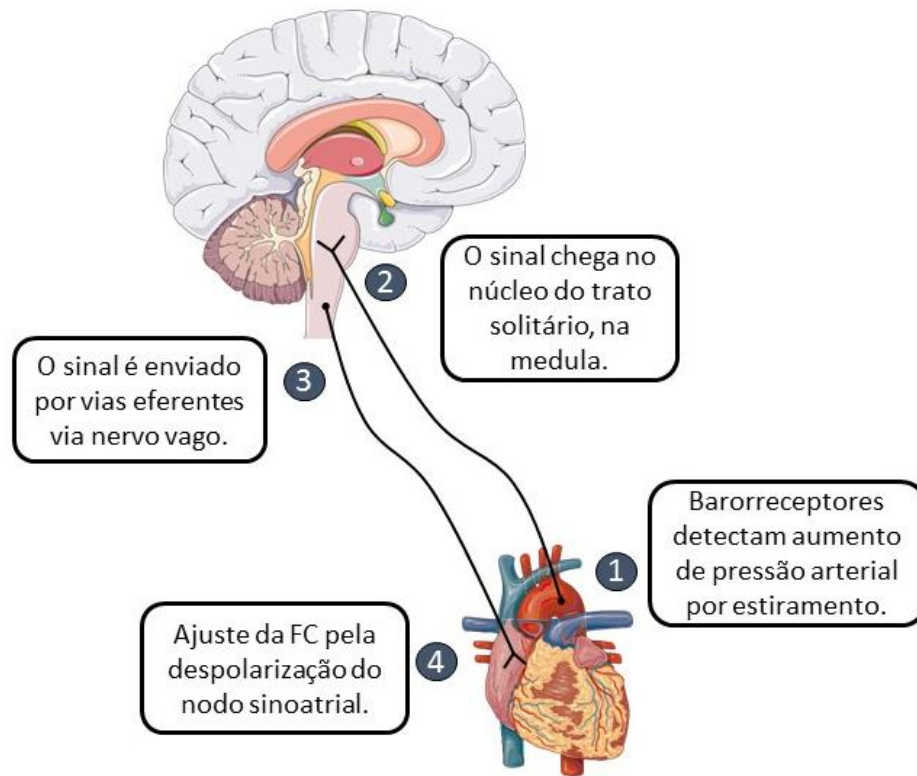
No processo de regulação autonômica, neurônios sensoriais localizados no coração, aferentes e motores, exercem uma função crítica na regulação fisiológica neurocardíaca. Esses neurônios enviam sinais mecânicos e químicos para o sistema nervoso intrínseco do coração, que posteriormente transmite sinais ao cérebro via medula espinal, passando pelos gânglios cardíacos extrínsecos e pelo nervo vago, após atravessarem o gânglio nodoso (Shen, 2021; Meng; Shivkumar; Ajijola, 2014; Wehrwein; Orer; Barman, 2016).

Entre os mecanismos de controle, os barorreceptores exercem função fundamental na regulação momento a momento da PA, FC e do volume sanguíneo. O barorreflexo arterial é um mecanismo de tamponamento crítico que previne a flutuações agudas da PA, através de receptores de estiramento (mecanorreceptores) localizados, principalmente, no seio carotídeo e no arco aórtico. Esses mecanorreceptores enviam informações sobre a distensão das paredes dos vasos através dos nervos glossofaríngeo e vago, respectivamente, para um grupo de núcleos especializados na medula espinal, que envia para o núcleo do trato solitário (NTS) na medula. O NTS, por sua vez, estimula os centros vagais e proporciona inibição do SNS e ativação do SNP, diminuindo a FC (Figura 3). Além disso, estudos de Malpas (2002) e Claude Julien (2005) evidenciaram que a atividade barorreflexa está intimamente relacionada às oscilações rítmicas da pressão arterial por meio das chamadas ondas de Mayer (~0,1 Hz), que são influenciadas tanto pela intensidade das perturbações desencadeadoras como pela sensibilidade dos barorreceptores, refletindo a interação entre os componentes simpático e parassimpático sobre a circulação.

Ainda sobre os barorreceptores, apesar de sua alta eficácia a curto prazo, eles apresentam um fenômeno adaptativo: quando a pressão arterial permanece elevada ou reduzida por longos períodos, o ponto de ajuste do reflexo se desloca. Isso explica por que o

barorreflexo não é um regulador tão eficaz da hipertensão arterial crônica, quanto é para a estabilidade momentânea da pressão arterial, por exemplo, em mudanças posturais rápidas ou durante o exercício (Kougias *et al.*, 2010).

Figura 3 - Controle autonômico da frequência cardíaca mediado pelos barorreceptores



Fonte: Autor, 2025.

Além dos barorreceptores, existem os receptores sensíveis à respiração, que induzem aumento da FC durante a inspiração por inibir o fluxo vagal, e durante a expiração ocorre a diminuição da FC por meio da restauração do fluxo vagal mediada pela acetilcolina (Shaffer; McCraty; Zerr, 2014). Ainda, existem outros mecanismos de controle autonômico mediados por outros tipos de receptores com inervações aferentes em outros tecidos corporais, a exemplo dos mecano e metaborreceptores localizados nos músculos esqueléticos, ou simplesmente ergorreceptores. Esses, emitem sinais aferentes para o centro integrador principalmente em situação de trabalho muscular, como no exercício físico, e induzem modificações nos sinais autonômicos eferentes para o coração em comparação com o repouso (Aimo *et al.*, 2021). Essa complexa interação dos mecanismos de controle neural, central e

periférico provoca uma diminuição da atividade parassimpática e principalmente um aumento da atividade simpática para o coração durante o exercício, pois pode ser atenuada pelo bloqueio dos receptores β 1-adrenérgicos (Fisher, 2014).

A importância do sistema muscular como um dos mecanismos de controle autonômico pode ser exemplificada também pelo fato de uma menor quantidade de massa muscular ser associada a uma disfunção autonômica. Um estudo realizado com mulheres submetidas à cirurgia bariátrica demonstrou que a melhora nas variáveis da VFC foi menos pronunciada em mulheres com baixa massa muscular (Carvalho *et al.*, 2023). Além disso, comunicações mediadas por substâncias secretadas pelos músculos e por outros tecidos periféricos, a exemplo das citocinas inflamatórias produzidas no tecido adiposo e hormônios secretados pelas células enteroendócrinas, incluindo o peptídeo-1 semelhante ao glucagon (GLP-1), são responsáveis por essa comunicação, que pode ser feita via corrente sanguínea ou sensibilização dos neurônios sensoriais vagais (Berthoud; Albaugh; Neuhube, 2021).

Deste modo, é possível inferir que um coração saudável não é um metrônomo. As oscilações são complexas e em variam constantemente, o que permite que o sistema cardiovascular se ajuste rapidamente a repentinos desafios físicos e psicológicos à homeostase corporal (Shaffer; Ginsberg, 2017). Então, a VFC indexa a função neurocardíaca e é gerada por interações coração-cérebro e processos dinâmicos não lineares do SNA (Shaffer; Ginsberg, 2017). O controle do SNA do coração é um processo dinâmico modulado pela inervação parassimpática e simpática (Malpas, 2010). A análise da VFC obtida de um eletrocardiograma, monitor Holter ou frequencímetro têm sido utilizados e validados para avaliar a função autonômica cardíaca por meio de índices no domínio do tempo, da frequência e não lineares, que quantificam a ativação parassimpática e simpática (Tiwari *et al.*, 2021).

Na obesidade infantil, ocorre a interrupção da maturação normal do controle autonômico cardíaco, uma vez que as evidências demonstram um padrão de desenvolvimento autonômico na infância, caracterizado por um aumento progressivo da atividade parassimpática em relação à atividade simpática e, em crianças com obesidade, ocorre redução da atividade parassimpática e aumento da atividade simpática (Eyre *et al.*, 2014). A idade e a obesidade influenciam de maneira significativa na VFC, e diversos fatores podem estar envolvidos, como o tamanho do coração em relação ao corpo, a frequência respiratória, a adiposidade, os hormônios sexuais e a aptidão física (Eyre *et al.*, 2014). Isso ocorre porque no início do desenvolvimento cerebral, estruturas do sistema límbico desenvolvem múltiplas

interconexões e conexões com os centros do SNA do tronco cerebral e moldam funções de alguns sistemas, como o cardiovascular. Ao longo da infância, essas conexões se fortalecem ou enfraquecem dependendo do ambiente, estresse e outras exposições (Mulkey; Plessis, 2019).

2.3.2. Variabilidade da frequência cardíaca

A detecção dos estágios iniciais da disfunção autonômica cardíaca e das causas relacionadas pode ser uma estratégia útil para identificar a presença de risco cardiovascular na obesidade infantil, e a VFC pode ser usada como um sinal de alerta de doenças cardíacas (Tiwari *et al.*, 2021; Jarczoc *et al.*, 2022; Van Biljon *et al.*, 2019). De modo geral, uma revisão sistemática com metanálise identificou que parâmetros mais baixos de VFC foram preditores significativos de maior mortalidade em diferentes idades, sexo, localizações geográficas e populações (Jarczoc *et al.*, 2022). Além disso, a VFC pode ser analisada em índices lineares como o domínio do tempo e da frequência e índices não lineares (Tabela 1).

As medições no domínio do tempo quantificam a variabilidade das medições dos intervalos entre os batimentos cardíacos sucessivos. Além disso, existe a possibilidade de expressar os valores em logaritmo ao invés dos valores originais, com a justificativa de obtenção de uma distribuição normal. Alguns dos índices no domínio do tempo mais utilizados são: SDNN (*standard deviation between NN intervals*), RMSSD (*square root of the mean of the square of the differences between consecutive NN intervals*), PNN50 (*percentage of adjacent NN with differences in duration greater than 50*) (Shaffer; Ginsberg, 2017).

No domínio da frequência ocorre a estimativa da distribuição da potência absoluta ou relativa em quatro bandas de frequência. As oscilações da frequência cardíaca são divididas em ULF (*ultra-low-frequency*, $\leq 0,003$ Hz), VLF (*very-low-frequency*, 0,0033–0,04 Hz), LF (*low frequency*, 0,04–0,15 Hz), HF (*high frequency*, 0,15–0,40 Hz), além da razão LF/HF. A potência absoluta é calculada em milissegundos (ms) ao quadrado dividido por ciclos por segundos (ms^2/Hz). A potência relativa é estimada como a porcentagem da potência total da VFC ou em unidades normais (nu), que divide a potência absoluta para uma banda específica pela potência absoluta somada das bandas LF e HF (Shaffer; Ginsberg, 2017).

Entre os índices de VFC há também os não lineares, que permitem a realização da quantificação da imprevisibilidade de uma série temporal. Alguns dos índices mais

importantes são SD1 (*standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph*), SD2 (*standard deviation of long-term continuous NN intervals*) e a razão SD1/SD2 (Stein; Reddy, 2005).

Tabela 1 - Principais índices de variabilidade da frequência cardíaca

Índice	Domínio	Unidade	Descrição	Parte do SNA avaliada
SDNN	Tempo	ms	Desvio padrão dos intervalos NN.	Atividade simpática e parassimpática.
RMSSD	Tempo	ms	Raiz quadrada média das diferenças sucessivas de intervalos RR.	Atividade parassimpática.
NN50	Tempo	bpm	Números de pares de intervalos NN adjacentes que diferem em mais de 50 ms em toda a gravação.	Atividade parassimpática.
pNN50	Tempo	%	Porcentagem de intervalos RR sucessivos que diferem em mais de 50 ms.	Atividade parassimpática.
LF	Frequência	ms ²	Potência absoluta da banda de baixa frequência (0,04–0,15 Hz).	Atividade simpática e parassimpática.
HF	Frequência	ms ²	Potência absoluta da banda de alta frequência (0,15–0,4 Hz).	Atividade parassimpática.
LF/HF	Frequência	%	Relação de potência LF para HF.	Comparação da atividade simpática e parassimpática.
LF	Frequência	nu	Potência relativa da banda de baixa frequência (0,04–0,15 Hz) em unidades normais.	Atividade simpática e parassimpática.
HF	Frequência	nu	Potência relativa da banda de alta frequência (0,15–0,4 Hz) em unidades normais.	Atividade parassimpática.
.SD1	Não linear	ms	Desvio padrão do gráfico de Poincaré perpendicular à linha de identidade.	Atividade parassimpática.
.SD2	Não linear	ms	Desvio padrão do gráfico de Poincaré ao longo da linha de identidade.	Atividade simpática e parassimpática.
SD2/SD1	Não linear	%	Razão de SD1 para SD2.	Comparação da atividade simpática e parassimpática.
DFA alfa 1	Não linear	-	Análise de flutuação sem tendência,	Reflexo barorreceptor.

descrevendo a flutuação de curto prazo.

DFA alfa 2	Não linear	-	Análise de flutuação sem tendência, descrevendo a flutuação de longo prazo.	Reflexo barorreceptor.
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Fonte: Autor, 2025.

A VFC é uma medida das mudanças naturais que ocorrem entre os batimentos consecutivos da frequência cardíaca, que representa o número de batimentos cardíacos por minuto (McCraty; Shaffer, 2015). A medida da VFC deve ser realizada em ciclos cardíacos normais (N), sem artefatos e batimentos ectópicos. Por esta razão, usamos a denominação intervalo N-N e não intervalo R-R (Ernst, 2017; Rodriguez-Nunez *et al.*, 2022). Um nível ideal de VFC reflete um funcionamento adequado, caracterizado por uma capacidade inerente de autorregulação, adaptabilidade ou resiliência. Em contraste, uma variação muito pequena indica esgotamento do sistema relacionado à idade, estresse crônico, patologia ou funcionamento inadequado em vários níveis de sistemas de controle autorregulatório (McCraty; Shaffer, 2015), pois condições patológicas podem produzir VFC. Quando as anormalidades da condução cardíaca elevam as medições da VFC, isso está fortemente relacionado ao aumento do risco de mortalidade, especialmente em idosos (Stein *et al.*, 2005).

Embora estudos que investigaram alterações na VFC de crianças com obesidade ainda sejam escassos, um estudo transversal com 110 crianças entre 7 e 10 anos classificadas como eutróficas, sobrepeso e obesidade relatou que crianças com obesidade tinham maior ativação simpática, embora a classificação nutricional de sobrepeso não parecesse afetar a função autonômica cardíaca (Campos *et al.*, 2022). Estudos anteriores também observaram que o estado nutricional de sobrepeso não causou disfunção do SNA quando comparado com crianças com peso normal (Kaufman *et al.*, 2007), bem como diminuição da atividade parassimpática e variabilidade global em crianças com obesidade, com diferença significativa nos valores de SD1 e SD2 (Vanderlei *et al.*, 2010). No entanto, alguns achados não corroboraram com os discutidos acima, o que pode estar relacionado ao tempo de exposição à obesidade (Vanderlei *et al.*, 2010; Rabbia *et al.*, 2003).

Alguns estudos têm investigado modificações na VFC após intervenções em crianças com obesidade e atestado benefícios, principalmente quando há um componente de treinamento físico (Li *et al.*, 2022; Brasil *et al.*, 2020; Gutin *et al.*, 1997). Li *et al.* (2022), investigaram um protocolo de treinamento físico com predominância aeróbica realizado

durante 16 semanas e observaram que houve melhoras nos índices RMSSD, SDNN, LF, HF, LF/HF. Brasil *et al.* (2020) realizaram um protocolo de treinamento de judô durante 12 semanas e observaram que houve melhora nos índices SDNN, RMSSD, PNN50, LF, HF e razão LF/HF.

2.4. DISBIOSE INTESTINAL E SUA RELAÇÃO COM A OBESIDADE, INFLAMAÇÃO E FUNÇÃO AUTONÔMICA

O microbioma intestinal é o ecossistema microbiano no intestino composto por bactérias, vírus, fungos e protozoários e seus metabólitos que interage diretamente com o hospedeiro, sendo, portanto, considerada parte integrante do corpo humano. Essa integração permite que essas bactérias, por exemplo, desempenhem funções importantes para a nossa saúde, como a metabolização de nutrientes, produção de moléculas sinalizadoras, formação da barreira intestinal, prevenção da invasão de patógenos e regulação imunológica. As bactérias da microbiota intestinal humana são divididas, principalmente, em quatro filos: Bacteroidota, Firmicutes, Actinobacteria e Proteobacteria e são relativamente estáveis. No entanto, na disbiose intestinal, ou seja, um desequilíbrio na composição e função do microbioma intestinal, existe maior probabilidade do surgimento de doenças, especialmente as cardiometabólicas (Zhang *et al.*, 2024; Geng *et al.*, 2022).

A microbiota intestinal é considerada extremamente influente no metabolismo do hospedeiro e no balanço energético. Estudos têm demonstrado uma associação positiva entre disbiose intestinal e obesidade, não só devido ao consumo excessivo de ultraprocessados e outros fatores, como o uso excessivo de antibióticos, álcool e baixa ingestão de fibras, que levam ao estresse e inflamação local, mas também pela disbiose afetar centros regulatórios do apetite, estimulando maior consumo calórico e obesidade (Liu *et al.*, 2021).

Um dos primeiros estudos a demonstrar a associação da obesidade com a microbiota intestinal observou que camundongos livres de germes que receberam uma dieta rica em gordura têm maior proteção contra obesidade em comparação com aqueles com microbiota. Além disso, os animais submetidos ao transplante de microbiota fecal de camundongos com obesidade apresentaram maior ganho de peso em comparação com animais submetidos ao transplante fecal de camundongos magros (Turnnaugh *et al.*, 2006). Em seguida, várias linhas

de investigação têm produzido evidências desta relação, inclusive em humanos, em que, por exemplo, uma baixa diversidade microbiana foi associada positivamente à adiposidade total e prejuízos no metabolismo da glicose (Chatelier *et al.*, 2013).

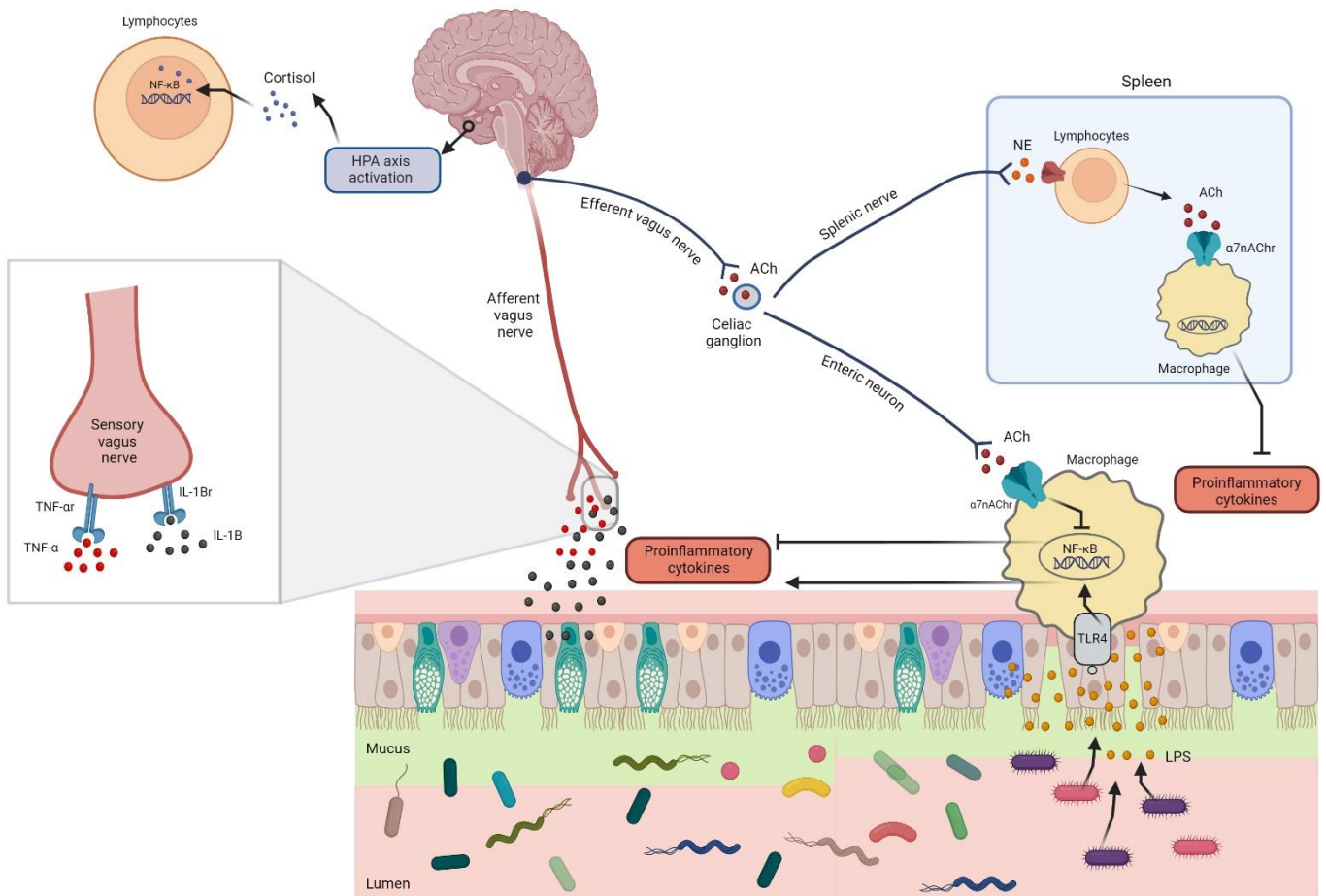
O metabolismo energético também sofre influência da microbiota intestinal, pois ela consegue modular a absorção e utilização de nutrientes e influenciar o desenvolvimento e função do tecido adiposo (de Cani *et al.*, 2024). Colangeli *et al.* (2023), abordaram os impactos que microbiota intestinal pode causar no metabolismo mitocondrial em adipócitos. Por exemplo, a microbiota intestinal tem a capacidade de estimular ou inibir o escurecimento do tecido adiposo branco, o que pode levar ao aumento do gasto energético e à melhora da saúde metabólica em animais (Chevalier *et al.*, 2015; Zamorano *et al.*, 2015). Neste sentido, a utilização de compostos com propriedades probióticas tem sido extensamente estudada por pesquisadores no enfrentamento da obesidade, inclusive com resultados ligeiramente positivos de acordo com uma revisão sistemática com metanálise conduzida por Sadegui *et al.* (2024).

Em relação à comunicação da microbiota intestinal com outros sistemas do hospedeiro, a produção de metabólitos bacterianos decorrentes da metabolização de nutrientes e de compostos bioativos exerce função de destaque (Geng *et al.*, 2022). Diversos metabólitos com diferentes funções são produzidos pela microbiota intestinal, tais como os ácidos graxos de cadeia curta (AGCC), metabólitos do triptofano (TMs), ácido gama-aminobutírico (GABA), LPS, e trimetilamina (TMA) (precursora de trimetilamina-N-óxido no fígado), (Zhang *et al.*, 2024). Na condição de disbiose intestinal, há maiores chances de translocação de moléculas nocivas ao hospedeiro do intestino para a circulação, o qual pode desencadear respostas inflamatórias e aumentar as chances de inflamação sistêmica (Vincenzo *et al.*, 2024).

Nesse sentido, a disbiose intestinal tem sido destacada como um dos fatores principais que levam à disfunção do SNA, tanto por vias neurais como humorais que tem a inflamação como ponto crucial neste processo. A produção de substâncias como LPS e citocinas pró-inflamatórias que interagem com células neurais localizadas nos centros de controle autonômico estimula, por via humoral, a neuroinflamação e consequente disfunção autonômica (Costa *et al.*, 2024). Além disso, uma má comunicação bidirecional vagal entre o intestino e o SNS pode influenciar centros cerebrais de controle do apetite, estimulando o consumo calórico excessivo e ganho de peso (Fox, 2013).

A figura 4, disponibilizada em um artigo publicado pelo nosso grupo de pesquisa (Costa et al., 2024) ilustra que, na disbiose, níveis aumentados de LPS no intestino estimulam o aumento dos níveis de citocinas pró-inflamatórias por meio da interação dessa endotoxina com receptores TLR4 expressos em células imunes e consequente ativação do complexo NF- κ B. As vias aferentes do nervo vago detectam o aumento de citocinas pró-inflamatórias e normalmente enviam sinais eferentes ao gânglio celíaco, liberando ACh. Este, por sua vez, envia sinais ao baço para liberar NE nos linfócitos, que liberam ACh para os macrófagos, inibindo a produção de citocinas pró-inflamatórias e prevenindo o aumento da inflamação sistêmica. Além disso, sinais são enviados do gânglio celíaco para as células imunes no intestino, onde a ACh usa o mesmo mecanismo para atenuar a inflamação local.

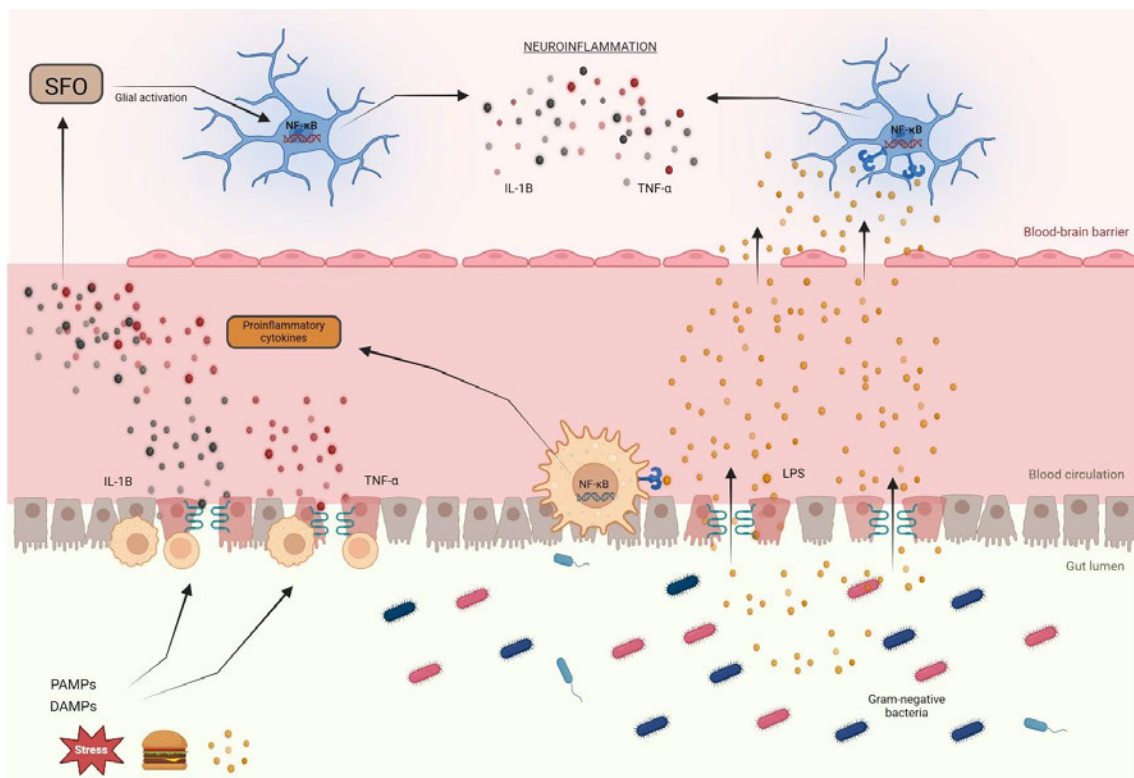
Figura 4 – Reflexo imune vagal estimulado pela microbiota intestinal



Fonte: Costa et al., 2024.

A inflamação sistêmica e a presença de endotoxinas na circulação sanguínea devido ao desequilíbrio da microbiota intestinal representa um risco para a função autonômica. Na figura 5, também proveniente do mesmo artigo publicado pelo nosso grupo de pesquisa (Costa et al., 2024) são representados possíveis mecanismos humorais envolvendo disbiose intestinal e neuroinflamação. A disbiose intestinal promove alterações na composição e diversidade da microbiota intestinal, reduz a camada de muco e aumenta a permeabilidade intestinal. Esse comprometimento da barreira intestinal torna o intestino suscetível à entrada de LPS na corrente sanguínea, causando inflamação periférica e promovendo respostas imunes centrais por meio de mecanismos subjacentes distintos. Danos à BHE permitem que o LPS entre no sistema nervoso central (SNC) e cause ativação da microglia. Isso ocorre porque a microglia possui receptores TLR-4 que, ao se ligarem ao LPS, ativam a via de sinalização NF- κ B, promovendo aumento da inflamação em regiões do SNC associadas ao controle autonômico. Outro mecanismo é que o SFO, uma região do SNC que não tem a proteção da BHE e o SFO, pode detectar um aumento nas citocinas pró-inflamatórias periféricas, projetar-se para o PVN e promover a ativação da microglia, ligando a inflamação sistêmica à neuroinflamação. Juntos, esses mecanismos causam neuroinflamação, especialmente nos núcleos autônomos, como o NTS-PVN.

Figura 5 – Mecanismos humorais que ligam disbiose e neuroinflamação



Fonte: Costa *et al.*, 2024.

Além do SNS e SNP, outro componente do SNA é o sistema nervoso entérico (SNE), que é considerado uma ponte neural que conecta a microbiota intestinal e o SNC (Geng *et al.*, 2022). O SNE é composto por neurônios e células gliais e se estende por todo o trato gastrointestinal, organizados em dois plexos principais, o plexo mioentérico (encontrado entre as camadas musculares longitudinal e circular do trato gastrointestinal) e o plexo submucoso (localizado na submucosa da parede intestinal) (Suman, 2024). O SNE, às vezes em conjunto com o SNA, desempenha um papel importante na coordenação de processos digestivos, a exemplo da secreção, fluxo sanguíneo e motilidade intestinal (Suman, 2024).

A discussão sobre a obesidade e suas consequências é importante para refletirmos possíveis caminhos para a atenuação deste enorme problema que acaba por diminuir a qualidade de vida das pessoas, havendo a necessidade da criação de mecanismos para que haja resolutividade. Desta maneira, é extremamente importante que haja a elaboração, investigação e implementação de protocolos de intervenção eficazes para o melhoramento de fatores relacionados à obesidade, como a composição corporal, variáveis cardiometabólicas e microbiota intestinal. Assim, estimular a prática regular de atividade física e a alimentação saudável são fatores essenciais dentro de um programa que tenha tal objetivo.

2.5. INTERVENÇÃO COM EXERCÍCIO FÍSICO E EDUCAÇÃO ALIMENTAR E NUTRICIONAL

Estratégias que estimulem a promoção da atividade física e a realização de atividades relacionadas a alimentação saudável parece ser ideal para o sucesso do protocolo estabelecido para prevenção e/ou tratamento da obesidade. As populações submetidas a pesquisas intervencionais podem ser de natureza diversa, a exemplo de escolares com obesidade, em que a literatura científica relata que o aumento da atividade física e melhor consumo alimentar durante o horário escolar está associado ao bem-estar físico, psicológico e social (Smedegaard *et al.*, 2016).

Aliar o estímulo à prática regular de atividade física e uma alimentação saudável em um protocolo de intervenção atende a duas das recomendações principais do relatório da comissão de combate à obesidade infantil, emitido pela Organização Mundial de Saúde

(OMS) em 2016. Isto ocorre, uma vez que o relatório trabalha na criação de programas para encorajar o consumo de alimentos saudáveis e desencorajar o consumo de alimentos não saudáveis, bem como na implementação de programas que promovam a atividade física, desencorajando o sedentarismo (OMS, 2016). Além disso, outras evidências destacam a necessidade encontrar estratégias para conter o avanço da obesidade infantil, principalmente àquelas com foco em exercício físico e nutrição (Koskinas *et al.*, 2024).

A EAN é conceituada como qualquer combinação de estratégias educacionais, acompanhadas de apoios ambientais, projetadas para facilitar a adoção voluntária de escolhas alimentares e outros comportamentos relacionados à alimentação e nutrição que conduzam à saúde e ao bem-estar (Contento, 2016). Nesse sentido, principalmente para estratégias realizadas no Brasil, o Marco de Referência de Educação alimentar e Nutricional para políticas públicas deve ser consultado (Brasil, 2012). As atividades de EAN objetivam melhorar os níveis de conhecimento nutricional, corrigir comportamentos nutricionais inadequados e promover hábitos alimentares saudáveis (Aktaş *et al.*, 2018). A EAN é uma ferramenta eficaz e extremamente relevante, pois fazer escolhas alimentares saudáveis necessárias para mudar o comportamento alimentar não é uma tarefa fácil de realizar. Contudo, uma mudança simples no estilo de vida pode ter um grande efeito na saúde pública de milhões de pessoas, principalmente as acometidas por doenças crônicas não transmissíveis (Sharma; Rani, 2016).

Um estudo investigou se um protocolo de intervenção de EAN induziria efeitos positivos sobre os hábitos alimentares de escolares. Nesta pesquisa, os autores observaram que a intervenção teve efeito positivo em aumentar a frequência de desjejum saudável dos estudantes. Ainda, os autores destacaram a necessidade de desenvolver políticas públicas baseadas em evidências e intervenções no ambiente escolar para promoção de um estilo de vida saudável e um comportamento nutricional adequado para uma melhor qualidade de vida (Elseifi; Abdelrahman; Mortada, 2020).

Além das intervenções de EAN, a atividade física regular compõe a maioria das intervenções realizadas neste contexto, e o exercício pliométrico surge como uma alternativa importante a ser utilizada, principalmente como um componente dentro de circuito de exercícios físicos. O exercício pliométrico é um tipo de exercício caracterizado pela realização de saltos de peso corporal utilizando ciclos musculares de alongamento e encurtamento, ou seja, enquanto as crianças, por exemplo, estão brincando de pular e saltitar

elas estão realizando um exercício pliométrico (Bedoya; Miltenberger; Lopez, 2015). Wang e Zhang (2016) resumem o ciclo de alongamento-encurtamento como um aprimoramento da capacidade dos sistemas neural e musculotendíneo de produzir força máxima no menor período de tempo.

Alguns autores propuseram que o exercício pliométrico poderia causar efeitos negativos quando aplicados na população infantil, argumentando que o risco de lesão aumentaria e que haveria atraso no crescimento (Lloyd; Meyers; Oliver, 2011). No entanto, o exercício pliométrico não induz mais risco de lesão do que outro tipo de atividade física em que crianças ou adolescentes participem normalmente, e quando as diretrizes são seguidas para a idade, o exercício pliométrico tem se mostrado benéfico também na população jovem. Alguns dos efeitos benéficos do exercício pliométrico são: aumento da função neuromuscular, maior facilidade no controle de peso, aumento do bem-estar psicossocial, aumento da densidade mineral óssea e diminuição do risco de lesões (Markovic; Mikulic, 2010). Inclusive, uma revisão sistemática com metanálise identificou que exercícios com saltos aplicados em crianças e adolescentes melhoram o conteúdo mineral, propriedades e estrutura óssea (Gòmes-Bruton *et al.*, 2017).

O exercício pliométrico também foi analisado no contexto da obesidade, mais especificamente nos parâmetros de inflamação e saúde óssea. Neste caso, os autores compararam os níveis circulantes de citocinas inflamatórias, adipocinas, osteocinas e marcadores ósseos em repouso e em resposta a uma única sessão de exercício pliométrico entre adolescentes com obesidade e com peso normal. Os resultados demonstraram que não houve diferença significativa de inflamação ou diferenças na reabsorção óssea, mas houve comprometimento da formação óssea quando os adolescentes foram comparados aos do grupo controle com peso normal (Kurgan *et al.*, 2020). É importante ressaltar também que, Nobre *et al.*, 2017 observaram efeitos benéficos de um programa de 12 semanas de treinamento pliométrico em crianças de 7 a 9 anos com obesidade. Eles observaram uma melhoria significativa dos componentes de aptidão física relacionados à saúde, bem como da aquisição da coordenação motora das crianças.

Outros estudos evidenciam a importância de exercícios desta natureza em um programa de exercícios, melhorando a força muscular e equilíbrio postural em crianças com paralisia cerebral (Elnaggar *et al.*, 2022) e aumentando a massa muscular e melhorando os níveis de glicose e leptina de mulheres jovens com obesidade (Racil *et al.*, 2016). Além disso,

o exercício pliométrico foi eficaz em induzir efeitos adaptativos positivos na pressão arterial e frequência cardíaca em meninos com sobrepeso e obesidade com idade entre 7 e 9 anos (Leandro *et al.*, 2021).

De acordo com Dabas e Seth (2018), as intervenções de prevenção e tratamento da obesidade podem ser classificadas como intervenções domiciliares, intervenções escolares e intervenções baseadas na comunidade e políticas. Neste sentido, uma revisão investigou intervenções realizadas em vários destes locais, e demonstrou a força de cada uma dessas abordagens. Ademais, sugeriu maior efetividade quando há combinação das abordagens (Foltz *et al.*, 2012).

Uma revisão conduzida por García *et al.* (2016), selecionou nas bases de dados *Web of Science*, *Scopus*, *Dialnet*, *PubMed*, *Eric*, *Sportdiscus* e *Psycinfo* 7 artigos que realizaram programas de atividade física e de EAN em ambientes escolares. Os autores observaram que as ações induziram modificações positivas na composição corporal em menos da metade dos estudos analisados, em contrapartida, a maioria dos programas de intervenção tiveram efeitos positivos sobre os hábitos alimentares e nível de atividade física.

As escolas podem fornecer um ambiente ideal para intervenções de mudanças de estilo de vida, uma vez que servem como um ponto focal para envolver alunos, famílias, educadores, administradores e membros da comunidade. Ao reunir indivíduos para apoiar uma visão comum, os recursos existentes podem ser usados para criar um ambiente escolar saudável e sustentável (Briggs; Mueller; Fleishhacker, 2010). Embora alguns autores defendam que existe uma dificuldade na alocação de recursos para os programas de prevenção da obesidade no ambiente escolar e que os estudos dessa natureza servirão para evidenciar a estratégia alimentar e de atividade física mais assertiva para determinada população (Bustos *et al.*, 2016).

Em muitos países, as abordagens baseadas na escola para a atividade física têm a vantagem óbvia de que as crianças que mais precisam são razoavelmente alcançáveis, devido ao fato de que a vasta maioria das crianças e adolescentes recebe sua educação primária em escolas públicas. Além disso, saúde e bem-estar são parte integrante do currículo da escola pública, o que significa que existem educadores qualificados e uma cultura existente para atividades de ensino e aprendizagem relacionadas à saúde, bem-estar e atividade física (Smedegaard *et al.*, 2016).

Diferentes pesquisadores utilizam intervenções desta natureza como uma importante ferramenta para trabalhar a prevenção e tratamento da obesidade infantil. O modelo proposto por Scherr *et al.* (2016) denominado *Shaping Healthy Choices Program* foi projetado para alunos do ensino fundamental no intuito de aumentar o conhecimento sobre nutrição; promover a disponibilidade e consumo de frutas e vegetais; melhorar os padrões alimentares e estimular a atividade física; promover mudanças positivas no ambiente escolar; e facilitar o desenvolvimento de uma infraestrutura para sustentar o programa. Logo, fundamentou-se em melhorar tanto os fatores individuais dos alunos bem como os ambientes doméstico e escolar, ao mesmo tempo em que criava um sistema de apoio baseado na comunidade. Neste estudo os autores observaram uma redução importante nos percentis do IMC.

Levando em consideração que a gordura corporal excessiva em uma idade jovem tende a persistir na idade adulta e está associada também a distúrbios psicossociais, que levam ao baixo desempenho cognitivo e escolar, torna-se importante destacar que os efeitos benéficos destes modelos de intervenção não se resumem a resultados de cunho físico. Um estudo demonstrou que intervenções baseadas na escola melhoraram o desempenho escolar, a função cognitiva e a memória de trabalho (Martin *et al.*, 2014).

Outra revisão, neste caso, sistemática com metanálise, analisou 85 estudos sobre intervenções em escolas. Segundo os autores, houve um impacto positivo significativo dessas intervenções no IMC e na pressão arterial das crianças. Além disso, os efeitos benéficos foram potencializados quando houve envolvimento dos pais (Oosterhoff; Joore; Ferreira, 2016). Em relação à adesão dos pais, Lanigan (2018) relata que os pais que percebem um alto risco de os filhos desenvolverem obesidade são mais propensos a se engajar em intervenções. Ainda, outros fatores como a oportunidade de comparação com outras pessoas e trabalhar em grupo encorajam a participação dos pais nestes programas. Murimi *et al.* (2020) também relatam que as intervenções que envolveram a presença dos pais, identificaram comportamentos específicos a serem modificados e garantiram a participação dos professores no processo foram mais eficazes.

Outro ponto relevante é a percepção equivocada do estado nutricional, pois ela pode dificultar o processo de prevenção da obesidade, sobretudo a infantil. Em uma intervenção de aproximadamente 3 meses, ocorreram sessões de educação em saúde para alunos e pais, monitoramento regular do peso dos alunos e fornecimento de feedback por meio de um aplicativo para smartphone. Após esse período, os autores observaram que a porcentagem de

percepção precisa dos alunos sobre seu próprio estado nutricional no grupo de intervenção aumentou de 49,0% para 59,2% da linha de base para três meses, enquanto diminuiu de 64,1% para 58,1% no grupo controle, havendo alteração estatisticamente significativa. No entanto a percepção dos pais sobre o estado nutricional dos filhos não melhorou significativamente (Shen *et al.*, 2020).

Um estudo de 16 semanas de intervenção composta por um programa de treinamento e aconselhamento nutricional realizado em crianças com obesidade observou resultados positivos. Houve melhoria significativa na composição corporal, no perfil metabólico e inflamatório. Segundo os autores, o aconselhamento nutricional reforçou os benefícios do exercício e transformou a intervenção em uma estratégia educacional poderosa (Cordellat *et al.*, 2020).

Segundo Kelley *et al.* (2019), os exercícios devem ser o tipo mais importante de intervenção para a saúde fisiológica geral dessa faixa etária, visto suas melhorias clinicamente relevantes nos desfechos de adiposidade e melhorias significativas em muitos desfechos secundários. No entanto, o exercício de maneira isolada pode não ser eficaz para redução do peso nas situações em que a alimentação está inadequada, a exemplo do consumo alimentar nos países ocidentais (ACSM, 2017).

Desta maneira, as evidências apresentadas acima reforçam que a adoção precoce de intervenções eficazes pode mitigar comorbidades e prevenir uma trajetória de obesidade persistente ao longo da vida. Sendo assim, percebe-se a importância de inserir a atividade física regular e alimentação saudável como estratégias de prevenção e tratamento da obesidade, que, assim como outras doenças, interage diretamente com outros sistemas do corpo humano, a exemplo da microbiota intestinal, e dos sistemas imune, neural e cardiovascular.

3. MATERIAL E MÉTODOS

3.1 DELINEAMENTO GERAL DO ESTUDO

O trabalho em questão é de caráter translacional, em que realizamos uma pesquisa com crianças com obesidade e em seguida, visando estudar os mecanismos biomoleculares envolvidos com as variáveis fisiológicas, executamos um protocolo semelhante com animais.

3.2 PROTOCOLO COM HUMANOS

3.2.1 Delineamento do estudo, participantes e aspectos éticos

Em relação ao trabalho com humanos, o estudo em questão é um recorte de um projeto de pesquisa maior, intitulada Treinamento físico e educação nutricional como estratégias para modular a microbiota intestinal e parâmetros cardiometabólicos em escolares com obesidade, financiado pelo Programa de Pesquisa para o SUS (PPSUS). Este trabalho se caracteriza como um ensaio clínico randomizado não controlado com intervenção com duração de 4 meses (REBEC: RBR-2mzmpxf), contendo também um recorte transversal na linha de base para recrutamento amostral e análises preliminares. O trabalho prático de coleta de dados foi realizado com crianças de 7 a 11 anos matriculadas em escolas públicas da cidade de João Pessoa - PB. Este estudo foi submetido e aprovado Comitê de Ética em Pesquisa em Seres Humanos da UFPB (CAAE: 37352720.6.1001.5188) e segue a resolução 466/12 do Conselho Nacional de Saúde.

O convite foi feito às escolas. Uma vez aceito, as crianças foram submetidas à avaliação do estado nutricional. Para o ensaio clínico, foram incluídas crianças com obesidade ($IMC/idade > 2,0$ escore Z), de 7 a 11 anos e de ambos os sexos, que foram assim randomizadas para receberem um protocolo de intervenção composto por um programa de EAN e um protocolo de exercício físico com componente pliométrico. Além disso, foram excluídas as crianças com doenças crônicas ou que não aceitaram participar do estudo.

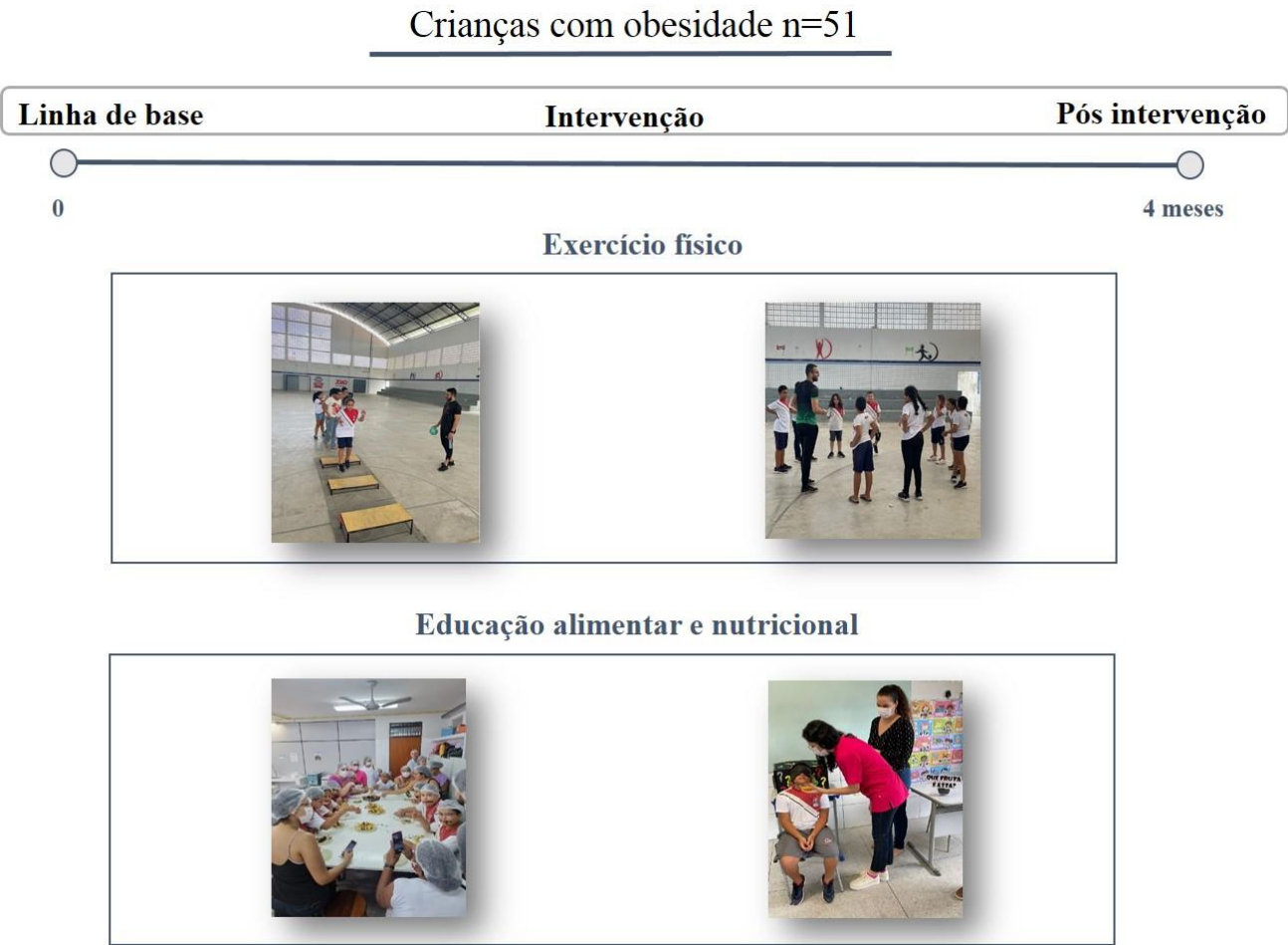
Segundo dados da Secretaria de Educação e Cultura do Município de João Pessoa, em 2020 havia 23.861 alunos regularmente matriculados em 97 escolas públicas de ensino fundamental do município de João Pessoa, distribuídos nas quatro regiões geográficas do município (norte, sul, leste e oeste). Para o cálculo do tamanho da amostra, foram considerados os seguintes parâmetros: tamanho da população de referência igual a 23.861 crianças do 1º ao 5º ano do ensino fundamental da rede pública, prevalência do desfecho obesidade igual a 13,3% (Arruda-Neta *et al.*, 2021), intervalo de confiança de 95% e poder do teste de 80%. Com base nesses parâmetros, o tamanho mínimo da amostra original foi estabelecido em 76 crianças, número que foi acrescido de 20% para compensar perdas e

recusas, totalizando um número amostral mínimo estimado em, no mínimo, 91 crianças para as análises transversais. Para o estudo longitudinal, apenas com crianças com obesidade, a amostra foi composta pelo número máximo (n=51) de crianças que quiseram participar da intervenção.

3.2.2 Intervenção com exercício físico e educação alimentar e nutricional

A intervenção foi composta por um protocolo de exercício físico e um programa de EAN. A intervenção teve duração de 4 meses e foi realizada com as crianças com obesidade (n=51) (Figura 6).

Figura 6 – Desenho experimental do protocolo de intervenção realizado com as crianças



Fonte: Autor, 2025

3.2.3.1 Exercício físico

Antes do início da intervenção, um profissional da educação física realizou um treinamento com toda a equipe para posterior execução do protocolo. O exercício físico foi caracterizado majoritariamente como exercício pliométrico dentro de um circuito de exercícios funcionais. O circuito teve duração média por sessão de 30 minutos e os outros 10 minutos compreenderam atividades lúdicas e de alongamento para diversificar as atividades e melhorar a adesão das crianças ao protocolo estabelecido. Além disso, a FC média dos indivíduos durante uma sessão de exercícios na fase de execução foi de 146 bpm, caracterizando-o como exercício de moderada a alta intensidade.

O exercício físico foi realizado semanalmente em dois dias não consecutivos (terças e quintas ou quartas e sextas-feiras), durante 4 meses em aproximadamente 28 sessões. Os grupos foram formados por no máximo 15 crianças e a quantidade de séries e repetições do componente pliométrico do circuito seguiu um aumento gradativo ao longo das semanas de treino, respeitando o princípio da sobrecarga (Almeida et al., 2021). Como descrito nas tabelas 2 e 3 e ilustrado na figura 7.

Tabela 2 - Distribuição do programa de exercício pliométrico segundo o tipo de salto

Tipo de salto	Séries	Repetições	Altura da plataforma (cm)
1 - Laterais	1	10	10
2 – Salto grupado	2	5	10
3 - Crescente	4	5	10, 20, 30, 30 e 40
4 - Decrescente	4	5	40, 30, 30, 20 e 10
5 - Monopedal	4	5	Sem plataforma
6 – Monopedal	4	5	Sem plataforma
7 – Crescente + grupado	2	5	10, 20, 30 e 40

Fonte: Autor, adaptado de Almeida *et al.*, 2021.

Descrição de cada exercício: **1** - Impulsão lateral com os dois pés, alternando o solo e a plataforma de 10 cm; **2** - Salto com os pés juntos após queda da plataforma de 10 cm; **3** - Saltos alternados entre o solo e as plataformas com alturas crescentes; **4** - Saltos alternados entre o solo e as plataformas com alturas decrescentes; **5** - Repetições de saltos máximos com o toque no solo com apenas o pé direito; **6** - Repetições de saltos máximos com o toque no solo com apenas o pé esquerdo; **7** - Saltos crescentes de 10, 20, 30 e 40 cm, seguidos de um salto grupado após a queda da plataforma de 40 cm.

Tabela 3. Descrição do programa de exercício pliométrico segundo a progressão de treino

Sessões	Séries	Repetições	Total de saltos
1 ^a – 4 ^a	5	5	25
5 ^a – 8 ^a	8	5	40
9 ^a – 12 ^a	10	5	50
13 ^a – 16 ^a	13	5	65
17 ^a – 20 ^a	17	5	85
21 ^a – 24 ^a	20	5	100
24 ^a – 28 ^a	22	5	110

Fonte: Autor, adaptado de Almeida *et al.*, 2021.

Descrição de alguns termos: **Sessões:** Número de dias em que ocorriam os treinos; **Séries:** Número de pausas entre os exercícios realizados durante o treino; **Repetição:** Número de saltos realizados em cada série; **Total de saltos:** Número total de saltos executados em cada dia de treino.

Figura 7 - Demonstração do exercício físico realizado em escolares com obesidade

Fonte: Autor, 2025

Além disso, as crianças foram orientadas a realizar todos os saltos no esforço máximo (altura e/ou distância máxima) e tempo mínimo de contato no solo, que justifica a pliometria. O solo foi forrado com colchões de poliuretano na espessura de 10 milímetros visando ao amortecimento do choque entre os saltos, e buscando prevenir algum tipo de lesão. Além do componente pliométrico, o circuito foi montado com auxílio de cones, círculos, bolas e outros materiais e foi executado entre cada série da pliometria.

3.2.3.2 Educação alimentar e nutricional (EAN)

O programa de EAN (Figura 8) foi conduzido durante um período de 4 meses paralelamente às atividades de exercício físico e foi planejado utilizando os princípios do Marco de Referência de EAN (BRASIL, 2012) e do Guia Alimentar para a População Brasileira (BRASIL, 2014). Para incentivar a presença das crianças nas atividades, foram entregues estrelas que totalizaram 10 pontos ao final da intervenção àqueles que compareceram em todas as sessões. Em cada encontro foi implementada uma atividade de EAN com um tema específico, entrega de material didático/lúdico e realizada discussão pertinente a fim de estimular o desenvolvimento crítico das crianças e/ou dos responsáveis sobre os hábitos alimentares, estimulando-os e incentivando-os a adotar ou permanecer com hábitos alimentares considerados saudáveis.

Figura 8 - Demonstração de uma atividade de educação alimentar e nutricional realizada com escolares com obesidade



Fonte: Autor, 2025

Foram realizadas 12 atividades, das quais 9 ocorreram de forma presencial, 1 atividade foi direcionada para os pais em casa (atividade 10), 1 atividade sobre os benefícios da educação física e redução do tempo de uso de telas foi realizada durante o treinamento físico (atividade 11) e por último, e a culminância consistiu de um passeio com as crianças na UFPB para execução de atividades lúdicas nos laboratórios e confecção de espetinhos de frutas no Laboratório de Dietética (atividade 12). Neste programa, foram trabalhados temas diversos, que estão descritos na tabela 4.

Tabela 4 - Descrição das atividades de educação nutricional realizadas com escolares com obesidade

Sessão	Nome da atividade
1	Que fruta é essa? (Jogo dos sentidos)
2	Semáforo dos alimentos
3	Alimentos <i>in natura</i> , processados e ultraprocessados + teor de açúcar, sal e óleo
4	Higiene dos alimentos + oficina de sucos
5	<i>My plate</i> : alimentação saudável

6	Cinema educativo – Sid, o Cientista • Uma Alimentação Saudável!
7	Piquenique com alimentos aproveitados integralmente + caça palavras
8	Horta portátil
9	De olho na lupa
10	Muito Além da Obesidade
11	Incentivo à atividade física e redução do uso de telas
12	Laboratório de Sabores

Fonte: Autor, 2025.

3.2.3 Análise antropométrica e da composição corporal

O peso corporal foi aferido com auxílio de uma balança eletrônica (Omron®, HBF-514C) e a estatura de um estadiômetro (alturaexata®). O estado nutricional, usando o IMC/I e sexo de acordo com os padrões da OMS, foi determinado usando *Anthro plus* (versão 1.0.4; OMS). As crianças foram classificadas em peso normal: ≥ -2 escore $z \leq +1$, sobrepeso: $>+1$ escore $z \leq +2$ e obesidade escore $z > +2$ (De Onis *et al.*, 2007).

Para medir a circunferência da cintura, foi utilizada uma fita métrica de aço flexível com escala de 0 a 200 cm e precisão de 0,1 mm (Sanny®). As dobras cutâneas foram medidas três vezes sempre no lado direito do corpo, utilizando um adipômetro científico com precisão de 0,1 mm (Sanny®). Os valores médios foram calculados e posteriormente utilizados para estimar o percentual de gordura corporal (Slaughter *et al.*, 1988).

3.2.4 Amostras de sangue e análise bioquímica e de citocinas

Amostras de sangue foram coletadas dos participantes após jejum de 12 horas e sem exercício extenuante nas últimas 24 horas por um profissional de enfermagem. Foi recomendado que os pais ou responsáveis estivessem com as crianças no dia da coleta para minimização do risco de eventos adversos durante a coleta. Foram utilizados tubos específicos para a separação do soro para análises posteriores. Além disso, a coleta ocorreu na escola em uma sala adaptada e com todos os materiais necessários como preconizam as normas de biossegurança. Ainda, o sangue foi armazenado em recipientes acondicionados e

imediatamente transportado para o Laboratório de Fisiologia, do Departamento de Fisiologia e Patologia da UFPB, onde foi processado e armazenado a uma temperatura de -80°C.

Os níveis séricos de alanina transaminase (ALT), aspartato aminotransferase (AST), gama-glutamil transferase (GGT), colesterol total (CT), colesterol de lipoproteína de baixa densidade (LDL-c), colesterol de lipoproteína de alta densidade (HDL-c), triglicerídeos (TG), glicemia de jejum, hemoglobina glicada (HbA1c), creatinina, ureia e proteína c-reativa de alta sensibilidade (hs-CRP) foram realizados usando um analisador automatizado (Lab-Max 240, Labtest, Lagoa Santa, MG, Brasil) e kits padronizados de acordo com as instruções do fabricante (Labtest, Lagoa Santa, MG, Brasil). A resistência à insulina foi avaliada usando a calculadora de avaliação do modelo de homeostase da resistência à insulina (HOMA-IR) fornecida por Oxford.

Amostras de soro foram utilizadas para dosagem de citocinas pela técnica Cytometric Bead Array (CBA). Os kits Th1/Th2/Th17 CBA da Becton Dickinson Biosciences foram usados para quantificar as citocinas IL-2, IL-4, IL-6, IL-10, IL-17^a, IFN- γ e TNF- α . O Becton Dickinson (BD)TM CBA usa uma série de partículas (microesferas ou grânulos) com diferentes intensidades de fluorescência para detectar simultaneamente citocinas solúveis através de uma superfície de captura. Cada grânulo é conjugado a um anticorpo específico e as citocinas são detectadas através do fluorocromo ficoeritrina. A intensidade de fluorescência de cada complexo revela a concentração de citocinas (pg/mL). Um citômetro de fluxo Accuri C6 BD[®] foi usado e a análise dos dados CBA foi realizada usando o software FCAP 1.0.1 (Neco *et al.*, 2017).

3.2.5. Variáveis cardiovasculares

Todos os indivíduos foram instruídos a não praticar atividade física intensa por no mínimo 24 horas antes dos exames. Além disso, os participantes também foram orientados a não consumir bebidas com cafeína ou qualquer bebida estimulante 36 horas antes do registro da pressão arterial e da aferição da VFC.

3.2.5.1. Mensuração da pressão arterial

A pressão arterial foi medida por um esfigmomanômetro digital (Omron Healthcare® HBP-1100) validado para uso em crianças de acordo com a Sociedade Brasileira de Cardiologia. Após um período inicial de estabilização (5 min), três medidas consecutivas foram feitas para cada criança com intervalos de 1 min e os valores médios foram usados na análise.

3.2.5.2 Análise da variabilidade da frequência cardíaca

Todos os testes foram realizados com o participante em repouso e na posição supina. As gravações foram realizadas pela manhã em uma sala silenciosa. Um registro de curta duração foi realizado por 10 minutos com as crianças em silêncio e respirando normalmente em volume corrente. Uma cinta elástica de tamanho ajustável foi posicionada confortavelmente ao redor do peito do participante e um dispositivo de transmissão (Polar modelo H10, Polar Electro, Finlândia; sistema de transmissão de 5 kHz codificado com Owncodes, Polar Electro) foi anexado na frente ao nível do processo xifóide. Por meio de transmissão sem fio, um smartphone (Iphone12) foi utilizado para registrar os intervalos RR em um APP (Elite HRV LLC, Asheville, NC, EUA, e Release 4.0.2, 2018). Em seguida, os dados foram exportados para um software (Kubius HRV) para o tratamento e obtenção dos resultados referentes a esta análise. A análise foi realizada com correção forte de batimentos ectópicos e foi considerada de boa qualidade quando a correção desses batimentos foi menor do que 5%.

Além disso, estudos anteriores demonstraram que o aplicativo de smartphone e a cinta torácica (polar) fornecem excelente complacência do eletrocardiograma para todas as variáveis no domínio do tempo, domínio da frequência e índices não lineares analisadas neste trabalho (Pereira *et al.*, 2020; Gamelin *et al.*, 2008).

3.2.6 Avaliação do teste respiratório de hidrogênio

Antes do teste respiratório, tanto as crianças quanto seus pais receberam instruções específicas. Eles foram solicitados a jejuar por 12 h, evitando leite ou laticínios, alimentos ricos em fibras, bebidas açucaradas, refrigerantes e sucos industriais por 24 h antes do teste.

Além disso, eles foram instruídos a não usar probióticos por 30 dias antes do teste. No dia do teste, as crianças receberam um kit de higiene oral contendo escova de dentes, pasta de dentes e fio dental para garantir a boa higiene oral. Durante o teste, eles sopraram lentamente em um dispositivo padronizado para estabelecer o nível basal de hidrogênio na respiração. Em seguida, eles ingeriram uma solução padronizada de glicose (composta de 50 g de glicose e 250 mg de aroma de morango em 200 mL de água), e amostras de respiração foram coletadas antes e depois de 15, 30, 60, 90 e 120 min após a ingestão de glicose (Lira *et al.*, 2020). O teste de hidrogênio na respiração foi realizado usando o EasyH2-P00401 (Dynamed, São Paulo, Brasil). Amostras de respiração foram imediatamente analisadas para H₂ usando o software EasyH2 versão 2.0 e a positividade foi definida como um aumento em H₂ de ≥ 20 em relação à linha de base (Rodriguez *et al.*, 2019). Apesar das limitações do teste de H₂, ele continua sendo uma ferramenta útil no diagnóstico de SIBO pediátrico devido à sua natureza prática e não invasiva (Rodriguez *et al.*, 2019).

3.2.7 Análise metabolômica

O soro foi analisado por metabolômica direcionada usando o kit MxP® Quant 500 (Biocrates Life Sciences AG, Innsbruck, Áustria). A análise metabolômica de todas as amostras foi realizada simultaneamente em um único kit, com execuções de padrões de controle interno espaçadas a cada 6 a 7 amostras biológicas. O kit MxP® Quant 500 permitiu a identificação dos metabólitos relacionados a aminoácidos, classes de lipídios e outras 14 classes de metabólitos. A preparação da amostra (usando 2 x 10 µL de soro), análises de instrumentos, medidas e verificações de controle de qualidade e quantificação de metabólitos foram realizadas de acordo com as instruções do fabricante. A análise foi realizada usando um sistema Waters ACQUITY PREMIER LC acoplado a um espectrômetro de massa triplo quadrupolo Waters XEVO-TQXS equipado com uma fonte de ionização por eletrospray (ESI) (Waters, Saint-Quentin-en-Yvelines, França). A separação cromatográfica dos metabólitos foi obtida usando uma coluna fornecida pela Biocrates. Os diferentes parâmetros de separação e detecção (fase móvel, gradiente de eluição, temperatura da coluna, fluxo, parâmetros de fonte) foram definidos pela Biocrates.

3.2.8 Análise da microbiota intestinal

O DNA genômico total das fezes foi extraído diretamente das amostras usando extração em coluna de sílica. O DNA foi eluído em água livre de DNase e RNase e diluído em concentrações de 1:10 e 1:100 (resultando em três diluições usadas para macerações subsequentes - original, 1:10 e 1:100). O DNA total extraído das amostras de fezes foi usado como um modelo para análise metataxonômica 16S conduzida pela Neopropecta Microbiome Technologies (Florianópolis, SC, Brasil) usando a plataforma Illumina NextSeq (Illumina, Inc, San Diego, CA, EUA). A preparação da biblioteca foi realizada usando o Protocolo Neopropecta NGS (Christoff *et al.*, 2017). A região hipervariável V3-V4 do gene 16S RNA foi amplificada com os primers 341F (CCTACGGGGRSGCAGCAG) e 806R (GGACTACHVGGGTWTCTAAT) (Caporaso *et al.*, 2012; Wang; Qian, 2009). As bibliotecas foram quantificadas usando o sistema fluorométrico Qubit e por qPCR a partir de curvas padrão usando o kit Colibri Library Quantification (Invitrogen, EUA). Os pools foram visualizados em um gel de agarose a 3%, bem como as amostras que passaram por qPCR para verificar os tamanhos dos fragmentos e garantir que nenhum dímero ocorreu. As amostras então prosseguiram para o sequenciamento usando o kit NextSeq P1 600 ciclos com 2 reações de extremidade pareada de 305 pb (Aguiar *et al.*, 2024).

3.2.9 Análise estatística

Quando necessário, a normalidade dos dados foi avaliada pelo teste de *Kolmogorov-Smirnov*. As estatísticas descritivas foram apresentadas como média (desvio padrão) ou mediana (mín-máx). A comparação da maioria das variáveis pré e pós intervenção foi realizada através do teste *T student* pareado ou seu equivalente não paramétrico. As correlações entre as variáveis foram analisadas por meio do teste de correlação de *Pearson* ou *Spearman* de acordo com a distribuição dos dados. A análise de metabolômica foi realizada no site MetaboAnalyst, considerando o pareamento dos dados. A análise de microbiota intestinal foi realizada no software R versão 4.4.2. A análise estatística foi realizada no *GraphPad Prism®* (versão 8) e houve significância estatística quando $p < 0.05$, e para as análises de metabolômica e microbiota intestinal os valores foram também apresentados corrigidos pela equação de Benjamini-Hochburg (FDR).

3.3. PROTOCOLO COM ANIMAIS

3.3.1. Delineamento do estudo, animais e aspectos éticos

Após finalização do protocolo com humanos, iniciamos o estudo com ratos *Wistar* com obesidade. A intervenção foi composta por exercício físico com duração de 6 semanas, após indução de obesidade via dieta rica em gordura (HFD) com adição de bebida açucarada (BA). Nesta pesquisa, analisamos variáveis antropométricas, pressão arterial, função autonômica, expressão gênica e microbiota intestinal.

Foram utilizados 30 ratos *Wistar* (*Rattus norvegicus*) provenientes da colônia do Biotério José Paulino, localizado no Centro de Ciências da Saúde da Universidade Federal de Pernambuco, aclimatizados por uma semana para posterior ensaio biológico no Laboratório de Nutrição Experimental, localizado no Departamento de Nutrição/ Centro de Ciências da Saúde da Universidade Federal da Paraíba.

O cálculo do tamanho amostral foi realizado com base no documento “Guia Brasileiro de Produção, Manutenção ou Utilização de Animais em Atividades de Ensino ou Pesquisa Científica” elaborado pelo Ministério da Ciência, Tecnologia e Inovação, publicado no ano de 2023 (BRASIL, 2023).

Conforme o documento supracitado e as características da pesquisa, para calcular o tamanho amostral foi realizada a equação:

$$E = N - T$$

Onde:

E: Tamanho da amostra;

N: Número total de sujeitos (grupos de animais);

T: Número de combinações de tratamentos.

Levando em conta que o presente projeto de pesquisa pretende utilizar três grupos experimentais, contendo três tratamentos distintos e 5 animais por grupo:

$$E = (5 \times 5) - 3 \quad E = 22$$

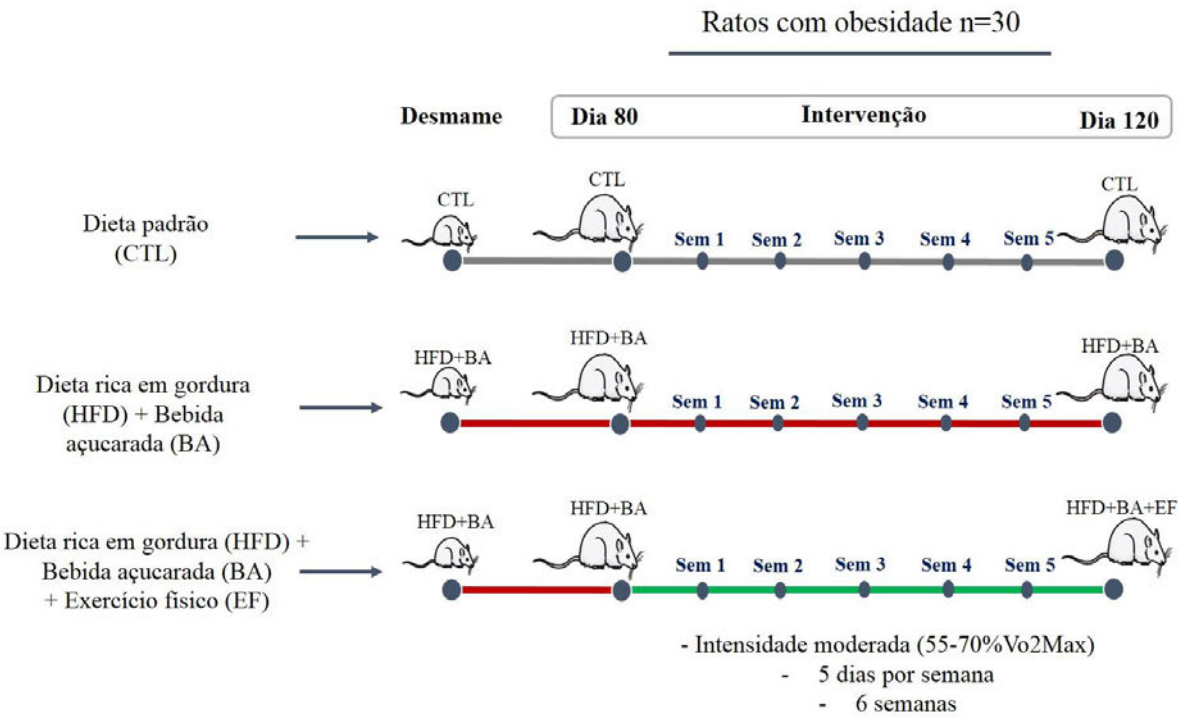
Por fim, esta pesquisa teve duas etapas de investigação e para a realização de cada etapa foram necessários um total de 15 animais (5 por grupo), totalizando 30 animais para todo o experimento.

Todos os ratos utilizados no experimento receberam água filtrada e dieta *ad libitum* e foram mantidos em gaiolas de polipropileno sob temperatura controlada ($21 \pm 1^{\circ}\text{C}$), com umidade entre 50–55% e ciclo claro-escuro de 12 horas. Ainda, todos os procedimentos experimentais foram submetidos e aprovados pela Comissão de Ética no Uso de Animais da Universidade Federal da Paraíba (CEUA/UFPB) número 4724190723 e seguiram as orientações do Conselho Nacional para o Controle de Experimentação Animal (CONCEA) e os Princípios Internacionais para Pesquisas Biomédicas Envolvendo Animais.

3.3.2. Randomização

Os ratos foram divididos aleatoriamente em três grupos: os que receberam dieta controle (CTL: n=10), os que receberam apenas HFD (HFD: n=10), os que receberam HFD e foram submetidos ao exercício físico durante o período de intervenção (HFD+EF: n=10) (Figura 9).

Figura 9 – Desenho experimental do protocolo utilizado com ratos com obesidade



Fonte: Autor, 2025. Sem: semanas; Vo2Max: volume de oxigênio máximo.

3.3.3. Dietas

A dieta controle (CTL) foi preparada de acordo com o American Institute of Nutrition – AIN-93M e a dieta hiperlipídica (HFD) foi elaborada no LANUCAM, da Universidade Federal da Paraíba (UFPB). A composição nutricional das dietas CTL e HFD que foram utilizadas está descrita na tabela 5.

Tabela 5 - Composição nutricional dietética (g/100g)

Nutrientes	Dieta controle	Dieta hiperlipídica
Amido	39,7	28
Amido dextrinizado	13,2	12
Caseína	20	16
Sacarose	10	10
Óleo de Soja	7	2
Gordura animal	0	15
Gordura vegetal não hidrolisada	0	9
Fibra	5	3
Mix mineral	3,5	3,5
Mix vitamina	1	1
L-cistina	0,3	0,3
Colina	0,25	0,25

*A caseína possui cerca de 85% de pureza (sendo 85g de caseína realmente, em cada 100g).

3.3.4. Exercício físico

O exercício físico de intensidade moderada (55 a 70% do VO₂máx) foi realizado em esteira motorizada 5 dias por semana durante 6 semanas. Nos três primeiros dias, houve a aclimação dos ratos com o exercício com duração de 10 minutos. Em seguida, os ratos progrediram gradativamente até uma velocidade de 15,0 m/s, sustentada por 60 minutos, conforme descrito em estudos anteriores e adaptado para o objetivo deste trabalho (Da Silva *et al.*, 2017; Mostarda *et al.*, 2012; Rodrigues *et al.*, 2007). O programa de exercício em detalhes pode ser encontrado na Tabela 6.

Tabela 6 – Programa de exercício físico aeróbio para os animais de acordo com a progressão do tempo de intervenção

	Semana 1	Semana 2	Semana 3	Semana 4	Semana 5	Semana 6
Segunda	15 min to 5 m/s	35 min to 13 m/s	45 min to 13 m/s	50 min to 14 m/s	55 min to 14 m/s	60 min to 15 m/s
Terça	20 min to 6 m/s	35 min to 13 m/s	45 min to 13 m/s	50 min to 14 m/s	55 min to 14 m/s	60 min to 15 m/s
Quarta	25 min to 7 m/s	35 min to 13 m/s	45 min to 13 m/s	50 min to 14 m/s	55 min to 14 m/s	60 min to 15 m/s
Quinta	27.5 min to 8 m/s	35 min to 13 m/s	45 min to 13 m/s	50 min to 14 m/s	55 min to 14 m/s	60 min to 15 m/s
Sexta	30 min to 9 m/s	35 min to 13 m/s	45 min to 13 m/s	50 min to 14 m/s	55 min to 14 m/s	60 min to 15 m/s

3.3.5. Mensuração do peso e consumo dietético

A pesagem dos animais foi realizada três vezes na semana durante todo o experimento segundo escala adequada (Modelo AS-1000; Marte, Santa Rita, Minas Gerais). E o consumo de ração também foi avaliado três vezes na semana por meio da pesagem da oferta dietética e do referente rejeito.

3.3.6. Determinação de parâmetros cardiovasculares

Os ratos foram anestesiados com cloridrato de cetamina (80 mg/kg, i.p.) e xilazina (10 mg/kg, i.p.) para inserção de cateteres de polietileno na artéria e veia femoral (canulação). Os cateteres foram tunelizados pela região dorsal e, em seguida, o cetoprofeno (5 mg/kg) foi administrado por via subcutânea (Cavalcante *et al.*, 2019). A PA e a FC foram registradas em animais conscientes 24 h após a cirurgia. Os registros de PA e FC foram feitos por meio da cânula arterial conectada a um transdutor de pressão (ML866/Pro, AD Instruments, Power Lab, Bella Vista, NSW, Austrália), conforme descrito anteriormente (De Brito Alves *et al.*, 2014). A PA pulsátil (PAP) e a FC foram registradas por 50-60 min em condições basais, e os valores de PA sistólica (PAS), PA diastólica (PAD), PA média (PAM) e FC foram calculados para uma seleção de 10 min para cada animal (LabChart™ Pro, ADInstruments, Bella Vista, NSW, Austrália) (De Brito Alves *et al.*, 2014).

3.3.7 Avaliação da função autonômica

Após 60 min de registros basais de PA e FC, drogas vasoativas foram administradas para avaliar a função autonômica dos animais. Para avaliar a função parassimpática, metilatropina (2 mg/kg iv; Sigma-Aldrich, St Louis, MO, EUA), um antagonista do receptor muscarínico, foi administrada para promover alterações na FC basal ($\Delta FC = FC$ pós-metilatropina e FC pré-metilatropina) (Cavalcante *et al.*, 2019). Após 15 min, cloridrato de propranolol (4 mg/kg, iv; Sigma-Aldrich, St. Louis, MO, EUA) foi administrado para avaliar o tônus simpático conforme avaliado por alterações na FC após a administração da droga ($\Delta FC = FC$ pós-propranolol - FC pré-propranolol) (Cavalcante *et al.*, 2019).

3.3.8 Extração de RNA e PCR quantitativo em tempo real (RT-qPCR)

O RNA total do cólon, tronco cerebral e coração foi extraído usando TRI-Reagent (Sigma Aldrich, ref #T9424), de acordo com as instruções do fabricante. Os cDNAs de primeira fita foram sintetizados a partir de 1 µg de RNAs totais usando TAKARA Prime Script™ RT Reagent (TAKARA Bio, ref #RR037A). A pureza e a concentração dos RNAs foram determinadas usando NanodropOne (Ozyme, Saint-Cyr-L'Ecole, França), e a qualidade foi verificada usando um Bioanalyser (Agilent Technologies, Santa Clara, Estados Unidos). Os ensaios de PCR em tempo real foram realizados com Rotor Gene Q (Qiagen, Hilden, Alemanha) usando SYBR® Premix Ex-Taq™ (TAKARA Bio, ref #RR420L). A proteína de ligação TATA-box (TBP) foi usada como gene de referência para normalizar os resultados, conforme relatado anteriormente (Naville *et al.*, 2019). Os primers utilizados estão listados na Tabela 7.

Tabela 7. Lista de primers utilizados

Gene	Forward	Reverse
TBP	TGGTGTGCACAGGAGCCAAG	TTCACATCACAGCTCCCCAC
Caspase 1	ATTCAGGCATGCCGTGGAGA	AATGTCCTCCAAGTCACAAG
MCP1	CTGGGCCTGTTGTTTCACAGTTG	GCCGACTCATTGGGATCATCTTG
Cldn1	TCGACTCCTTGCTGAATCTG	TGCTGTGGCCACTAATGTCTG
Ffar3	CAACACTCTGCATCTGTGAC	CAGCTTGTTTCACGAAGACCA

Gpx2	GCTTCCCTTGCAACCAGTTC	CATAAGGGTAGGGCAGCTTG
IL-1 β	CTCTCCACCTCAATGGACAG	TTGGGATCCACACTCTCCAG
Muc2	ATTACCCCCACAGTGGACAA	GGGATGTCCACCACAAAGTT
NLRP3	GAAAAGCCTGAGCTGACTGT	CTTTGCTCCAGACCCTACAC
NTRK2	GTGGTGATTGCCTCTGTGG	TTGGAGATGTGGTGGAGAGG
Ocln	CCACCTATCACTTCAGATCAAC	TCAGCAGCAGCCATGTACTC
Sod2	TCATGCAGCTGCACCACAGC	CCATTGAACTTCAGTGCAGG
Tjp1 (Zo1)	ACTGCCAGCTTTAAGCCTCC	AGGTTTCTGAGGCTCTGGG
TLR2	ATTGGGTGGAGAACCTCATGG	ATGGCCGCGTCGTTGTTCTC
TLR4	CTGGCTGGGACTCTGATCAT	GAAGCTCAGATCTAGGTTCT
TNF- α	CCAGACCCTCACACTCAGATC	CTTGGTGGTTTGCTACGACG
RGS6	GCACAGATTGACAGACATTG	CCCATAAGGTGATGTCATCG
Calca	CCTCCAGGCAGTTCCTTTGA	TCCAGCTCCCTGACTTTTCAT
Fos	GGCAAAGTAGAGCAGCTATC	TGGCAATCTCGGTCTGCAAC
Nos1	ATGTCCTCAAAGCCATCCAG	TGAAGGCGATGGACTCAGAT
VIP	CTCTGGGCAGAGCTGAAGAT	AGCCTTTCACGAGCTAAAGA
IL-18	CTGTGACCCTATCTGTGAAG	TGTCCTGGCACACGTTTCTG
RAMP1	AAGACTCTGTGGTGTGACTG	GTGGTGGACAGCAATGAAGA

TBP: TATA-box binding protein; MCP1: monocyte chemotactic protein 1; Cldn1: claudin-1; Ffar3: free fatty acid receptor 3; Gpx2: glutathione peroxidase 2; IL-1 β : Interleukin 1 β ; Muc2: Mucin 2; NLRP3: NLR family pyrin domain containing 3; NTRK2: Neurotrophic Receptor Tyrosine Kinase 2; Ocln: Occludin; Sod2: superoxide dismutase 2; Tjp1: tight junction protein 1; TLR2: toll like receptor 2; TLR4: toll like receptor 4; TNF- α : tumour necrosis factor α ; RGS6: regulator of G protein signaling 6; Calca: calcitonin related polypeptide alpha; Fos: fos proto-oncogene; Nos1: nitric oxide synthase 1; VIP: vasoactive intestinal peptide; IL-18: interleukin 18; RAMP1: receptor Activity Modifying Protein 1.

3.3.9 Análise da microbiota intestinal

A análise da microbiota intestinal no final do experimento foi realizada por sequenciamento do rRNA 16S bacteriano em fezes pela plataforma genEPII (Lyon, França). O DNA purificado foi extraído de 15 mg de fezes com o kit de micropreparação de DNA Zymobiomics (Zymo Research, ref #D4301). 40 ng de DNA foram usados para a geração de bibliotecas visando as regiões V3-V4 (Quick-16S NGS Library Prep Kit, Ozyme) e então sequenciadas no padrão Illumina MiSeq v3. As sequências foram “desmultiplexadas” usando o software Bcl2fastq (v2.17.1.14) e então limpas com cutadapt (v1.9.1). Variantes de sequência de amplicon (ASVs) foram identificadas usando Qiime2 DADA2 com uma abordagem OTU de ruído zero. Finalmente, uma atribuição taxonômica foi feita com a base

Greengenes-13.8-nr99. As diversidades alfa e beta e demais análises foram calculadas no RStudio (v2021.09.1 build 372) usando as bibliotecas phyloseq (v1.34.0) e metagenomeseq (1.32.0).

3.3.10 Análise estatística

Quando necessário, a normalidade dos dados foi avaliada pelo teste de *Kolmogorov-Smirnov*, a homogeneidade de variâncias pelo teste de Levene e a independência de resíduos pelo teste de Durbin Watson. As estatísticas descritivas foram apresentadas como média (desvio padrão) ou mediana (mín-máx). A análise de microbiota intestinal foi realizada no software R versão 4.4.2 e no *GraphPad Prism*® (versão 8) e houve significância estatística quando $p < 0.05$, e para as análises de microbiota intestinal os valores foram também apresentados corrigidos pela equação de Benjamini-Hochburg (FDR).

4. RESULTADOS

Este trabalho gerou produtos importantes, como artigos científicos publicados e submetidos em revistas de elevado impacto científico e trabalhos apresentados em congressos de alta relevância na comunidade científica na área da pesquisa.

O primeiro artigo derivado da tese é intitulado “*Cardiac autonomic dysfunction is associated with low-grade inflammation in children with obesity living in the northeast Brazilian region*” foi publicado no periódico **Jornal de Pediatria** (Qualis A3; Fator de impacto: 2.8). Nesse artigo, foi observado que as crianças com obesidade têm maior disfunção autonômica em comparação com as crianças com peso normal, fato observado pela menor VFC encontrada em crianças com obesidade nos domínios do tempo e frequência e também nos índices não lineares. Além disso, crianças com obesidade produziram menos IL-10 e mais IL-2, o que indica um prejuízo do sistema inflamatório nessa condição. Ainda, a inflamação crônica de baixo grau parece ser uma condição importante para esse distúrbio cardiovascular, uma vez que houve associação entre algumas das variáveis analisadas.

Após entender que as crianças com obesidade que estávamos trabalhando tinham disfunção autonômica, tive a ideia de que poderíamos estabelecer pontos de corte de frequência cardíaca que poderiam ser usados na atenção básica do sistema único de saúde para a triagem de risco de disautonomia a partir da utilização de equipamentos de baixo custo, como um relógio ou um frequencímetro. Neste sentido, publicamos o segundo artigo derivado da tese na **Revista Paulista de Pediatria** (Qualis B1; Fator de impacto: 1.4) intitulado “*Analysis of heart rate as a predictor of changes in heart rate variability in children*”, que estabeleceu diferentes pontos de corte de levando em consideração o sexo e estado nutricional das crianças.

No terceiro e último artigo proveniente de análises transversais, buscamos entender se de acordo com o SIBO havia diferenças em relação ao consumo de ultraprocessados e inflamação. No artigo que tem como título “*Assessment of Biochemical, Inflammatory Biomarkers and Ultra-Processed Food Consumption in Children with Small Intestinal Bacterial Overgrowth: A Cross-Sectional Study*”, publicado na **Nutrients** (Qualis A1; Fator de impacto: 4.8), demonstramos não só o percentual de 13% de SIBO em crianças de João Pessoa, mas também a relação do consumo de ultraprocessados com o SIBO, provavelmente por intermédio da inflamação.

Antes de iniciarmos a intervenção, buscamos na literatura o apoio necessário para realizar a melhor estratégia possível, consultando e analisando boas referências e observando as lacunas. Esta análise nos permitiu construir uma revisão sistemática visando entender se existia um tipo de exercício mais eficaz para crianças com disfunção autonômica e assim, utilizar a melhor opção no protocolo de exercício. Sendo assim, podemos inferir de acordo com o quarto artigo derivado da tese, ainda em revisão por pares na revista **Sport Sciences for Health** (Qualis B1; Fator de impacto: 1.3), que todos os exercícios são benéficos para melhorar disfunção autonômica em crianças com obesidade, mas ainda não é possível afirmar se existe um tipo mais eficaz.

O quinto artigo derivado da tese está submetido à revisão por pares na revista **The Journal of Clinical Endocrinology and Metabolism** (Qualis A1; Fator de impacto: 5.1) e contém os resultados obtidos com a intervenção realizada nas crianças com obesidade, tendo como título “*Multicomponent intervention combining physical exercise and food and nutritional education modulate gut microbiome and improve cardiac autonomic function in childhood obesity*”. Neste estudo, observamos, principalmente, que a intervenção modulou positivamente o microbioma intestinal e melhorou a inflamação e função autonômica dessas crianças, além da composição corporal. Desta maneira, os resultados nos deixaram curiosos para entender melhor sobre os mecanismos biomoleculares que dão suporte aos resultados fisiológicos encontrados.

Sendo assim, realizamos um protocolo animal com exercício físico que foi pensado principalmente para elucidar alguns dos mecanismos envolvidos nos resultados encontrados no estudo com humanos. Desta pesquisa, estamos em processo final de construção do sexto artigo derivado da tese, que será submetido para revisão por pares na revista **Microbiome** (Qualis A1; Fator de impacto: 13.8) e tem como título “*Aerobic exercise modulates gut microbiota and improves inflammation and autonomic function in rats with obesity*”. Nele, observamos que o exercício físico melhorou a função autonômica e pressão arterial, reduziu genes relacionados com a atividade simpática no tronco cerebral, aumentou a expressão de genes relacionados com a atividade parassimpática no cólon, reduziu a expressão de genes relacionados com inflamação no cólon e tronco cerebral e melhorou a microbiota intestinal e a expressão de genes de barreira intestinal, provavelmente pela maior perfusão sanguínea e redução da inflamação.

Além disso, três resumos com dados preliminares dos artigos citados acima foram apresentados no XX Congresso Brasileiro de Obesidade e Síndrome Metabólica. Eles são intitulados: *Evaluation of heart rate as a predictor of changes in heart rate variability indices*; *Alterations in heart rate variability and inflammatory biomarkers in children with obesity*; *Plyometric training and nutritional education improve anthropometric variables, blood pressure, and heart rate variability in children with obesity* (Apêndice 7). Além disso, outro resumo foi apresentado no I Brazilian Conference on Nutrition, Gut Microbiome, Health and Disease, intitulado *Physical training improves cardiac autonomic function: role of gut microbiota modulation and inflammation in childhood obesity in a translational study*. Esta apresentação oral me rendeu o prêmio de segundo melhor trabalho deste congresso (Apêndice 8).

Por fim, desenvolvi sob orientação do Prof. Dr. José Luiz de Brito Alves cooperações com laboratórios de diferentes centros de pesquisas. Essas cooperações resultaram em produtos na forma de artigos que foram publicados, majoritariamente, em revistas classificadas nos quatros estratos superiores de acordo com a classificação *Qualis CAPES* 2017-2020. São elas a **Pharmacological Research** (A1 – 1 artigo publicado), **Critical Reviews in Food Sciences and Nutrition** (A1 – 2 artigos publicados), **Nutrients** (A1 - 3 artigos publicados), **International Journal of Environmental Research and Public Health** (A1 – 1 artigo), **Nutrition, Metabolism and Cardiovascular Diseases** (A2 - 1 artigo), **Foods** (A2 - 3 artigos publicados), **International Journal of Mental Health and Food Addiction** (A2 – 1 artigo publicado), **Food Bioscience** (A2 – 1 artigo publicado), **Journal of Developmental Origins of Health and Disease** (A3 – 1 artigo publicado), **World Journal of Diabetes** (A4 – 1 artigo publicado) e **Journal of the Endocrine Society** (B1 – 1 artigo) (Apêndices 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 e 24). Outros produtos foram gerados e publicados na forma de capítulos de livro, no livro *Probiotics for Human Nutrition in Health and Disease*, de circulação internacional (Apêndice 25).

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APÊNDICES

APÊNDICE 1 - ARTIGO 1 DERIVADO DA TESE

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ORIGINAL ARTICLE

Evaluation of cardiac autonomic function and low-grade inflammation in children with obesity living in the Northeast Brazilian region



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Abstract

Objective: Evaluate autonomic function and low-grade inflammation and characterize the correlation between these variables in schoolchildren with obesity living in the Brazilian northeast region.

Methods: 84 children with obesity and 41 with normal weight were included in this cross-sectional study. Anthropometry, body composition, blood pressure (BP), inflammatory biomarkers, and heart rate variability (HRV) indexes were analyzed in children aged 7 to 11 years.

Results: children with obesity had increased systolic ($p = 0.0017$) and diastolic ($p = 0.0131$) BP and heart rate ($p = 0.0022$). The children with obesity displayed significantly lower SDNN, RMSSD, NN50, HF (ms), HF (nu), SD1, SD2, and higher LF (ms), LF (nu), LF/HF, SD1/SD2, DFA- α 1, and DFA- α 2, compared to normal weight. A lower and higher capacity for producing IL-10 ($p = 0.039$) and IL-2 ($p = 0.009$), respectively, were found in children with obesity compared to children with normal weight. Although IL-2, IL-4 and IL17A did not correlate with HRV parameters, IL-6 was positively correlated with SDNN, LF (ms) and SD2, TNF- α was positively correlated with LF/HF and SD1/SD2 ratio, and IFN- γ was positively correlated with SDNN, RMSSD, NN50, LF (ms), HF (ms), SD1, and SD2.

Conclusions: The findings suggest that children with obesity have impaired autonomic function and systemic low-grade inflammation compared to children within the normal weight range, the

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inflammatory biomarkers were correlated with HRV parameters in schoolchildren living in the northeastern region of Brazil.

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Introduction

Childhood obesity is a global health issue associated with infancy and lifelong adverse health consequences, including an increased risk of suffering or developing cardio-metabolic diseases.¹ In Brazil, the prevalence of childhood obesity in the last three decades was 8.2%, increasing with age, decade, and in more developed regions.²

Traditional factors, such as insulin resistance, hypertension, and reduced high-density lipoprotein cholesterol (HDL-C) are linked to cardiovascular disease. However, the detection of the early stages of cardiac autonomic dysfunction and related causes could be a useful strategy to identify the presence of cardiovascular risk in childhood obesity, and heart rate variability (HRV) could be used as a warning sign of cardiac diseases.³

Autonomic nervous system (ANS) control of the heart is a dynamic process modulated by parasympathetic and sympathetic innervation.⁴ RR-variation or HRV obtained from an electrocardiogram, Holter monitor or chest strap has been a commonly used and validated test to assess cardiac autonomic function through time, frequency, and non-linear domains indices that quantify parasympathetic (RMSSD, NN50, HF, and SD1), and sympathetic (LF, LF/HF and SD2) modulation.⁵

Although an early study carried out with German children has not found significant differences in HRV parameters between children with normal weight and obesity,⁶ cardiac autonomic dysfunction and reduced HRV has been reported in children with obesity.^{7,8} Concomitantly, chronic low-grade inflammation and obesity have been reported in children.⁹ In this condition, excess adipose tissue dysregulates the immune system, affecting the balance and levels of cytokines and increasing the levels of pro-inflammatory interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF α), and C-reactive protein (CRP).^{10,11}

Regarding the association of autonomic impairment with chronic low-grade inflammation in children with obesity, The authors found only one cross-sectional study with a reduced sample size demonstrating that impaired HRV and low-grade chronic inflammation coexist in childhood obesity in the United States of America, but no significant relationships were found between any of the measured HRV variables and cytokines levels.⁹ Considering that inflammation and autonomic function may be modulated by the environment and lifestyle and may have ethnic-specific implications, and the scarcity of studies linking inflammation and autonomic function in children, this study aimed to evaluate autonomic function and low-grade inflammation and to characterize the correlation between these variables in schoolchildren with obesity living in the Northeastern region of Brazil. The hypotheses tested are that children with obesity have lower HRV and higher low-grade inflammation compared to normal-weight children and that there is a positive association between autonomic dysfunction and chronic low-grade inflammation in children living in the Northeast region of Brazil.

Methods

Ethical aspects

This cross-sectional study was conducted in agreement with the declaration of Helsinki. The study was approved by a Human Research Ethics Committee (Health Sciences Center, Federal University of Paraíba – João Pessoa – PB – Brazil; Reference number protocol n° 4.676.103) and all procedures were conducted in agreement with Resolution 466/2012 of the National Health Council. All parents received information about the study and gave written informed consent before data collection.

Participants and study design

According to data from the Department of Education and Culture of the Municipality of João Pessoa (SEDEC), in 2020 there were 23.861 students regularly enrolled in 97 primary public schools in the municipality of João Pessoa (PB, Brazil), distributed in the four geographic regions of the municipality (north, south, east and west). To calculate the sample size, the following parameters were considered: the size of the reference population equal to 23.861 children from the 1st to the 5th year of primary public schools, the prevalence of obesity outcome equal to 13.3%,¹² 95% confidence interval, test power of 80%, and design effect (deff) equal to 1. Based on these parameters, the minimum size of the original sample was established at 76 children, a number that was plus 20% to compensate for losses and refusals, resulting in a final sample of 125 children.

Children between 7 and 11 years of age, of both sexes, enrolled in public primary schools were included in the study, and recruitment and data collection were conducted in schools. Children were excluded if they presented any of the following: physical inability to perform the anthropometric measures, psychological or behavioral disturbances, use of any medication, or having a disease compromising any analysis.

In the study, 125 children were allocated to two groups: 84 children in the obesity (OB) group and 41 in the normal weight (NW) group. Children were evaluated for anthropometry, body composition, and cardiovascular parameters, evaluating blood pressure, HRV, and cytokines levels. All collections were performed at the school.

Anthropometry and body composition

The body weight was measured with an electronic scale (Omron®, HBF-514C) and height was assessed using a stadiometer (alturaexata®). Nutritional status, using body mass index for age (BMI/A) and sex according to WHO standards was determined using Anthro plus (version 1.0.4; WHO)

Children were classified in normal weight: ≥ -2 z-score $\leq +1$, overweight: $> +1$ z-score $\leq +2$, and obesity z-score $> +2$.

To measure the waist circumference, a flexible steel measuring tape with a scale of 0–200 cm and precision of 0.1 mm (Sanny®) was used. Skinfolts were measured three times always on the right side of the body, using a scientific adipometer with a precision of 0.1 mm (Sanny®). The average values were calculated and subsequently used to estimate the body fat percentage.

Blood pressure measurement

Blood pressure was measured with a digital sphygmomanometer (Omron healthcare® HBP-1100) validated for use in children by the Brazilian Society of Cardiology. After an initial stabilization period (5 min), three consecutive measurements were taken for each child at 1 min intervals, and the average values were used in the analysis. The measurement was performed with the child in a seated position and with an adequate cuff. In addition, the arm was free and positioned at heart level, resting on a surface and with the palm facing up.

Heart rate variability analysis

It was recommended that children do not engage in vigorous physical activity or drink caffeinated beverages for at least 24 h prior to HRV analysis. Recordings were made in the morning in a quiet room. A short-term recording was performed with the children at rest, in the supine position, and breathing normally at tidal volume. HRV analysis was performed in 10 min record, according to previous studies.^{13–15} A smartphone (Iphone12) was used to record the RR intervals on an APP (Elite HRV LLC, Asheville, NC, USA, and Release 4.0.2, 2018). An elastic belt of adjustable size was positioned comfortably around the participant's chest and a transmission device (Polar model H10, Polar Electro, Finland; 5 kHz transmission system coded with Owncodes, Polar Electro) was attached on the front at the level of the xiphoid process. The raw data recorded was exported as a text file and analyzed by Kubios HRV Standard software (version 3.2.0; Biosignal Analysis and Medical Image Group, Department of Physics, University of Kuopio, Kuopio, Finland). The interpolation of the series was performed by cubic spline, and frequency values of 4 Hz were set. The R-wave time instants were automatically detected by applying a built-in QRS detection algorithm of Kubios software and when ectopic beats exceeded 5% of the total analyzed, the measurement was repeated. Early studies have demonstrated that smartphone apps and chest strap (Polar) provide excellent electrocardiogram compliance for all variables in the time domain, frequency domain, and nonlinear indexes.^{16,17} The following measures of HRV analysis were determined; 1) time-domain parameters: the standard deviation between the duration of NN intervals (SDNN), the square root of the mean of the sum of the squares of the successive differences between adjacent normal-to-normal beats (RMSSD), adjacent NN with differences in duration greater than 50 (NN50) low-frequency band (LF), high-frequency band (HF), standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph (SD1), standard deviation

of long-term continuous NN intervals (SD2) and detrended fluctuation analysis (DFA).

Blood samples and cytokines measurements

Blood samples were collected by a qualified nurse after 12 h fasting and without strenuous exercise in the last 24 h. Serum samples were used for the measurement of cytokines through the Cytometric Bead Array (CBA) technique. Th1/Th2/Th17 CBA kits from Becton Dickinson Biosciences were used to quantify the cytokines IL-2, IL-4, IL-6, IL-10, IL-17a, IFN- γ , and TNF- α . The Becton Dickinson (BD)™ CBA uses a series of particles (microspheres or beads) with different fluorescence intensities to simultaneously detect soluble cytokines through a capture surface. Each bead is conjugated to a specific antibody and cytokines are detected by the fluorochrome phycoerythrin. The fluorescence intensity of each complex reveals the cytokine concentration (pg/mL). An Accuri C6 BD® flow cytometer was used, and analysis of CBA data was performed using FCAP 1.0.1 software.

Statistical analysis

The data normality was assessed by the Kolmogorov-Smirnov test. Descriptive statistics are presented as mean (standard deviation) or median (min-max). Comparison intergroup (NW and OB) was performed using Student's t-test in the case of the parametric data or the Mann-Whitney test for non-parametric data. The relationships between variables were analyzed using Pearson or Spearman correlation test according to data distribution. The statistical analysis was performed using GraphPad Prism® (version 6.01) and the significance was maintained at $p < 0.05$. An a posteriori power analysis was performed to assess differences between children with obesity and normal weight. Power was calculated using a two-sided 95% confidence interval, a prevalence of 63.51% of children with obesity having a heart rate above the mean, a prevalence of 35.48% of children with normal weight having a heart rate above the mean, a prevalence ratio of 1.8; and a prevalence difference of 28.03. The statistical power was performed using OpenEpi software (version 3.01) and the value obtained was 75.75%. Lastly, the effect size between-groups differences were calculated using Cohen's d test and classified as small ($d \geq 0.20$ and < 0.50), medium ($d \geq 0.50$ and < 0.80), and large ($d \geq 0.80$).

Results

A total of 1.957 children were evaluated and classified according to BMI, with 1.009 males and 948 females. Of the total number of children assessed, 79 (4.04%) were classified as underweight, 1.249 (63.82%) as normal weight, 328 (16.76%) as overweight, and 301 (15.38%) as obese. One hundred and twenty-five of these children were included in this study, 41 with NW and 81 with obesity.

The anthropometric and cardiovascular characteristics of children included in the study are presented in Table 1. The groups were similar in terms of sex ($p = 0.702$) and age ($p = 0.205$). The obesity group had higher BMI/A ($p < 0.0001$), waist-hip ratio (WHR) ($p < 0.0001$), body fat (BF) ($p < 0.0001$), SBP ($p = 0.0017$), DBP ($p = 0.0131$), and

Table 1 Anthropometric and cardiovascular characteristics of children with normal weight (NW) and obesity (OB).

Variables	NW (n = 41)	OB (n = 84)	p-value	Effect size Cohen's d
Male – n (%)	17 (41.5)	39 (46.4)	0.702	-
Female – n (%)	24 (58.5)	45 (53.6)		
Age (years)	9.6 (1.2)	9.2 (1.3)	0.205	–0.31
BMI/A (z-score) †	–0.18 (–1.53–1.00)	2.70 (2.01–5.66)	< 0.000	4.13
WHR	0.82 (0.04)	0.88 (0.06)	< 0.000	1.10
BF (%) †	21.30 (13.70–34.50)	36.60 (22.75–68.20)	< 0.000	2.30
SBP (mmHg)	103.0 (10.44)	110.7 (13.05)	0.001	0.62
DBP (mmHg) †	58.50 (43.00–85.00)	62.50 (40.00–94.00)	0.013	0.50
HR (bpm)	87.0 (65.0–111.00)	95.0 (63.00–121.00)	0.002	0.67

Values are expressed as n (%), mean (SD) or median (min-max), and applied chi-square, T test or † Mann–Whitney, respectively.

BMI, Body mass index for age; WHR, waist hip ratio; BF, body fat; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HR, heart rate.

heart rate (HR) ($p = 0.0022$) when compared to the NW group, with the medium effect size for SBP, DBP and HR.

As reported in Table 2, children with obesity had increased IL-2 levels ($p = 0.009$) and decreased IL-10 levels ($p = 0.039$) when compared to the NW group (Table 2), with a medium effect size for the IL-2 and small effect size for the IL-10. Serum levels of IL-4, IL-6, IL-17A, TNF- α and IFN- γ were similar between groups (Table 2, $p > 0.05$).

Regarding the HRV analysis, children with obesity had a predominance of sympathetic ANS in the detriment of parasympathetic. In the time domain, children with obesity had decreased SDNN ($p = 0.002$), RMSSD ($p = 0.001$), and NN50 ($p = 0.008$) compared to the NW children, with a larger effect size for SDNN and RMSSD (Table 3). In time-frequency, children with obesity had increased LFnu band ($p = 0.001$) and LF/HF ratio ($p = 0.003$) and decreased HFnu band ($p = 0.004$) compared to NW children, with the medium effect size for the three variables (Table 3). In non-linear indices, children with obesity had decreased SD1 ($p < 0.000$) and SD2 ($p = 0.004$), and increased SD2/SD1 ratio ($p = 0.001$), DFA- α 1 ($p = 0.002$), and DFA- α 2 when compared to NW children, with medium effect size for SD2/SD1 and DFA- α 1 and larger effect size for DFA- α 2 (Table 3).

Correlations between HRV and inflammatory variables are shown in Table 4. Although IL-2, IL-4, and IL17A did not correlate with HRV parameters, IL-6 showed a positive

correlation with SDNN, LF, and SD2, and IL-10 with LF (ms). TNF- α was positively associated with LF/HF ratio and SD1/SD2 ratio. IFN- γ showed a positive correlation with SDNN, RMSSD, NN50, LF, HF, SD1, and SD2.

Discussion

This study identified that children with obesity had higher levels of SBP, DBP, HR, and higher serum levels of IL-2 and a lower serum level of IL-10 than NW children. The children with obesity also had autonomic dysfunction, as observed in linear and non-linear indices. The authors also found a positive correlation between HRV indices and inflammatory biomarkers, suggesting that autonomic and inflammatory dysfunction may occur simultaneously in children.

Some important variables related to obesity and cardiovascular disease were also different in these children, such as the increase in SBP, DBP, and HR. There is strong evidence of greater sympathetic activation in obesity, including changes in resting BP and HR, which is consistent with the results found in this study in children with obesity. It is important to highlight that, despite the existing differences according to nutritional status, the values of BP and HR were within the normal limits proposed by the Brazilian Guidelines of Hypertension and III Guidelines of the Brazilian

Table 2 Comparison of the values of cytokines between children with normal weight (NW) and obesity (OB).

Variables	NW (n = 41)	OB (n = 84)	p-value	Effect size Cohen's d
IL-2 (pg/mL) †	7.15 (0.78–13.95)	8.34 (0.50–39.59)	0.009	0.63
IL-4 (pg/mL) †	3.41 (0.43–11.90)	3.73 (0.33–28.92)	0.734	0.17
IL-6 (pg/mL) †	10.14 (0.05–80.32)	9.82 (0.21–95.43)	0.794	0.16
IL-10 (pg/mL) †	5.22 (0.82–14.94)	2.70 (0.17–14.80)	0.039	–0.41
IL-17A (pg/mL) †	52.69 (2.32–111.6)	50.12 (2.97–157.70)	0.808	0.02
TNF- α (pg/mL) †	4.14 (0.15–10.64)	4.62 (0.11–29.31)	0.591	0.15
IFN- γ (pg/mL) †	9.74 (1.59–15.38)	7.40 (0.23–34.18)	0.101	–0.08

Values are expressed as median (min-max). Mann-Whitney test.

IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; IL-10, interleukin-10; IL-17A, interleukin-17A; TNF- α , tumor necrosis factor- α ; IFN- γ , interferon- γ .

Table 3 Assessment of heart rate variability in children with normal weight (NW) and obesity (OB).

Variables	NW (n = 41)	OB (n = 84)	p-value	Effect size Cohen's d
Time domain				
SDNN (ms) †	47.9 (20.8–119.8)	37.9 (12.7–84.9)	0.002	–0.82
RMSSD (ms) †	42.5 (15.3–140.6)	30.1 (7.2–92.5)	0.001	–0.89
NN50 (beats) †	151.0 (4.0–459.0)	76 (0.0–357.0)	0.008	–0.69
Frequency domain				
LF (ms ²) †	1000 (58.0–4435)	650.0 (100.0–3555)	0.0053	–0.67
HF (ms ²) †	942.0 (107.0–7470)	383.0 (18.0–2833)	0.0005	–0.89
LF (nu)	50.9 (15.7)	60.53 (13.8)	0.001	0.67
HF (nu)	47.6 (13.6)	39.4 (13.8)	0.004	–0.59
LF/HF †	1.105 (0.417–7.762)	1.509 (0.295–5.908)	0.003	0.68
Non-linear measurements				
SD1 (ms) †	30.1 (10.8–99.5)	21.3 (5.1–65.5)	< 0.000	–0.90
SD2 (ms) †	57.2 (25.5–137.3)	48.3 (17.3–100.2)	0.004	–0.77
SD2/SD1 (ms) †	1.77 (1.03–3.55)	2.15 (1.22–3.57)	0.001	0.61
DFA- α 1	1.02 (0.21)	1.15 (0.20)	0.002	0.65
DFA- α 2	0.45 (0.14)	0.57 (0.13)	< 0.000	0.90

Values are expressed as mean (SD) or median (min-max); T test or † Mann–Whitney, respectively.

SDNN, standard deviation between NN intervals; RMSSD, square root of the mean of the square of the differences between consecutive NN intervals; NN50, adjacent NN with differences in duration greater than 50; LF, low frequency; HF, high frequency; SD1, standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph; SD2, standard deviation of long-term continuous NN intervals; DFA, detrended fluctuation analysis; ms: milliseconds; nu: normalized units.

Society of Cardiology on Analysis and Issue of Electrocardiographic Reports, respectively.^{18–20}

Inflammation in obesity occurs due to functional changes in adipose tissue, resulting in the secretion of pro-inflammatory cytokines produced by activated macrophages residing in adipose tissue, and decreased secretion of protective adipokines, such as adiponectin.²¹ The mechanisms underlying this dysfunction occur in response to the structural changes in adipose tissue and may involve endoplasmic reticulum stress, hypoxia, and cellular senescence.²¹ Chronic low-grade inflammation results from a persistent failure to organize the inflammatory response. The interaction of

macrophages and monocytes and other inflammatory mediators has been studied in the pathogenesis of obesity.²²

This study was the first to evaluate a broad cytokine profile in children and showed that although IL-4, IL-6, IL17A, TNF- α and IFN- γ were similar between groups, children with obesity had increased serum levels of IL-2 and decreased serum levels of IL-10 compared to NW children, suggesting that children with obesity may have a loss of ability to regulate the monocyte-mediated immune response to produce cytokines.²³ Physical inactivity can, among other things, lead to obesity, impair immune system function, and increase the risk of low-grade chronic inflammation. A

Table 4 Linear correlation coefficients between heart rate variability measures and inflammatory variables.

Variables	IL-2	IL-4	IL-6	IL-10	IL-17A	TNF- α	IFN- γ
SDNN (ms) †	0.161	0.071	0.246*	0.206	0.033	0.134	0.401*
RMSSD (ms) †	0.148	0.086	0.161	0.125	–0.044	0.015	0.299*
NN50 (ms) †	0.116	0.118	0.115	0.071	–0.043	0.010	0.229*
LF (ms) †	0.0856	0.0853	0.2924*	0.2317*	0.1037	0.1957	0.4713*
HF (ms) †	0.1147	0.0600	0.1798	0.1528	–0.0111	0.0061	0.3163*
LF (nu)	–0.012	0.024	0.088	0.048	0.155	0.166	0.113
HF (nu)	0.006	–0.026	–0.089	–0.041	–0.149	–0.167	–0.106
LF/HF †	–0.023	–0.010	0.034	0.023	0.115	0.239*	0.024
SD1 (ms) †	0.134	0.083	0.161	0.136	–0.041	0.010	0.308*
SD2 (ms) †	0.151	0.062	0.256*	0.217	0.052	0.167	0.425*
SD1/SD2 (ms) †	–0.071	–0.034	0.029	0.019	0.173	0.219*	–0.024
DFA- α 1	–0.030	0.036	0.118	0.077	0.162	0.196	0.139
DFA- α 2	0.033	–0.037	–0.068	–0.093	0.010	–0.006	–0.177

SDNN, standard deviation between NN intervals; RMSSD, square root of the mean of the square of the differences between consecutive NN intervals; NN50, adjacent NN with differences in duration greater than 50; LF, low frequency; HF, high frequency; SD1, standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph; SD2, standard deviation of long-term continuous NN intervals; DFA, detrended fluctuation analysis; ms: milliseconds; nu: normalized units.

† = nonparametric data.

higher concentration of IL-2-producing cells and a lower capacity to produce IL-10 have previously been reported in sedentary children compared to active children.²⁴ It has been demonstrated that obesity alters immune function and that the imbalance between pro- and anti-inflammatory mediators in obesity may induce the establishment or progression of cardiovascular complications and dysautonomia.^{10,25}

HRV is a measure of the natural changes occurring between beats in a row in heart rate, which represents the number of heart beats per minute.²⁶ The HRV measurement must be carried out on normal cardiac cycles (N), without artifacts and ectopic beats. For this reason, The authors used the denomination N-N interval and not the R-R interval in this study.⁷ An optimal HRV level reflects adequate functioning, characterized by an inherent capacity for self-regulation, adaptability, or resilience. In contrast, a very small variation indicates age-related system depletion, chronic stress, pathology, or improper functioning at various levels of self-regulatory control systems.²⁶

HRV analysis using linear and non-linear methods has been used to better understand complex and dynamic heart rhythms. In 2010, cross-sectional studies conducted in Brazilian children with obesity aged between eight and 12 years showed a reduction in non-linear HRV measures and decreased LF and HF indices, suggesting a reduction in both sympathetic and parasympathetic activities.^{27,28} Another study conducted in Brazilian children showed increased LFnu band and LF/HF ratio in children with obesity when compared to children within the normal weight range but without changes in non-linear HRV measures.⁸

The present study showed that children with obesity had decreased HRV measures in the time domain, increased LFnu and LF/HF ratio in the frequency domain, and increased SD2/SD1 and DFA- α 1 and DFA- α 2 in non-linear measures. The results may indicate unresolved cardiac stress characterized by parasympathetic withdrawal and sympathetic hyperactivation in obese children.²⁹ Although it is not possible to determine the underlying causes involved in the fluctuations in HRV variables in children with obesity, it is reasonable to suggest that lifestyle changes over the last decade, particularly evidenced by the increase in consumption of ultra-processed and hypercaloric foods, associated with reduced physical activity, may have played a key role in altering HRV indices. This reaffirms the importance of the present study, which is essential to understand and identify the potential risk of autonomic dysfunction in children, as well as to propose effective intervention strategies to prevent cardiovascular disease later in life.

Regarding the contribution of hormonal, reflex, metabolic, endothelial, and inflammatory mechanisms in cardiovascular homeostasis mediated by the autonomic nervous system,¹⁸ the main mechanisms to explain alterations of the ANS in obesity are sympathetic overactivity, dysfunction of arterial baroreceptor properties, a key mechanism involved in the control of sympathetic and vagal tonus,¹⁸ and impairment of the respiratory-cardiovascular coupling, which may result in sympathetic activity and autonomic dysfunction.¹⁸

The positive correlations found between HVR indices in the time, frequency, and non-linear domains with biomarkers of inflammation are consistent with available

evidence regarding the association between inflammation and ANS activity.^{18,30} An early study found a positive association between HRV and pro-inflammatory markers, although a meta-analysis had shown that a negative correlation between HRV and pro-inflammatory markers is more common.³⁰ In this sense, an important point to highlight is the fact that vagal hyperactivity is positively correlated with first-response inflammatory mediators, such as TNF- α and IFN- γ , through the elimination of the parasympathetic nervous system via acetylcholine, which may partially explain the positive correlation of RMSSD, NN50, HF and SD1 with IFN- γ . On the other hand, the positive correlation of LF and SD2 with IL-6 indicates a slower action, characteristic of the sympathetic nervous system via epinephrine and norepinephrine. Furthermore, parameters that reflect total HRV (e.g., SDNN) are influenced by both mechanisms, which explains the positive correlation with IL-6 and IFN- γ .³⁰

This study contributes to a better understanding of dysautonomia and low-grade chronic inflammation in children, demonstrating the coexistence of these conditions in children with obesity from the Northeast region of Brazil. The positive correlation between HRV indices and inflammation provides valuable information and encourages mechanistic studies to better elucidate this issue. In addition, this study may help to establish reference values for HRV indices in children, which could be an essential screening tool for cardiovascular risk in health outpatient clinics.

This study has some limitations. First, the cross-sectional design allows only the study of associations between variables, not causal relationships. Second, HRV and inflammatory biomarkers may differ by sex, and stratification by sex could be examined in further studies; and third, a broad assessment of metabolic variables panel could help to better understand the cardiometabolic risk in children with obesity in a post-pandemic scenario. These limitations will be addressed in future studies.

Conclusion

The results of this study show several cardiovascular risk factors in obese children compared to children in the normal weight range. At such a young age, obesity is already associated with lower HRV and low-grade chronic inflammation, which reflects and reinforces the need to develop effective strategies to prevent and combat childhood obesity. Furthermore, the correlations between inflammatory markers and HRV indices underscore the importance of elucidating the mechanisms by which such interactions may occur.

Conflicts of interest

The authors declare no conflicts of interest.

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APÊNDICE 2 - ARTIGO 2 DERIVADO DA TESE

ORIGINAL ARTICLE

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Analysis of heart rate as a predictor of changes in heart rate variability in children

Análise da frequência cardíaca como preditor de alterações na variabilidade da frequência cardíaca em crianças

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Conflict of interests

The authors declare that there is no conflict of interests.

Author's contributions

Study design: Costa PCT, Arruda Neta ACP, Alves JLB. **Data collection:** Costa PCT. **Data analysis:** Costa PCT, Arruda Neta ACP, Alves JLB. **Manuscript writing:** Costa PCT, Arruda Neta ACP. **Manuscript revision:** Martins VJB, Alves JLB. **Study supervision:** Martins VJB, Alves JLB.

Declaration

The database that originated the article is available with the corresponding author.

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ABSTRACT

Objective: To evaluate the predictive validity and cut-off point of heart rate and blood pressure on heart rate variability (HRV) changes in children with and without obesity.

Methods: This study included 125 children, of whom 41 were normal weight and 84 were obese. Anthropometry, blood pressure, heart rate, and HRV were measured using an electronic scale and stadiometer, a sphygmomanometer, and HRV monitor. In addition, the receiver operating characteristic (ROC) curve was obtained by statistical analysis of the data.

Results: Heart rate proved to be a good predictor for changes in the square root of the mean of the square of the differences between consecutive NN intervals (RMSSD) parameter in students of both sexes for the normal-weight group (ROC 0.89; 95%CI 0.77-1.00) and obesity (ROC 0.90; 95%CI 0.83-0.97). In addition, the heart rate cut-off point for alterations in the RMSSD parameter for normal-weight boys was 93 bpm (sensitivity 100.00% and specificity 87.50%) and for boys with obesity, the established cut-off point was 91 bpm (sensitivity 94.74% and specificity 63.64%). Heart rate also proved to be a good predictor considering low-frequency/high-frequency ratio (LF/HF) and standard deviation of long-term continuous NN intervals /standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph ratio (SD2/SD1). Systolic and diastolic blood pressures were good predictors in more specific stratifications and, therefore, can be used in some cases.

Conclusions: The predictive validity of heart rate was shown to be at a good level, with high sensitivity and acceptable specificity for the cut-off points according to the different analyses stratified by gender and nutritional status. In this sense, health professionals will be able to use heart rate to estimate cardiovascular risk in children of different sexes and nutritional status.

Keywords: Heart rate; Autonomic nervous system; Obesity; Children; Prediction.

RESUMO

Objetivo: Avaliar a validade preditiva e o ponto de corte da frequência cardíaca e da pressão arterial nas alterações da variabilidade da frequência cardíaca (VFC) em crianças com e sem obesidade.

Métodos: Foram incluídas 125 crianças neste estudo, sendo 41 com peso normal e 84 com obesidade. Antropometria, pressão arterial, frequência cardíaca e VFC foram medidas por meio de balança eletrônica e estadiômetro, esfigmomanômetro e monitor de VFC. Além disso, a curva característica de operação do receptor (ROC) foi obtida pela análise estatística dos dados.

Resultados: A frequência cardíaca mostrou-se um bom preditor de alterações no parâmetro de raiz quadrada da média do quadrado das diferenças entre os intervalos NN consecutivos (RMSSD) em escolares de ambos os sexos para o grupo de peso normal (ROC 0,89; IC95% 0,77-1,00) e obesidade (ROC 0,90; IC95% 0,83-0,97). Além disso, o ponto de corte da frequência cardíaca para alterações no parâmetro RMSSD para meninos com peso normal foi de 93 bpm (sensibilidade 100,00% e especificidade 87,50%), e para meninos com obesidade, o ponto de corte estabelecido foi de 91 bpm (sensibilidade 94,74% e especificidade 63,64%). A frequência cardíaca também se mostrou um bom preditor considerando os índices da relação baixa frequência/alta frequência (LF/HF) e razão desvio padrão de intervalos NN contínuos de longo prazo/desvio padrão da variabilidade instantânea de intervalos NN contínuos na relação gráfica de Poincaré (SD2/SD1). As pressões arteriais sistólicas e diastólicas foram bons preditores em estratificações mais específicas e, portanto, podem ser utilizadas em alguns casos.

Conclusões: A validade preditiva da frequência cardíaca mostrou-se em bom nível, com alta sensibilidade e especificidade aceitável para os pontos de corte, conforme as diferentes análises estratificadas por sexo e estado nutricional. Desta forma, os profissionais de saúde poderão utilizar a frequência cardíaca para estimar o risco cardiovascular em crianças de diferentes sexos e estado nutricional.

Palavras-chave: Frequência cardíaca; Sistema nervoso autônomo; Obesidade; Crianças; Predição.

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Heart rate predicts changes in heart rate variability

INTRODUCTION

Childhood obesity currently affects 39 million children worldwide and its prevalence has increased at an alarming rate, with the highest incidence observed in economically disadvantaged countries such as Brazil.^{1,2} Obesity is considered a risk factor for the development of cardiovascular and metabolic diseases and, once established in childhood, tends to persist into adulthood.³⁻⁶

Children with overweight or obesity exhibit an increased risk of cardiovascular disorders early in life, as evidenced by sympathetic overactivity, vagal withdrawal, increased blood pressure (BP), and resting heart rate (HR).⁷⁻⁹ Heart Rate Variability (HRV) is a non-invasive method that quantifies the variation between consecutive heartbeats and is a commonly used and validated measure to assess cardiac autonomic function using time, frequency, and non-linear domain indices to quantify parasympathetic and sympathetic modulation.¹⁰

Increased HRV represents efficient autonomic activity. On the other hand, decreased HRV generally represents an abnormal and inadequate adaptation of the autonomic nervous system, which may indicate the presence of autonomic dysfunction.^{11,12} In children, reduced HRV has been associated with obesity^{13,14} and its complications¹⁵ proving to be a risk factor for cardiometabolic diseases and potentially early mortality.¹³ Therefore, regular monitoring of the HRV measurements may allow the identification of early physiological warning signs of cardiovascular disease.

Electrocardiogram Holter monitor, or chest strap is commonly used to check HRV.¹⁶⁻¹⁸ However, its use is not very accessible and has been mainly limited to scientific research. Furthermore, its application is not practical and requires data processing and analysis by specialists. In this sense, the use of HR or BP measurements can offer a convenient and low-cost screening method for regular monitoring of the autonomic nervous system health in children. However, the predictive validity of HR or BP for HRV measures has not been previously evaluated. Therefore, the aim of the present study was to evaluate the predictive validity and cut-off point of HR and BP in HRV changes in children with and without obesity. Using the predictive value or cut-off point for HR or BP, health professionals will be able to identify the potential risk of autonomic dysfunction in children.

METHOD

This cross-sectional study was based on data from the project "Physical training and nutrition education as strategies to

modulate the gut microbiota and metabolic, inflammatory, and cardiovascular parameters in schoolchildren with obesity: a cooperation for a multicenter study" (NUTRO Study). The main objective of NUTRO is to evaluate the effectiveness of a multicomponent intervention on cardiometabolic parameters in children with obesity.

The sample size was calculated using the following parameters: size of the reference population equal to 23,861 children from the 1st to the 5th year of Fundamental I (elementary school), prevalence of the obesity outcome equal to 13.30%,¹⁹ 95% confidence interval (CI), test power of 80.00%, and design effect (deff) equal to 1. Based on these parameters, the minimum size of the original sample was set at 76 children. Potential losses were compensated by increasing the sample size by 20.00%.

The sample was decided for convenience. Ten schools were selected from the four geographical regions of the community (North, South, East, and West). A nutritional screening of all children enrolled in the school was conducted to determine children for the study. To diagnose the nutritional status, weight and height measurements were taken and then the students were classified according to the World Health Organization (WHO) cut-off points.²⁰

The study included normal-weight and obese children, aged 7–11 years, of both sexes, and who were duly enrolled in the municipal education network of João Pessoa, Paraíba (PB), Brazil. However, children who had any motor or psychological disorders that made it impossible for them to perform the tests were excluded.

The body weight was measured with an electronic scale (Omron® HBF-514C) and height was assessed using a stadiometer (alturaexata®). Nutritional status, using body mass index for age (BMI/A) and sex according to WHO standards, was determined through Anthro Plus (version 1.0.4; WHO). Children were classified into normal weight (≥ -2 Z score $\leq +1$); overweight ($> +1$ Z score $\leq +2$); and obesity (Z score $> +2$).²⁰

BP and HR were measured with a digital sphygmomanometer (Omron healthcare® HBP-1100) validated for use with children according to the Brazilian Society of Cardiology. After an initial stabilization period (5 minutes), three consecutive measurements were taken for each child at 1-minute intervals, and the average values were used in the analysis.

All tests were conducted with the participant at rest and in the supine position. The recordings were performed in the morning in a quiet room. Short-term recording was completed in ten minutes with the children in a quiet state and breathing normally at tidal volume. Through wireless transmission,

a smartphone (iPhone12) was used to record the RR intervals on an application (Elite HRV LLC, Asheville, NC, USA, Release 4.0.2, 2018). An elastic belt of adjustable size was placed comfortably around the participant's chest and a transmitter (Polar model H10, Polar Electro, Finland) was attached to the front at the level of the xiphoid process. Previous studies have shown that the smartphone app and chest strap (Polar) provide excellent electrocardiogram compliance for all variables in the time domain, frequency domain, and nonlinear indices.^{18,21}

The predictive power of HR for changes in HRV was assessed using receiver operating characteristic (ROC) curves. First, the total area under the ROC curve between HR, systolic blood pressure (SBP), and diastolic blood pressure (DBP), and markers of changes in HRV were determined. The larger the area under the ROC curve, the greater the predictive power of HR for changes in HRV. Next, the cut-off points for HR, SBP, and DBP that obtained significant areas under the ROC curve were identified with their respective sensitivity and specificity values. The criterion for obtaining cut-off points for HR as a predictor of changes in HRV was considered as more balanced sensitivity and specificity.

In this study, HR, SBP, and DBP were considered good predictors when their CIs were greater than 0.50 and the area under the ROC curve was greater than 0.70. The CI determines whether the predictive ability of HR, SBP, and DBP is not due to chance and its lower limit must not be less than 0.50. A 95%CI was used in this study.

This cross-sectional study was conducted in agreement with the Declaration of Helsinki. The Human Research Ethics Committee of the Health Sciences Center, Federal University of Paraíba, João Pessoa (PB), Brazil, protocol nº 4.676.103, approved the study. All parents received information about the study and gave written informed consent before data collection.

RESULTS

A total of 1,957 children were evaluated and classified according to BMI, with 1,009 males and 948 females. Of the total number of children, 79 (4.04%) were classified as underweight, 1,249 (63.82%) as normal weight, 328 (16.76%) as overweight, and 301 (15.38%) as obesity. This study included 125 of these children, of which 41 were normal weight and 81 were obese.

HR proved to be a good predictor of changes in the square root of the mean of the square of the differences between consecutive NN intervals (RMSSD) parameter in children of both sexes for the normal weight group (ROC 0.89; 95%CI 0.77–1.00)

and obesity (ROC 0.90; 95%CI 0.83–0.97) (Table 1). HR was also a good predictor of changes in the RMSSD parameter in boys (ROC 0.97; 95%CI 0.88–1.00) and girls (ROC 0.84; 95%CI 0.62–1.00) from the normal-weight group, as well as in boys (ROC 0.91; 95%CI 0.80–1.00) and girls (ROC 0.90; 95%CI 0.79–1.00) from the obesity group (Table 1).

The HR cut-off point for changes in the RMSSD parameter was 93 bpm for normal-weight boys (sensitivity 100.00% and specificity 87.50%) and 91 bpm for boys with obesity (sensitivity 94.74% and specificity 63.64%). In girls, the established cut-off was 94 bpm for both normal weight (sensitivity 100.00% and specificity 70.00%) and obesity (sensitivity 85.00% and specificity 83.33%).

Regarding changes in the low-frequency/high-frequency ratio (LF/HF) parameters, HR proved to be a good predictor for the normal weight students (ROC 0.88; 95%CI 0.74–1.00) and obesity group (ROC 0.77; 95%CI 0.65–0.89), regardless of gender. When considering gender, HR proved to be a good predictor for both boys (ROC 1.00; 95%CI 1.00–1.00) and girls (ROC 0.77; 95%CI 0.50–1.00) for the normal weight group, as for boys (ROC 0.72; 95%CI 0.52–0.92) and girls (ROC 0.81; 95%CI 0.66–0.96) of the obesity group (Table 1).

The HR cut-off points for changes in LF/HF were 102 bpm (100.00% sensitivity and 100.00% specificity) for normal-weight boys and 91 bpm (85.00% sensitivity and 50.00% specificity) for obese boys. In girls, the established cut-off point was 94 bpm for girls with normal weight (sensitivity 85.71% and specificity 75.00%) and obesity (sensitivity 82.35% and specificity 66.67%) (Table 1).

As for alterations in the parameter standard deviation of long-term continuous NN intervals /standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph ratio (SD2/SD1), HR proved to be a good predictor in the group of normal-weight students (ROC 0.91; 95%CI 0.81–1.00) and in the group with obesity (ROC 0.85; 95%CI 0.75–0.94), regardless of gender. When considering gender, HR proved to be a good predictor for both boys (ROC 0.97; 95%CI 0.89–1.00) and girls (ROC 0.84; 95%CI 0.62–1.00) for the normal weight group, as for boys (ROC 0.87; 95%CI 0.71–1.00) and girls (ROC 0.87; 95%CI 0.74–0.99) of the obesity group (Table 1).

The HR cut-off points for changes in SD2/SD1 were 90 bpm (sensitivity 100.00% and specificity 85.71%) for boys with normal weight and 91 bpm (95.24% and specificity 77.78%) for boys with obesity. In girls, the established cut-off point was 90 bpm for girls with normal weight (sensitivity 100.00% and

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Heart rate predicts changes in heart rate variability

Table 1. The area under the receiver operating characteristic curve and 95% confidence interval, cut-off point, sensitivity, and specificity of heart rate as predictors of changes in parameters in heart rate variability indices in students from João Pessoa (PB), 2023.

Heart rate	ROC curve (95%CI)	Cut-off point (bpm)	Sensitivity (%)	Specificity (%)
Normal weight (girls+boys)				
RMSSD (ms)	0.89 (0.77–1.00)	93	100.00	77.78
LF/HF Ratio	0.88 (0.74–1.00)	94	90.91	87.50
SD2/SD1 Ratio	0.91 (0.81–1.00)	90	100.00	73.33
Normal weight (boys)				
RMSSD (ms)	0.97 (0.88–1.00)	93	100.00	87.50
LF/HF Ratio	1.00 (1.00–1.00)	102	100.00	100.00
SD2/SD1 Ratio	0.97 (0.89–1.00)	90	100.00	85.71
Normal weight (girls)				
RMSSD (ms)	0.84 (0.62–1.00)	94	100.00	70.00
LF/HF Ratio	0.77 (0.50–1.00)	94	85.71	75.00
SD2/SD1 Ratio	0.84 (0.62–1.00)	90	100.00	62.50
Obesity (girls+boys)				
RMSSD (ms)	0.90 (0.83–0.97)	93	84.62	78.26
LF/HF Ratio	0.77 (0.65–0.89)	94	75.68	68.00
SD2/SD1 Ratio	0.85 (0.75–0.94)	91	94.44	57.69
Obesity (boys)				
RMSSD (ms)	0.91 (0.80–1.00)	91	94.74	63.64
LF/HF Ratio	0.72 (0.52–0.92)	91	85.00	50.00
SD2/SD1 Ratio	0.87 (0.71–1.00)	91	95.24	77.78
Obesity (girls)				
RMSSD (ms)	0.90 (0.79–1.00)	94	85.00	83.33
LF/HF Ratio	0.81 (0.66–0.96)	94	82.35	66.67
SD2/SD1 Ratio	0.87 (0.74–0.99)	94	86.67	64.71

ROC: receiver operating characteristic; 95%CI: 95% confidence interval; area under the ROC curve that demonstrates discriminatory power for changes in heart rate variability parameters (lower limit of 95%CI > 0.50); RMSSD: square root of the mean of the square of the differences between consecutive NN intervals; LF/HF: low frequency/high frequency ratio; SD2/SD1: standard deviation of long-term continuous NN intervals/standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph ratio.

specificity 62.50%) and 94 bpm for obesity (sensitivity 86.67% and specificity 64.71%) (Table 1).

SBP proved to be a good predictor for changes in the RMSSD parameter in both sexes (ROC 0.81; 95%CI 0.64–0.97) and in boys (ROC 0.87; 95%CI 0.63–1.00), and for changes in the SD2/SD1 parameter in girls (ROC 0.77; 95%CI 0.51–1.00), in the group of normal-weight students. However, SBP did not prove to be a good predictor for the other parameters in the group of normal-weight students, as well as for any parameter in the group of students with obesity (Table 2). On the other hand, DBP proved to be a good predictor only for changes in the RMSSD parameter in normal-weight girls (ROC 0.81; 95%CI 0.56–1.00) (Table 3).

The SBP cut-off points for screening changes in the RMSSD parameter for both sexes was 105 mmHg (sensitivity 88.89% and specificity 66.67%) and for normal-weight boys, it was 114 mmHg (sensitivity 100% and specificity 87.50%). For the SD2/SD1 parameter in normal-weight girls, the SBP cut-off point as a screening tool was 106 mmHg (sensitivity 85.71% and specificity 75.00%). The DBP cut-off point for screening changes in the RMSSD parameter in normal-weight girls was 62 mmHg (sensitivity 100.00% and specificity 80.00%).

The results of general and by sex analysis of area under the ROC curve of HR to RMSSD and LF/HF indices in children are available in Figure 1.

Table 2. The area under the receiver operating characteristic curve and 95% confidence interval, cut-off point, sensitivity, and specificity of systolic blood pressure as predictors of changes in parameters in heart rate variability indices in students from João Pessoa (PB), 2023.

Systolic blood pressure	ROC curve (95%CI)	Cut-off point (mmHg)	Sensitivity (%)	Specificity (%)
Normal weight (girls+boys)				
RMSSD (ms)	0.81 (0.64–0.97)	105	88.89	66.67
LF/HF Ratio	0.60 (0.37–0.83)	104	81.81	50.00
SD2/SD1 Ratio	0.64 (0.43–0.86)	106	75.00	66.67
Normal weight (boys)				
RMSSD (ms)	0.87 (0.63–1.00)	114	100.00	87.50
LF/HF Ratio	0.64 (0.21–1.00)	114	75.00	75.00
SD2/SD1 Ratio	0.53 (0.13–0.93)	114	60.00	71.43
Normal weight (girls)				
RMSSD (ms)	0.70 (0.42–0.97)	104	100.00	50.00
LF/HF Ratio	0.57 (0.25–0.89)	104	85.71	50.00
SD2/SD1 Ratio	0.77 (0.51–1.00)	106	85.71	75.00
Obesity (girls+boys)				
RMSSD (ms)	0.53 (0.37–0.68)	106	76.92	43.48
LF/HF Ratio	0.57 (0.41–0.72)	106	75.68	44.00
SD2/SD1 Ratio	0.59 (0.44–0.74)	105	80.56	46.15
Obesity (boys)				
RMSSD (ms)	0.51 (0.26–0.75)	106	84.91	45.45
LF/HF Ratio	0.50 (0.24–0.75)	106	85.00	40.00
SD2/SD1 Ratio	0.59 (0.31–0.85)	106	85.71	44.44
Obesity (girls)				
RMSSD (ms)	0.54 (0.33–0.75)	106	65.00	50.00
LF/HF Ratio	0.60 (0.39–0.79)	106	64.71	53.33
SD2/SD1 Ratio	0.58 (0.37–0.78)	106	66.67	52.94

ROC: receiver operating characteristic; 95%CI: 95% confidence interval; area under the ROC curve that demonstrates discriminatory power for changes in heart rate variability parameters (lower limit of 95%CI >0.50); RMSSD: square root of the mean of the square of the differences between consecutive NN intervals; LF/HF: low frequency/high frequency ratio; SD2/SD1: standard deviation of long-term continuous NN intervals/standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph ratio.

DISCUSSION

The results of this study suggest that HR may be a feasible measure for predicting HRV changes and that established cut-off points may be useful in the clinical practice of pediatricians and health care professionals to identify children at risk for cardiac autonomic dysfunction. In addition, the findings showed that SBP and DBP can also be used as important screening parameters, but there are differences according to the stratifications. The use of HRV as a tool for estimating cardiovascular risk on a large scale has become unfeasible so far due to the need for more sophisticated equipment that is not yet available in basic public health units in Brazil. On the other hand, the use of HR as a cardiovascular risk marker and predictor of metabolic

dysfunction in children²² may be interesting given its relative practicality of measurement.

The established cut-off point determines the sensitivity and specificity of the analyzed tool, i.e., if the cut-off point is low, many patients with low cardiovascular risk could be identified as having cardiovascular risk, leading to low sensitivity and specificity. On the other hand, if the cut-off point is high, some of the patients with cardiovascular risk may be diagnosed as having low risk, characterizing the test as having low sensitivity and high specificity. Therefore, it is essential to select the cut-off point with high sensitivity and specificity to reduce the risk of false-positive and false-negative diagnoses.^{23,24}

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Table 3. The area under the receiver operating characteristic curve and 95% confidence interval, cut-off point, sensitivity and specificity of diastolic blood pressure as predictors of changes in parameters in heart rate variability indices in students from João Pessoa (PB), 2023.

Diastolic blood pressure	ROC curve (95%CI)	Cut-off point (mmHg)	Sensitivity (%)	Specificity (%)
Normal weight (girls+boys)				
RMSSD (ms)	0.61 (0.39–0.83)	62	88.89	61.11
LF/HF Ratio	0.50 (0.27–0.72)	62	63.64	50.00
SD2/SD1 Ratio	0.55 (0.32–0.79)	62	66.67	53.33
Normal weight (boys)				
RMSSD (ms)	0.34 (0.02–0.67)	59	100.00	12.50
LF/HF Ratio	0.25 (0.00–0.54)	59	100.00	12.50
SD2/SD1 Ratio	0.30 (0.00–0.62)	59	100.00	14.29
Normal weight (girls)				
RMSSD (ms)	0.81 (0.56–1.00)	62	100.00	80.00
LF/HF Ratio	0.71 (0.40–1.00)	58	85.71	50.00
SD2/SD1 Ratio	0.75 (0.47–1.00)	61	85.71	62.50
Obesity (girls+boys)				
RMSSD (ms)	0.52 (0.37–0.66)	56	82.05	17.39
LF/HF Ratio	0.56 (0.41–0.70)	56	81.08	16.00
SD2/SD1 Ratio	0.60 (0.45–0.74)	56	86.11	23.08
Obesity (boys)				
RMSSD (ms)	0.51 (0.28–0.74)	57	84.21	18.18
LF/HF Ratio	0.57 (0.35–0.79)	56	85.00	10.00
SD2/SD1 Ratio	0.58 (0.36–0.81)	56	90.48	22.22
Obesity (girls)				
RMSSD (ms)	0.51 (0.30–0.72)	59	75.00	25.00
LF/HF Ratio	0.54 (0.34–0.75)	59	76.47	20.00
SD2/SD1 Ratio	0.60 (0.39–0.80)	59	80.00	29.41

ROC: receiver operating characteristic; 95%CI: confidence interval; area under the ROC curve that demonstrates discriminatory power for changes in heart rate variability parameters (lower limit of 95%CI >0.50); RMSSD: square root of the mean of the square of the differences between consecutive NN intervals; LF/HF: low frequency/high frequency ratio; SD2/SD1: standard deviation of long-term continuous NN intervals/standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph ratio.

It is recognized that resting HR is associated with BP and metabolic dysfunctions in children and adolescents.^{22,25} To our better understanding, this is the first report demonstrating that HR and BP can be used to predict HRV change in children. Considering that HRV is a prediction of autonomic dysfunction²⁶ and cardiovascular events,²⁷ the findings of the present study suggest that normal ranges of HR established for children in systematic reviews of observational studies²⁸ should be interpreted with caution in clinical practice with regard to its effect on HRV.

Early studies evaluated other variables as predictors of cardiovascular risk in children and established cut-off points. Hand grip strength normalized for body mass was evaluated

as a cut-off point for cardiovascular risk in Chilean children. In this case, the authors established 0.33 and 0.40 as cut-off points for boys and girls, respectively.²⁹ Another study evaluated the serum concentrations of vitamin D and determined the cut-off points for cardiovascular risk in children. According to the authors, vitamin D had a good predictive capacity for cardiovascular risk, with the cut-off point for predicting this risk being 32 ng/ml.³⁰ The use of these different variables evaluated in clinical practice can provide a more robust diagnosis of cardiovascular risk for children.

The cross-sectional design does not provide support for causality statements. Therefore, prospective studies on children are needed to better characterize the relationship between

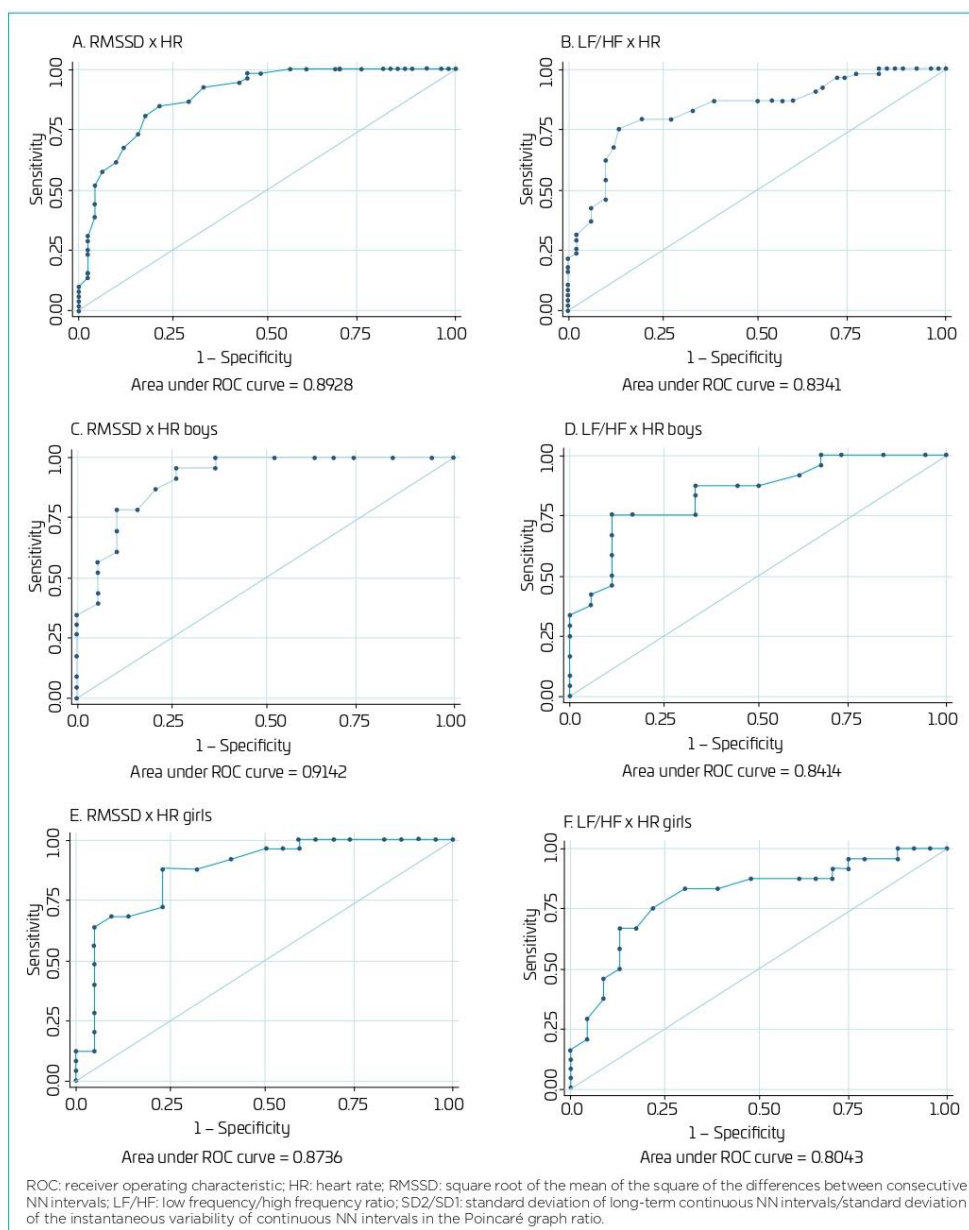


Figure 1. Receiver operating characteristic (ROC) curve for general and by sex analyses of the heart rate with RMSSD and LF/HF in children from João Pessoa (PB), 2023.

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HR and HRV, and the convenience sample is a limitation of the present study. In summary, we conclude that the predictive validity of HR was shown to be at a good level with high sensitivity and acceptable specificity for the cut-off points according to the different analyses stratified by gender and

nutritional status. Health professionals will be able to use the HR to estimate cardiovascular risk in children of different sexes and nutritional status. Furthermore, it is necessary to test the predictive validity of HR for cardiovascular risk in another population.

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APÊNDICE 3 - ARTIGO 3 DERIVADO DA TESE



Article

Assessment of Biochemical, Inflammatory Biomarkers and Ultra-Processed Food Consumption in Children with Small Intestinal Bacterial Overgrowth: A Cross-Sectional Study

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Abstract: Objective: This study evaluated anthropometric, biochemical, and inflammatory biomarkers, as well as dietary intake in Brazilian children diagnosed with small intestinal bacterial overgrowth (SIBO) and compared them with their counterparts without SIBO. Methods: This was a cross-sectional study with 106 children aged 7 to 10 years. A glucose-hydrogen breath test was performed to diagnose small intestinal bacterial overgrowth (SIBO). Anthropometric and dietary characteristics were assessed. Blood samples were collected and serum biochemical parameters and cytokines were measured. Results: The occurrence of SIBO was 13.2%. Age, BMI, BMI/age WC, BFP, sex and biochemical markers were similar between SIBO-positive and SIBO-negative children ($p > 0.05$). High consumption of ultra-processed foods tended to be higher in SIBO-positive compared to SIBO-negative children (47.8 ± 8.2 vs. 42.6 ± 9.5 , $p = 0.06$). Serum levels of IL-17 were higher in SIBO-positive than in SIBO-negative children [69.5 (5.4–125.7) vs. 53.4 (2.3–157.7), $p = 0.03$], while serum levels of IL-10 were lower in SIBO-positive than in SIBO-negative children [2.3 (0.6–7.2) vs. 5.7 (0.5–30.8), $p = 0.04$]. Finally, in a logistic regression adjusted for sex, BMI and age, consumption of ultra-processed foods ($p = 0.03$) and IL-6 levels ($p = 0.003$) were found to contribute to the occurrence of SIBO. Conclusion: this study identified for the first time an occurrence of 13% of SIBO in children living in the northeastern region of Brazil and showed that consumption of ultra-processed foods and serum levels of IL-6 may influence the occurrence of the SIBO in the pediatrics population.

Keywords: children; small intestinal bacterial overgrowth; inflammation; biochemical parameters; food consumption

1. Introduction

Impaired lifestyle has been associated with adverse health outcomes, impaired quality of life, and increased risk of gastrointestinal, metabolic, and inflammatory diseases in the pediatric population [1–3]. Hypercaloric diets, high consumption of ultra-processed foods, and low intake of fruits, natural vegetables, and fiber have been associated with obesity and related gastrointestinal, metabolic, and inflammatory diseases [4–6].

Early findings have shown that high consumption of ultra-processed foods may deleteriously affect the gut microbiota, disrupting intestinal homeostasis and promoting intestinal and systemic low-grade inflammation [7–9]. Excessive consumption of ultra-processed foods may be a risk factor for an overgrowth of certain bacteria and a decrease in beneficial bacteria in the gut [10].

Small intestinal bacterial overgrowth (SIBO) is a heterogeneous disorder characterized by excessive growth of selected microorganisms in the small intestine [11,12]. SIBO can disrupt host physiology and lead to gastrointestinal and non-gastrointestinal symptoms and complications [11,12]. The prevalence of SIBO in children has been studied in a wide range of clinical contexts and has been implicated as the cause of non-specific gastrointestinal symptoms such as chronic abdominal pain, bloating, diarrhea, and flatulence [11].

SIBO may be a risk factor for bacterial translocation, low-grade inflammation, and metabolic disorders in children [11]. However, the small number of studies has limited the understanding of the impact of SIBO on metabolic and inflammatory outcomes in children [13]. Therefore, this study evaluated biochemical and inflammatory biomarkers and dietary intake in children diagnosed with SIBO and compared them to their counterparts without SIBO.

2. Methods

2.1. Ethical Concerns

Following the Helsinki Declaration, this cross-sectional study was carried out. Approval was obtained from a Human Research Ethics Committee at the Federal University of Paraíba Health Sciences Center in João Pessoa, Brazil (Protocol number 4.676.103). All parents were informed about the study and provided written informed consent before data collection, following the guidelines outlined in Resolution 466/2012 of the National Health Council. The study received ethical approval on 28 April 2021.

2.2. Members and Study Design

The study included children aged 7 to 10 years, of both genders, who were enrolled in public primary schools. Data collection and recruitment took place in schools from April 2022 to September 2023. Children were excluded if they had physical limitations preventing anthropometric measurements, psychological or behavioral disorders, were taking medications, or had any condition that could interfere with the analysis. A total of 106 children underwent hydrogen breath testing to diagnose small intestinal bacterial overgrowth (SIBO). Additionally, the children were assessed for anthropometry, body composition, and blood samples.

2.3. Hydrogen Breath Test Assessment

Before the breath test, both the children and their parents received specific instructions. They were required to fast for 12 h, avoiding milk or dairy products, high-fiber foods, sugary drinks, soft drinks, and industrial juices for 24 h before the test. Additionally, they were instructed not to use probiotics for 30 days leading up to the test. On the test day, the children were provided with an oral hygiene kit containing a toothbrush, toothpaste, and dental floss to ensure clean teeth. During the test, they blew slowly into a standardized device to establish the baseline breath hydrogen level. Following this, they ingested a standardized glucose solution (composed of 50 g of glucose and 250 mg of strawberry flavoring in 200 mL of water), and breath samples were collected before and after 15, 30, 60, 90, and 120 min after glucose intake [12]. Hydrogen breath testing was performed using the EasyH2-P00401 (Dynamed, São Paulo, Brazil). Breath samples were immediately analyzed for H₂ using EasyH2 version 2.0 software and positivity was defined as an increase in H₂ of ≥ 20 over the baseline [11]. Despite the limitations of the H₂ test, it remains a useful tool in the diagnosis of pediatric SIBO due to its practical and non-invasive nature [11].

2.4. Anthropometry and Body Composition

In this study, we assessed anthropometry and body composition. Body weight was measured using an electronic scale (Omron®, model HBF-514C, São Paulo, Brazil), while height was determined using a stadiometer (alturaexata®, Belo Horizonte, Brazil). Nutritional status, based on body mass index for age (BMI/A) and sex, followed WHO standards and was determined using Anthro plus (version 1.0.4; WHO).

Waist circumference was measured using a flexible steel tape with a scale ranging from 0 to 200 cm and a precision of 0.1 mm (Sanny[®], São Paulo, Brazil). Skinfold thickness was measured three times, always on the right side of the body, using a scientific adipometer with a precision of 0.1 mm (Sanny[®]). The average values from these measurements were then used to estimate body fat percentage. All assessments were conducted by a trained investigator.

2.5. Assessment of Food Consumption

To assess the food consumption of the children, we used a 2-day food record—a questionnaire administered on both a weekday and a weekend day. During the interviews carried out by trained nutritionists, a photographic album of Global Diet was used to clearly define portion sizes. Following the input of the 24-h data, a quality control procedure was carried out. Subsequently, the data were entered into the BRASIL NUTRI[®] Software (Version 1), which was specifically designed for the 2017–2018 Family Budget Survey (known by its Portuguese acronym, POF). The foods were categorized into four groups based on the NOVA food classification: (1) unprocessed or minimally processed food, (2) processed culinary ingredients, (3) processed food, and (4) ultra-processed food. We calculated the average energy consumption (in kilocalories) over the two 24-h periods (R24h) based on the degree of food processing. By categorizing foods, we were able to calculate the energy intake specifically from unprocessed, processed, and ultra-processed foods. The sum of energy intake from groups 1, 2, 3, and 4 was used to obtain total caloric consumption. The energy of groups 1 and 2 were categorized together as unprocessed foods. Each relative proportion to total caloric consumption was determined as a percentage for unprocessed, processed, and ultra-processed foods.

2.6. Blood Samples and Biochemical and Cytokines Measurements

A qualified nurse collected blood samples from participants after a 12-h fasting period, during which they refrained from strenuous exercise in the preceding 24 h. Using an automated analyzer (Lab-Max 240, Labtest, Lagoa Santa, MG, Brazil) and standardized kits following the manufacturer's instructions (Labtest, Lagoa Santa, MG, Brazil), the serum concentrations of various biomarkers were assessed. These included albumin, alanine transaminase (ALT), aspartate aminotransferase (AST), cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), fasting glucose, glycated hemoglobin (HbA1c), and c-reactive protein (c-RP). Additionally, cytokines were measured using the Cytometric Bead Array (CBA) technique, as described in a previous study [2]. We employed Th1/Th2/Th17 CBA kits from Becton Dickinson Biosciences to quantify cytokines, including IL-2, IL-4, IL-6, IL-10, IL-17a, IFN- γ , and TNF- α . The fluorescence intensity of each complex corresponds to the cytokine concentration (measured in picograms per milliliter, pg/mL). For analysis, we utilized an Accuri C6 BD[®] flow cytometer, and the CBA data were processed using FCAP 1.0.1 software.

2.7. Statistical Analysis

We evaluated data normality using the Kolmogorov-Smirnov test. Descriptive statistics were reported as either mean (standard deviation) or median (minimum–maximum). Group comparisons were conducted using Student's *t*-test for parametric data and the Mann-Whitney test for non-parametric data. Additionally, logistic regression analysis was employed to further explore the relationships between small intestinal bacterial overgrowth (SIBO), consumption of ultra-processed foods, and serum cytokine levels. All statistical analyses were performed using GraphPad Prism[®] (version 8.01), and significance was maintained at $p < 0.05$.

3. Results

The study found a SIBO occurrence of 13.2%. After classifying individuals into positive and negative SIBO, we performed comparative analyses on several variables. Age, BMI, BMI/A, Waist circumference (WC), body fat percentage (BFP) and gender showed no statistically significant differences ($p > 0.05$, Table 1). In addition, biochemical markers of lipid and liver function, glucose metabolism, albumin, and c-RP were similar between SIBO-positive and negative children ($p > 0.05$, Table 1).

Table 1. Assessment of anthropometric and biochemical variables, age, and sex in children negative and positive for small intestinal bacterial overgrowth (SIBO).

Variables	Negative ($n = 92$)	Positive ($n = 14$)	p -Value
Age (years)	9.1 $\pm$ 0.9	8.9 $\pm$ 0.9	0.28
BMI (kg/m^2)	22.3 $\pm$ 5.3	23.1 $\pm$ 5.5	0.57
BMI/A	2.3 (−1.5–5.6)	2.5 (−1.0–4.3)	0.38
WC (cm)	72.9 $\pm$ 12.3	75.8 $\pm$ 16.2	0.42
BFP (%) †	36.6 (13.7–127.3)	44.7 (21.0–108.7)	0.28
Girls (n(%))	50 (54.3)	8 (57.1)	
Boys (n (%))	42 (45.7)	6 (42.9)	0.77
Albumin (g/dL)	4.5 $\pm$ 0.4	4.7 $\pm$ 0.3	0.25
AST (U/L)	37.5 $\pm$ 9.2	33.3 $\pm$ 7.2	0.11
ALT † (U/L)	22.0 (9.0–33.0)	16.5 (7.0–28.0)	0.09
Cholesterol † (mg/dL)	162 (121–278)	165 (144–226)	0.63
LDL-c † (mg/dL)	90 (52–146)	89 (58–137)	0.51
HDL-c(mg/dL)	48.5 $\pm$ 11.4	54.1 $\pm$ 13.0	0.10
FG (mg/dL)	82 $\pm$ 10	79 $\pm$ 10	0.31
HbA1c † (%)	5.6 (4.3–9.6)	5.5 (5.2–5.9)	0.31
HOMA-IR †	2.6 (0.4–7.7)	2.8 (0.8–5.1)	0.74
c-RP † (mg/dL)	1.3 (0.1–15.6)	1.2 (0.1–7.1)	0.88

Abbreviations: BMI: body mass index; BFP: body fat percentual; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDL-c: low-density lipoprotein cholesterol; FG: fasting glucose; HDL-c: high-density lipoprotein cholesterol; HbA1c: Glycated hemoglobin; HOMA-IR: homeostatic model assessment of insulin resistance; c-RP: c-reactive protein. Data are presented as mean and standard deviation or median (minimum–maximum). † non-parametric data.

The children tested as positive for SIBO had a similar consumption of calories, unprocessed or processed foods when compared to their SIBO-negative counterparts ($p > 0.05$, Table 2). However, children with a positive SIBO test had a higher consumption of ultra-processed foods, as indicated by a strong trend ($p = 0.06$, Table 2). Sweet biscuits, sugar added to food, industrialized chocolate milk products, salty cream cracker biscuits, industrialized juice powder, soft drinks, corn snacks, sausage, mortadella, and ham.

Table 2. Assessment of food consumption in children negative and positive for small intestinal bacterial overgrowth (SIBO).

Variables	Negative ($n = 92$)	Positive ($n = 14$)	p -Value
Kcal	2101 $\pm$ 439	2015 $\pm$ 535	0.51
Unprocessed, (%)	46 $\pm$ 10	41 $\pm$ 12	0.20
Processed, (%)	17 $\pm$ 10	17 $\pm$ 9	0.90
Ultra-processed, (%)	37 $\pm$ 9	42 $\pm$ 9	0.06

Data are presented as mean and standard deviation or median (minimum–maximum).

Serum concentrations of INF- γ , TNF α , IL-6, IL-4 and IL-2 were similar between SIBO positive and negative children ($p > 0.05$, Table 3). However, SIBO-positive children showed higher serum concentrations of IL-17 and a lower IL-10 compared to their SIBO-negative counterparts ($p < 0.05$, Table 3).

Table 3. Assessment of cytokines in children negative and positive for small intestinal bacterial overgrowth (SIBO).

Variables	Negative (n = 92)	Positive (n = 14)	p-Value
IL-17 †	53.4 (2.3–157.7)	69.5 (5.4–125.7)	0.03
INF- γ †	9.7 (0.9–34.2)	8.3 (0.2–13.1)	0.29
TNF α	4.8 $\pm$ 2.7	4.7 $\pm$ 3.2	0.85
IL-10 †	5.7 (0.5–30.8)	2.3 (0.6–7.2)	0.04
IL-6 †	10.1 (0.2–95.4)	17.5 (1.6–352.5)	0.08
IL-4 †	4.8 (0.3–28.9)	5.3 (0.4–11.4)	0.09
IL-2 †	7.6 (0.5–39.6)	8.2 (1.9–15.0)	0.80

Abbreviations: IL-17: interleukin 17; INF- γ : interferon-gamma; TNF α : tumor necrosis factor alpha; IL-10: interleukin 10; IL-6: interleukin 6; IL-4: interleukin 4; IL-2: interleukin 2. Data are presented as mean and standard deviation or median (minimum–maximum). † non-parametric data.

Logistic regression analysis was performed to determine the influence of variables on SIBO. Two models were used, one analyzing the anti-inflammatory cytokine IL-10 and the other analyzing the inflammatory cytokine IL-6. The models also included other variables such as consumption of ultra-processed foods, gender, BMI and age. In the second model ($p = 0.01$), consumption of ultra-processed foods ($p = 0.03$) and IL-6 levels ($p = 0.003$) were found to contribute to the occurrence of SIBO (Table 4).

Table 4. Logistic regression analysis predicting small intestinal bacterial overgrowth (SIBO) with predictors of ultra-processed foods and cytokines serum levels in children.

Variables	B	SE	t	p-Value	p-ANOVA	Adjusted R Square
Model 1						
Age	−0.05	0.03	1.51	0.13		
Sex	0.007	0.06	0.11	0.91		
IL-10	−0.01	0.007	1.82	0.07	0.117	0.037
BMI	0.001	0.006	0.27	0.78		
Ultra-processed	0.006	0.003	1.85	0.06		
Model 2						
Age	−0.04	0.03	1.01	0.31		
Sex	0.001	0.06	0.02	0.98		
IL-6	0.002	0.0008	2.97	0.003	0.01	0.09
BMI	0.001	0.006	0.28	0.77		
Ultra-processed	0.007	0.003	2.09	0.03		

Abbreviations: IL-10: interleukin 10; IL-6: interleukin 6; BMI: body mass index.

Figure 1 shows the experimental design and the main results.

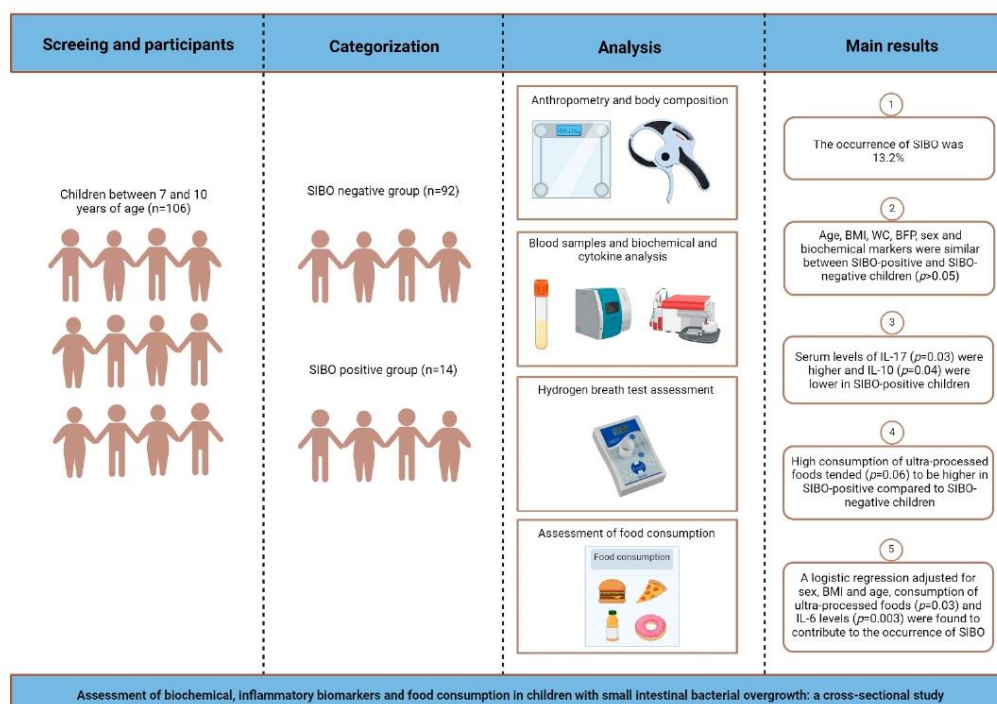


Figure 1. Schematic drawing showing the experimental design and main results obtained in cross-sectional study.

4. Discussion

The study found an occurrence of 13.2% for SIBO in children living in the Northeast of Brazil. In this study, the consumption of ultra-processed foods tended to be higher in SIBO-positive children. In addition, SIBO-positive children had increased levels of IL-17 and decreased levels of IL-10, and logistic regression adjusted for sex, BMI and age showed that consumption of ultra-processed foods and IL-6 levels may be significant predictors of SIBO.

The prevalence of SIBO in the general population can vary from 2.5% to 22%, with age and the occurrence of comorbidities having a positive influence on the risk of SIBO [14]. The data on the epidemiology of SIBO in children are limited due to the relatively small number of studies. The prevalence of SIBO varies according to the clinical context studied, ranging from 8.9% in children using proton pump inhibitors [15] to 91% in children with chronic abdominal pain [16].

Previous studies suggest an association between body composition and the occurrence of SIBO, which can occur in both obesity and underweight. Increased body fat percentage were associated with a greater likelihood of SIBO in children. A study with a small sample of 36 Italian children with excess of adipose tissue found a prevalence of 72.2% of SIBO-positive in children with obesity and overweight [13]. In Brazil, studies carried out in children and adolescents from the south-eastern region found an incidence of 31.5% of SIBO and showed that patients with SIBO had a lower weight-for-age Z score and increased intestinal permeability than those without SIBO [17,18]. In the present study, the BMI was not a predictor for SIBO.

Ultra-processed foods are characterized by being high in energy density, fat, sugar and salt, but low in fiber [19]. Using data from nationally representative dietary surveys conducted between 2004 and 2014, ultra-processed foods accounted for 25% of all calories consumed by children and adolescents in Brazil [20]. About 10 years later, we found an alarming consumption of ultra-processed foods in children, accounting for more than 40% of all calories consumed. We also found a trend towards increased consumption of ultra-processed foods in children with SIBO. A review of meta-analyses showed that greater exposure to ultra-processed foods was associated with the occurrence of several disorders, including gastrointestinal and cardiometabolic disorders [21]. In addition, another meta-analysis of five studies found that the increased risk of SIBO in individuals with obesity did not reach statistical significance, but was significant when only studies from Western countries were included [22], where ultra-processed food consumption is higher [5], which highlights the importance of the present study in discussing the effects of ultra-processed food consumption on SIBO.

High consumption of ultra-processed foods may affect the gut microbiota and its interaction with the gut mucosa, causing structural and functional changes in the gut, leading to local oxidative and inflammatory responses, which may contribute to negative modulation of the gut microbiota and potentially lead to SIBO [23]. Consumption of ultra-processed foods has been identified as one of the factors that may induce stimulation of inflammatory pathways in the gut, which has been identified as one of the causes of SIBO [24–26].

There is evidence that lipopolysaccharide production and its increased translocation into the bloodstream can induce chronic systemic inflammation in people with SIBO [27]. This study showed for the first time that serum concentrations of IL-17 were increased while serum levels of IL-10 were decreased in children with SIBO, suggesting a dysfunction of the systemic inflammatory state in these children. The relationship between inflammation and SIBO is a gap that needs further investigation. While one study has shown that the presence of SIBO is associated with elevated concentrations of IL-1 β in duodenal fluid [28], others have shown that measures of systemic inflammation do not differ between SIBO-positive and negative children [13,25].

Considering the above, this study provides an innovative perspective on the understanding that ultra-processed food consumption, together with an altered systemic inflammatory parameter, may contribute to the occurrence of SIBO in children. This statement is supported by the results found in this study, which show that the high consumption of ultra-processed foods in combination with high levels of IL-6 in a model adjusted for sex, age and BMI can induce SIBO in these children. A randomized, placebo-controlled trial showed that the administration of a probiotic containing *Saccharomyces boulardii* CNCM I-745 at a dose of 250 mg twice daily for 3 months was effective and safe in eradicating SIBO in decompensated cirrhosis [20]. Whether probiotic therapy is effective in eradicating SIBO in children remains to be investigated. A potential limitation of this study is that symptoms such as abdominal pain, bloating, diarrhea, and constipation were not recorded. Small intestinal cultures could be performed in children who test positive for SIBO on the hydrogen test. In addition, 16S rRNA sequencing could be used for further investigation.

This study identified for the first time an occurrence of 13% of SIBO in children living in the northeastern region of Brazil and showed that consumption of ultra-processed foods and serum levels of IL-6 may influence the occurrence of SIBO in the pediatric population. Although there are currently no guidelines regarding the diagnosis or treatment of SIBO in children, antibiotics remain the first-line approach to SIBO management. However, further studies are needed to know whether dietary interventions, such as low FODMAP (Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols) or probiotic therapy could be useful in treating SIBO in children.

Author Contributions: Conceptualization and design: P.C.T.C., D.B.D. and J.L.d.B.A. Formal analysis and investigation: P.C.T.C., D.B.D. and J.L.d.B.A. Data acquisition: P.C.T.C., D.B.D., T.A.F.V.d.N., R.C.S.d.M., L.d.L.T.T., A.d.C.P.d.A.N. and V.J.B.M. and J.L.d.B.A. Writing—original draft preparation: P.C.T.C. and J.L.d.B.A. Writing—review and editing: D.B.D., T.A.F.V.d.N., R.C.S.d.M., L.d.L.T.T., V.J.B.M. and J.L.d.B.A. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted following the Declaration of Helsinki and approved by the Ethics Committee of Lauro Wanderly University Hospital (Protocol number 4.676.103, approved on 28 April 2021) for studies involving humans.

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

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

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32 **APÊNDICE 4 – Artigo sob revisão no Sport Sciences for Health (ARTIGO 4)**

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**Effects of different types of physical training on heart rate variability in children
with obesity: A systematic review**

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Abstract

Purpose: This systematic review aimed to determine the effects of different types of physical training to improve heart rate variability in children with obesity. **Methods:** We carried out a search strategy in MEDLINE (via Pubmed), Embase, and Web of Science databases, considering four cores of keywords related to the PICOS strategy (exercise, heart rate, child, and clinical trials), with the aid of the boolean operators AND/OR". The study complies with PRISMA, 2020 and a previous protocol has been published in PROSPERO (CRD42022349347). **Results:** Heart rate variability-derived parameters constituted the primary outcome analyzed. We found that all analyzed types of physical exercise have beneficial effects on heart rate variability in children with obesity, mainly by increasing of parasympathetic indices, such as RMSSD, SDNN and HF, and decreasing of LF/HF, a sympathetic index. Furthermore, positive effects on body fat and heart rate were also observed in this population. **Conclusion:** This study is important to encourage the practice of any type of physical exercise to improve HRV in children with obesity, which reduces the risk of future cardiovascular complications. Also, this study highlights the need to create strategies for investigating this issue in children with obesity and its complications.

Keywords: Childhood Obesity. Autonomic Nervous System. Systemic inflammation. Physical Exercise. Cardiovascular diseases.

1. Introduction

Obesity is one of the most serious public health problems of this century, affecting millions of people around the world and promoting negative health consequences. Obesity is a disorder that can be caused by several factors, including biological, psychological, environmental, unhealthy lifestyle, and social factors (1).

Childhood obesity is a growing and alarming global health problem that puts children at high risk of morbidity and mortality in their adult lives. There is evidence that 50% of girls and 30% of boys between 6 and 11 years old will be people with obesity in adult life. In addition, this condition is associated with several adverse effects on the cardiovascular system, such as increased blood pressure and cardiac autonomic dysfunction (1, 2).

Heart rate variability (HRV) is a measure that reflects the variations in the intervals between successive heartbeats and has been used extensively to assess cardiac autonomic function (3, 4). Reduced HRV has been reported in children with obesity (5) and reflects autonomic dysfunction or inadequate functioning of self-regulatory control, adaptability, and resilience of the cardiovascular system (6).

Lifestyle modifications with increased physical activity and structured exercises can lead to improvements in HRV-related parameters (7). Previous systematic reviews with meta-analysis have demonstrated that physical training improved HRV-derived parameters in patients with type 2 diabetes mellitus (8), as also in healthy children (9) and in children and adolescents with chronic diseases including subjects with obesity, type-1 diabetes, and cerebral palsy (10).

Considering that the effectiveness of individualized physical training protocol for a specific population can guide best practices and implementation of physical training as an HRV-modulating intervention (11) and that HRV varies with age (12), gender (13),

physical activity, and disease condition (14, 15), this systematic review was conducted to establish the effects of different types of physical training to improve HRV specifically for children with obesity, due to the adaptive physiological particularities of this phase of life.

2. Methods

2.1. Ethics statement

Ethical approval and consent for individuals to participate in the study did not apply to this systematic review. The personal data of the participants were not included in this systematic review. Furthermore, all authors agreed to publish the results of this study. The study complies with PRISMA, 2020 and a previous protocol has been published in PROSPERO (CRD42022349347).

2.2. Search strategy

We reviewed studies reporting the effects of different types of training (considering intensity) on heart rate variability in children with obesity and excluded studies performed in animal models. We carried out a search strategy in MEDLINE (via Pubmed), Embase, and Web of Science databases, considering four cores of keywords related to the PICOS strategy (exercise, heart rate, child, and clinical trials), in which the separation of intracore terms occurred by the Boolean operator "OR" and between cores by the Boolean operator "AND". The following search strategy was used in PubMed: (Physical Exercise) OR (Training, Exercise) OR (Resistance Training) OR (Aerobic Exercise) OR (Plyometric Exercise) AND (Heart Rate Variability) OR (Autonomic Nervous System) (Heart Rate Determination) OR (Heart Rate Control) AND (Obese

Child) OR (Child Welfare) AND ((randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized [tiab] OR clinical trials as topic[mesh: noexp] OR randomly[tiab] OR trial[ti] OR placebo[tiab]) NOT ((animals [mh]) NOT humans [mh])). Furthermore, the search was not limited to specific years and there was also no language restriction. The search was adapted to other databases as needed.

2.3. Study selection, inclusion and exclusion criteria

Two authors (P.C.T.C. and M.L.A.S.) conducted the selection of the studies based on the selection criteria, decided the suitability of the articles for inclusion, and extracted the data. A third author (J.L.B.A.) resolved the disagreements (16). To be included, studies had to describe previously established primary outcome variables, i.e. HRV measurements before and after training in children with obesity. We excluded studies that evaluated physical training in combination with other strategies, such as dietary intervention, with or without a control group, corresponding to the group that did not receive physical training. The Rayyan® software (<https://www.rayyan.ai>) was used to remove the duplicates.

2.4. Data extraction

HRV indices in time- and frequency-domain constituted the primary outcome analyzed. The parameters analyzed in the time domain were SDNN (standard deviation of NN intervals), pNN50 (percentage of adjacent NN intervals varying by more than 50 milliseconds) and RMSSD (root mean square of successive NN-intervals differences). The indices investigated in the frequency domain were LF (low frequency), HF (high frequency) and LF/HF ratio (4). The analysis of the methods used in each study to obtain HRV data was also carried out. Furthermore, secondary outcomes included heart rate,

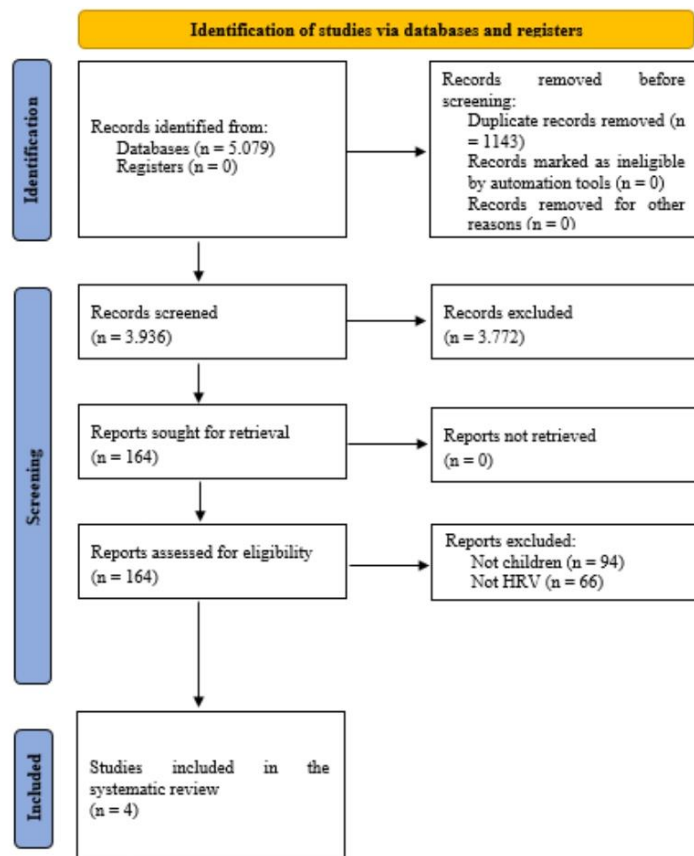
training characteristics (exercise modalities such as aerobic, moderate and high intensity), and clinical and biological parameters.

2.5. Risk of bias assessment

To assess the quality of the studies we used the Cochrane Risk of Bias Tool: Rob 2 (17). The overall judgment of the studies for the level of bias was made by ranking them low, with some concerns and a high risk of bias. The low-risk classification occurred when the study is considered to have a low risk of bias for all the domains analyzed. We consider that study had some concerns about the risk of bias if there were some concerns in at least one are for the outcome, but does not had a high risk of bias in any domain. Finally, when there is a high risk of bias in at least one domain for this result or when the study is considered to have many concerns in several domains, the study was classified as high risk of bias (17).

3. Results

An initial search yielded 5.079 articles, 1.143 of which were duplicates. After removing duplicates, all possible articles were manually checked by the authors. The use of selection criteria reduced the number of articles reporting the effect of training on HRV in children with obesity to four manuscript in the systematic review. The flow diagram according to the PRISMA guidelines can be found in more detail in Figure 1.

Figure 1. Flow diagram of studies selection

3.1. Included studies

The studies included in the systematic review had intervention and control groups and performed physical training as an intervention lasting three to four months. Among them, three studies were conducted with mostly female participants, aged between 9 and 11 years, and without associated comorbidities (Table 1).

The variables RMSSD, SDNN, PNN50, LF, HF, and the LF/HF ratio were evaluated in at least one of the included studies. The study by Gutin et al. (18) performed high-intensity physical training for 40 minutes a day, five days a week, and found that there was an increase in RMSSD and a decrease in LF/HF. Gutin et al. (19) with the same protocol, again demonstrated an improvement in the RMSSD. More recent studies with training protocols at lower intensities also demonstrated improvements in HRV indices. Brasil et al. (20) identified an increase in HF band and a decrease in the LF/HF ratio, and Li et al. (21) showed that aerobic exercise can increase SDNN, RMSSD, and HF, and decrease LF/HF ratio (Table 1). Furthermore, there was also an improvement in the fat percentage and HR of these children, as described in Table 1.

3.2. Quality assessment

According to the risk of bias assessment of the selected articles (n=4), none was considered to be at low risk of bias. With regard to the judgment of some concerns, one study fit this conception, as the allocation sequence was not random, considering that eutrophic children were automatically assigned to the control group and obese children to the case group (which was object of study), in addition to making clear the existence of differences between the groups. Three studies were classified as having a high risk of bias, as they do not inform whether the evaluators were aware of the study and, therefore, whether they knew about the intervention of each group. As well as whether the assessment of the outcome may have been influenced or likely to have been influenced by knowledge of the intervention. One of these articles was also judged to have a high risk of bias in domain 2, due to lack of information about the participants, regarding their knowledge of their intervention and who carried out the intervention, if they were aware of the assigned intervention. In addition to not informing which type of analysis was used

(by protocol or by intention to treat) and whether there was an impact of the failure to analyze the participants of the group to which they were randomized. The risk of bias was detailed in Table 2.

4. Discussion

This review showed that physical training as a non-pharmacological intervention to improve HRV in children is still little explored. The included studies demonstrate that aerobic and moderate and high-intensity training are effective in improving some HRV indices in the time and frequency domains in children with obesity. In addition, it was observed that physical training in this population improves parameters related to HRV, decreasing the percentage of body fat, leptin concentration, and heart rate. We identified also that studies are still scarce, highlighting the need for new clinical trials that evaluate the effect of physical exercise on HRV in children with obesity.

4.1. Physical training, inflammation and cardiovascular disease

Physical training can be classified as high-intensity, low-intensity, or interval training (22-24). The beneficial effects of physical training on different pathological conditions have been demonstrated, and due to its importance, several international guidelines have recommended at least 150 minutes of moderate-intensity aerobic exercise per week or 75 minutes of high-intensity physical activity per week and strength training muscle for adults (24). More specifically, for overweight children it should be adjusted according to additional criteria such as age, co-existing illnesses and comorbidities, medication history, and general physical condition (25).

Low-grade inflammation is considered a key point for the development of cardiovascular diseases by upregulating toll-like receptor 4 (TLR4)/NF- κ B pathways and exacerbating the production of pro-inflammatory cytokines IL-6 and TNF- α (26-28). Studies in rodents have shown that the TLR4/NF- κ B pathway influences the autonomic response of heart rate, leading to greater sympathetic activation and decreased vagal activity (29-31). It is recognized that exercise plays a key role in losing and maintaining weight loss, as well as preventing weight gain and related diseases (23). In addition, exercise has been shown to down-regulate TLR4, reduce low-grade inflammation and improve cardiac function (28).

Regarding the role of physical training on TLR4, regular training is believed to trigger anti-inflammatory adaptations, but the mechanisms by which this occurs are still not fully understood. Physical exercise decreases TLR4 expression in peripheral blood mononuclear cells, as well as decreases the concentration of TLR4 ligands in circulation, such as LPS and saturated FFAs (32). In addition, during exercise, there is an increase in anti-inflammatory cytokines, such as IL-10, which can suppress the increase in pro-inflammatory cytokines mediated by TLR4, such as TNF- α . Furthermore, the concentration of IL-10 is inversely related to the expression of TLR4, which suggests that this cytokine may regulate the expression of this receptor. There is evidence that this occurs through the negative regulation of essential adapter proteins, such as MyD88, inducing a reduction in NF- κ B expression (32, 33). In this sense, it is essential to investigate the expression of genes and proteins related to the pathways discussed above to better understand the mechanisms by which exercise can improve inflammation and consequently reduce cardiovascular risk.

4.2. Heart rate variability

Detection of early stages of cardiac autonomic dysfunction and related causes may be a useful strategy to identify the presence of cardiovascular risk in childhood obesity, and HRV may be used as a warning sign of heart disease (3, 34, 35). HRV indexes neuro-cardiac function and is generated by heart-brain interactions and non-linear dynamic ANS processes (4).

HRV has been a commonly used and validated test to assess cardiac autonomic function using time-domain, frequency-domain, and non-linear indices that quantify parasympathetic and sympathetic activation (3). Some of these indexes were evaluated by the articles included in this review, such as RMSSD, SDNN, PNN50, LF, HF, and the LF/HF ratio.

HRV is a measure of the natural changes that occur between consecutive heart rate beats, which represents the number of heartbeats per minute (6). An ideal HRV level reflects adequate functioning, characterized by an inherent capacity for self-regulation, adaptability, or resilience. In contrast, a very small variation indicates age-related system depletion, chronic stress, pathology, or improper functioning at various levels of self-regulatory control systems (6). In this way, the oscillations are complex and vary constantly, which allows the cardiovascular system to quickly adjust to sudden physical and psychological challenges to body homeostasis (4).

As shown in this systematic review, physical training in its various classifications can improve the HRV of children with obesity by improving vagal tone, and this change can positively affect the health of these individuals (36). This change is possible because HRV is susceptible to change through changing lifestyle habits (3), a fact that demonstrates the importance and needs to refine the optimization of existing strategies for different audiences, the example of children with obesity studied in this review.

Improving HRV through interventions such as exercise is clinically relevant because it is associated with a reduced risk of arrhythmias, hypertension, insulin resistance, and systemic inflammation, conditions commonly present in the metabolic phenotype of pediatric obesity (37). In addition, improvement in HRV may represent an increase in physiological resilience and the body's ability to adapt to stress, which may be an early indicator of therapeutic success in cardiovascular rehabilitation and prevention programs (38). Therefore, monitoring and promoting HRV should be considered valuable clinical strategies in the management of childhood obesity.

In addition to being pioneers in trying to understand which type of exercise works best to improve HRV in children with obesity, we were also the first to evaluate only children with obesity. There are a meta-analyses that have evaluated the effects of physical exercise on HRV in children and adolescents with obesity (39, 40). However, we have limited this review to children with obesity in order to better understand the characteristic effects of exercise at this stage of life, which is characterized by specific physiological and molecular adaptations.

4.3. Secondary outcomes

In addition to the effects of training on HRV, according to the included studies, other parameters of extreme relevance were improved in children with obesity, including a reduction in the percentage of body fat and a decrease in resting heart rate.

Increased body fat is related to greater production of pro-inflammatory cytokines to anti-inflammatory cytokines, which can lead the individual to chronic low-grade inflammation (41). Furthermore, systemic inflammation can cause a shift in autonomic control by the hypothalamus through activation of the NF- κ B pathway and induce greater sympathetic activation and elevated heart rate ((42)). Therefore, this study demonstrates

the importance of different types of training in characterized by different intensities promoting metabolic and cardiovascular health in children with obesity.

5. Conclusion

Systematic review showed that physical training at different intensities improved HRV-related parameters in children with obesity, including RMSSD, LF and HF, which may reflect autonomic improvement. The results obtained so far serve to encourage the practice of any type of physical exercise to improve HRV in children with obesity, which reduces the risk of future cardiovascular complications. In addition, variables related to HRV such as body fat percentage and heart rate were improved in children with obesity after all kind of physical training. Also, this study highlights the need to create new strategies for a better investigation and understanding of this very important issue in coping with obesity and its complications.

Author contribution: Each author contributed to the conception of the study, acquisition, analysis, and interpretation of data, drafted the manuscript and revised it critically for important intellectual content, approved the version to be published, and agreed to be accountable for all aspects of the study.

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Declarations

Conflict of interest: The authors declare no competing interests.

Ethical approval: Ethical approval was not required since this was a literature review.

Informed consent: For this type of study, informed consent is not required.

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Table 1. Summary of included studies

Reference	Groups	Intervention	Intensity of exercise	% female	Age	Comorbidities	HRV analysis methods	Measures	Findings
Gutin et al. 1997	Obesity group intervention (n= 17)	Physical training during 4 months	5 days a week for 40 min/session of high intensity	65%	9.6 ± 0.2	No comorbidities associated	Electrocardiogram	RMSSD, LF, HF, LF/HF	Exercise increased RMSSD, decreased LF/HF ratio and % BF
	Obesity group without intervention (n= 18)	No physical training	-	70%	9.5 ± 0.3				
Li et al. 2022	Obesity group intervention (n= 18)	Physical training during 16 weeks	Moderate- to high	100%	11.38 ± 0.27	No comorbidities associated	Portable heart rate monitor (Polar)	RMSSD, SDNN, LF, HF, LF/HF ratio and leptin	Exercise increased SDNN, RMSSD, HF, decrease LF/HF ratio and improved leptin concentration
	Obesity group without intervention (n= 18)	No physical training	-	100%					
Gutin et al. 2000	Obesity group intervention (n= 38)	Physical training during 4 months	5 days a week for 40 min/session of high intensity	67%	9.5 ± 1.0	No comorbidities associated	Electrocardiogram	RMSSD	Exercise increased RMSSD and decreased HR rest
	Obesity group without intervention (n= 38)	No physical training	-						
Brasili et al. 2020	Obesity group intervention (n=20)	Physical training during 12 weeks	Moderate- to high	50%	11.1 ± 1.1	No comorbidities associated	Portable heart rate monitor (Polar)	SDNN, RMSSD, pNN50, LF, HF, LF/HF ratio, and anthropometric and cardiorespiratory indices	Exercise increased HF and decreased LF/HF ratio, HR rest, and %BF
	Normal weight group without intervention (n= 15)	No physical training	-	47%	10.7 ± 1.6				

RMSSD: root mean square of successive NN-intervals differences; SDNN: (standard deviation of NN intervals); pNN50 (percentage of adjacent NN intervals varying by more than 50 milliseconds); LF: low frequency; HF: high frequency; HR: heart rate; BF: body fat.

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168 **Table 2.** Risk of bias assessment

Reference	D1	D2	D3	D4	D5	Overall Bias
Gutin et al. 1997	!		+		+	
Li et al. 2022	+	!	+		+	
Gutin et al. 2000	!	!	+		+	
Brasil et al. 2020	!	+	+	+	+	!

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 Low risk

 High risk

 Some concerns

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173 D1: Bias resulting from the randomization process; D2: Bias due to deviations from intended interventions;

174 D3: Bias due to lack of outcome data; D4: Bias in outcome measurement; D5: Bias in the selection of the

175 reported result.

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APÊNDICE 5 – Artigo sob revisão no The Journal of Clinical Endocrinology & Metabolism (ARTIGO 5)

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A multicomponent intervention combining physical exercise and food and nutrition education modulates gut microbiome and improves cardiac autonomic function in childhood obesity: a pre-post trial

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Abstract

Context: Childhood obesity is associated with gut microbiome dysbiosis, inflammation, and cardiac autonomic dysfunction. Objective: Evaluate a multicomponent intervention with diet and exercises for childhood obesity and related metabolic disorders. Methods: Anthropometry, gut microbiota, metabolomics, systemic inflammation, biochemical parameters, and cardiovascular parameters were assessed before and after a 4-month physical exercise intervention associated with food and nutrition education in 51 children with obesity, aged 7 to 10 years. Results: The intervention reduced body fat percentage and inflammatory IL-17A ($p=0.02$) and TNF- α ($p=0.01$) serum levels, and increased serum levels of albumin ($p<0.0001$) and HDL cholesterol ($p<0.0001$), and decreased triglycerides ($p=0.027$) and urea ($p=0.004$). Also, serum levels of metabolites, including sum of phosphatidylethanolamines, phosphatidylglycerols, choline and sum of BCAA were improved after the intervention. The relative abundance of the Proteobacteria phylum and anaerobic bacterial genera such as *Alistipes*, *Faecalibacterium*, *Escherichia-Shigella* and *Odoribacter* were decreased in the fecal microbiota after intervention. In addition, the multicomponent intervention reduced SBP, DBP, and other indices related to HRV. *Roseburia* was negatively correlated with BCAA, ADMA and TMAO, variables linked to cardiovascular health impairment that positively correlated with *Dialister*, *Odoribacter* and *Monoglobus*. Furthermore, high levels of BCAA were strongly positively correlated with HRV indices representing sympathetic activity and negatively correlated with indices representing parasympathetic activity. Conclusion: Findings reported here underscore the beneficial effects of food and nutrition education associated with exercises during four months in children with obesity considering changes in gut microbiome, inflammation and autonomic function. Although additional mechanistic studies should be performed to elucidate these relationships, this study provides novel information for the benefit of lifestyle modification strategies in the management of childhood obesity.

Keywords: Childhood obesity; Physical exercise; Nutrition; Microbiome; Autonomic control

1. Introduction

Obesity is a disease with multifactorial causes that include socioeconomic, genetic, and environmental factors and is recognized as a risk factor for health complications (1). The increasing prevalence of childhood obesity worldwide is a public health concern (2). In Brazil, the prevalence of childhood obesity reached 8-9% over the last three decades, increasing with age and in more developed regions (2, 3). In part, the excessive consumption of calories, ultra-processed foods, and sedentary lifestyles have contributed to the increasing prevalence of childhood obesity (4, 5).

Childhood obesity increases the risk of low-grade inflammation (6), hypothalamic inflammation (7), and associated cardiometabolic abnormalities, such as insulin resistance, high blood pressure, and cardiac autonomic dysfunction (8). Although the mechanism linking childhood obesity, chronic inflammation and cardiac autonomic dysfunction is complex, it is recognized that obesity is associated with gut microbiome dysbiosis (9). This is a condition characterized by alterations in composition and diversity of gut microbiota, reduction of the gut mucosal layer protection, and increased translocation of lipopolysaccharides (LPS), free fatty acids, and pro-inflammatory cytokines into the bloodstream (10), which could contribute to a state of low-grade inflammation.

This state of low-grade inflammation has been recognized as one of the causes of neuroinflammation (11), and is related with sympathetic overactivity and vagal withdrawal (6), alterations that contribute to cardiac autonomic function impairment (12), elevated blood pressure (BP) and lower heart rate variability (HRV) (13-15). Taken together, it is reasonable to infer that cardiac autonomic dysfunction in the pediatric population may be related to the inflammatory state induced by gut microbiome dysbiosis.

It is well known that lifestyle changes with regular physical activity and nutritional education programs are the cornerstone of the management of obesity-related metabolic disorders in the pediatric population (16, 17). Physical exercise can improve cardiac autonomic function by several neural mechanisms, as well as by modulating the arterial baroreflex, cardiopulmonary baroreceptors, and peripheral chemoreflex (18). Recently, it has been shown that physical exercise can quantitatively and qualitatively

alter gut microbiota biodiversity and impair inflammatory pathways in children with obesity (19).

Although it is known that physical exercise affects the microbiome-gut-brain axis pathways through vagal communication between the gut and the brain (20), there is no evidence that physical exercise can improve cardiac autonomic function by modulating the gut microbiome in childhood obesity. Therefore, this study aimed to investigate the effects of a 4-month multicomponent protocol consisting of physical exercise and food and nutrition education (FNE) on the gut microbiome and cardiac autonomic function in schoolchildren with obesity. The hypothesis tested is that the multi-component protocol will favorably alter the composition and functionality of the gut microbiota, thereby reducing the inflammatory status and cardiac autonomic dysfunction in children with obesity.

2. Methods

2.1 Ethical aspects

This study was conducted in agreement with the declaration of Helsinki, and was approved by the Human Research Ethics Committee (Health Sciences Center, Federal University of Paraíba – João Pessoa – PB – Brazil; Reference number protocol nº 4.676.103). All procedures were conducted in agreement with Resolution 466/2012 of the National Health Council. All parents received information about the study and gave written informed consent before data collection. For ethical reasons and because the intervention could potentially help children with obesity from regions with poor access to health care, all participants were submitted to the intervention. This clinical trial was recorded and approved by Brazilian Registry of Clinical Trials (ReBEC, number RBR-2mzmpxf).

2.2 Participants and study design

This study is part of a broader research initiative titled “Physical training and nutritional education as strategies to modulate intestinal microbiota and cardiometabolic parameters in schoolchildren with obesity” (NUTRO). Children with chronic conditions other than obesity were excluded. Schools were invited to participate voluntarily in the trial, and upon acceptance, the children underwent an evaluation of their nutritional status. For the clinical trial, children aged 7 to 10 years, of both genders, with obesity (BMI-for-age > 2.0 Z-score) were included to follow an intervention protocol comprising an FNE program and a physical exercise practice.

According to the Department of Education and Culture of João Pessoa, in 2020, a total of 23,861 students were officially enrolled in 97 public primary schools across the municipality, distributed among its four geographical regions (North, South, East, and West). To determine the sample size for the NUTRO project, several parameters were taken into account, including the reference population of 23,861 children in primary grades 1–5 within the public school system, an obesity prevalence rate of 13.3% (21), a 95% confidence interval, and a statistical power of 80%. For the intervention study, the sample consisted of a maximum of 51 children with obesity who were available to participate. Due to missing data from the same participants at both time points, the analyses of gut

microbiota and metabolomics were conducted with smaller groups of 20 and 40 participants, respectively.

2.3 Multicomponent Intervention Protocol

The multicomponent intervention consisted of a physical exercise protocol and an FNE program. It lasted four months and was performed on a group of children with obesity.

2.3.1 Physical exercise

The physical exercise was characterized by a circuit containing plyometric exercises, as the children performed this type of training for 30 minutes out of a total of 40 minutes. The other 10 minutes comprised playful and stretching activities to diversify activities and improve children's adherence to the established protocol. Between the sessions of plyometric exercise, the children also did this exercise to vary the activity and to make it more fun. Circuit exercise was performed weekly on two non-consecutive days for four months in 28 sessions (Table 1). The groups were formed with a maximum of 15 children, and the number of sets and repetitions gradually increased over the training weeks, respecting the overload principle (22) (Table 2). In addition, the children were instructed to perform all jumps at maximum effort (maximum height and/or distance) and minimum contact time on the ground, which justifies plyometrics. The ground was lined with polyurethane mattresses 10 millimeters thick to dampen the impact between jumps and to prevent injury.

Table 1. Distribution of the plyometric exercise program according to the jump type

Jump type	Series	Repetitions	Platform height (cm)
1 - Sides	1	10	10
2 – Group jump	2	5	10
3 - Ascending	4	5	10, 20, 30, 30 e 40
4 - Descending	4	5	40, 30, 30, 20 e 10
5 - Monopedal	4	5	No platform
6 – Monopedal	4	5	No platform
7 – Ascending + grouped	2	5	10, 20, 30 e 40

Description of each exercise: 1 - Lateral thrust with both feet, alternating the ground and the 10 cm platform; 2 - Jump with feet together after falling 10 cm from the platform; 3 - Alternating jumps between the ground and platforms with increasing heights; 4 - Alternating jumps between the ground and platforms with decreasing heights; 5 - Repetitions of maximum jumps with the touch on the ground with only the right foot; 6 - Repetitions of maximum jumps with the touch on the ground with only the left foot; 7 - Increasing jumps of 10, 20, 30 and 40 cm, followed by a grouped jump after the 40 cm drop from the platform.

Table 2. Description of the plyometric exercise program according to the exercise progression

Sessions	Series	Repetition	Total jumps
1 ^a – 4 ^a	5	5	25
5 ^a – 8 ^a	8	5	40
9 ^a – 12 ^a	10	5	50
13 ^a – 16 ^a	13	5	65
17 ^a – 20 ^a	17	5	85
21 ^a – 24 ^a	20	5	100
24 ^a – 28 ^a	22	5	110

Description of some terms: Sessions: Number of days in which exercise took place; Series: Number of pauses between exercises performed during exercise; Repetition: Number of jumps performed in each series; Total Jumps: Total number of jumps performed on each exercise day.

2.3.2 Food and nutrition education

The FNE program was conducted over four months in parallel with physical training activities. 12 activities were carried out, of which 9 took place in person, one activity was aimed at parents at home (activity 10), one activity on the benefits of physical education and reducing screen time was carried out during physical training, and last (activity 11), and the culmination consisted of a walk with the children. In this program, several themes were worked on, which are described in Table 3.

Table 3. Description of food and nutrition education activities carried out with children with obesity

Session	Activity name
1	Which fruit is this? (Game of the Senses)

2	Food traffic light
3	Fresh, processed, and ultra-processed foods + sugar, salt, and oil content
4	Food hygiene + juice workshop
5	<i>My plate</i> : healthy food
6	Educational film – Sid the Scientist • Healthy Eating!
7	Picnic with fully used food + word search
8	Portable vegetable garden
9	With an eye on the magnifying glass
10	Much More Than Obesity
11	Encouraging physical activity and reducing the use of screens
12	Flavors Laboratory

2.4 Anthropometry and body composition

Body weight was recorded using an electronic scale (Omron®, HBF-514C), while height was measured with a stadiometer (alturaexata®). Nutritional status was assessed based on body mass index-for-age (z-score) (BMI-Z) and sex, following WHO standards, using Anthro Plus (version 1.0.4; WHO).

Waist circumference was determined using a flexible steel tape (Sanny®) with a measurement range of 0–200 cm and an accuracy of 0.1 mm. Skinfold thickness was measured three times, consistently on the right side of the body, using a scientific adipometer (Sanny®) with a precision of 0.1 mm. The mean values obtained were then used to estimate body fat percentage (BFP), calculated according to the Slaughter equation (23).

2.5 Gut microbiota Assessment

Total genomic DNA was directly extracted from fecal samples using silica column-based extraction. The DNA was eluted in DNase- and RNase-free water and diluted at 1:10 and 1:100 concentrations, resulting in three sample dilutions (original, 1:10, and 1:100) for subsequent steps. The extracted DNA served as the template for 16S metataxonomic analysis performed by Neopropecta Microbiome Technologies (Florianópolis, SC, Brazil) using the Illumina NextSeq platform (Illumina, Inc., San

Diego, CA, USA). Library preparation followed the Neoprosecta NGS Protocol. The hypervariable V3-V4 region of the 16S RNA gene was amplified using primers 341F (CCTACGGGRSGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) (24, 25). Library quantification was performed using the Qubit fluorometric system and qPCR, based on standard curves generated with the Colibri Library Quantification kit (Invitrogen, USA). Sample pools were visualized on a 3% agarose gel, along with qPCR-processed samples, to verify fragment sizes and confirm the absence of dimers. Sequencing was subsequently conducted using the NextSeq kit P1 600 cycles, employing 2×305 bp paired-end reactions. All bioinformatics procedures followed the methodology described in a previous study (26). Briefly, data quality was assessed using FastQC (v0.11.9), after which filtering was applied via Trimmomatic 0.39 using the SE option (single-end reads, minimum length of 300 bp) (27). Chimeric sequences were identified across various sequence categories using UCHIME2 algorithms (28). High-quality reads were then utilized for taxonomic assignment using the Quantitative Insights into Microbial Ecology (QIIME) software, version 1.9.0 (29). Taxonomic classification was performed against the SILVA 132 database (30, 31). Following the sequencing of gut microbiota samples, sequences were imported into R software for statistical analysis, using specialized packages. Sequences with fewer than 10,000 reads were excluded due to the rarefaction process. Alpha and beta diversity analyses were conducted, with beta diversity evaluated using the Unifrac and Bray-Curtis methods. Taxonomic analysis at the phylum, family, and genus levels was performed using the Maaslin2 package and Kruskal-Wallis tests with Dunn's post-test, considering a significance threshold of $p < 0.05$. The sequences have been registered in the National Center for Biotechnology Information (NCBI) under the identifier PRJNA1255541.

2.6 Metabolomic analysis

All metabolomics analyses were conducted at the METANUTRIBIOTA Metabolomics platform in Lyon, France. Serum samples were processed in a randomized sequence using the commercial MxP Quant 500 XL kit (Biocrates Life Science AG, Innsbruck, Austria, MxP Quant 500 XL (accessed on February 22, 2024)), on a XEVO TQ-XS® instrument integrated with a UPLC system (Waters, Milford, Massachusetts, USA).

This targeted assay was designed to detect and quantify up to 1,000 metabolites spanning 39 biochemical classes, employing two MS methods. It is important to highlight that the kit, including all consumables, reagents, methodologies, and instructions, was applied exactly as specified, without any modifications. The kit provides detailed guidelines and precise settings for liquid chromatography (LC) and flow injection analysis (FIA) MS/MS, operating in both positive and negative ionization modes, with acquisition and quantification methods tailored to specific instruments. Prior to analysis, wash solvents and mobile phases were prepared according to the manufacturer's specifications. Serum samples, stool extracts, external standards, and quality control samples were placed onto patented 96-well filter plates preloaded with internal standards (included in the kit). The analytical workflow encompassed derivatization, extraction, and dilution, strictly following the kit's instructions.

Metabolites such as alkaloids, amine oxides, amino acids, amino acid-related compounds, bile acids, biogenic amines, carboxylic acids, cresols, fatty acids, hormones, indole derivatives, nucleobase-related compounds, vitamins, and cofactors were identified using MS after chromatographic separation via the MxP Quant 500 column. Additionally, acylcarnitines, lysophosphatidic acids, phosphatidic acids, lysophosphatidylcholines, phosphatidylcholines, lysophosphatidylethanolamines, phosphatidylethanolamines, lysophosphatidylglycerols, phosphatidylglycerols, lysophosphatidylinositols, phosphatidylinositols, lysophosphatidylserines, phosphatidylserines, sphinganine, sphingosine, sphinganine and sphingosine phosphates, sphingomyelins, ceramides, dihydroceramides, hexosylceramides, dihexosylceramides, trihexosylceramides, cholesteryl esters, monoglycerides, diglycerides, and triglycerides were quantified using FIA-MS/MS methodology.

Metabolite quantification was performed using either a seven-point LC-MS/MS calibration or a one-point FIA-MS/MS calibration with isotopically labeled internal standards, or semi-quantitatively with internal standards of similar chemical properties, as predetermined in the kit's quantification software. Sample preparation (using 2×10 μ L of serum), instrument analyses, quality control procedures, and metabolite quantification were conducted in strict adherence to the manufacturer's instructions. Data were acquired via MassLynx® software (Waters, Milford, Massachusetts, USA) and analyzed using Biocrates WebIDQ™ software (Biocrates Life Science AG,

Innsbruck, Austria). The final results are expressed in $\mu\text{mol/L}$. WebIDQ™ enables the computation of more than 400 quantifiable metabolic indicators (sums and ratios), facilitating data interpretation.

2.7 Biochemical and cytokines measurements

Serum concentrations of alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides, fasting glucose, glycated hemoglobin (HbA1c), creatinine, urea, and high-sensitivity C-reactive protein (hs-CRP) were determined using an automated analyzer (Lab-Max 240, Labtest, Lagoa Santa, MG, Brazil) and standardized kits, following the manufacturer's instructions (Labtest, Lagoa Santa, MG, Brazil).

Cytokine levels were assessed using the Cytometric Bead Array (CBA) technique, as described in a previous study (6). Th1/Th2/Th17 CBA kits from Becton Dickinson Biosciences were employed to quantify the cytokines IL-2, IL-4, IL-6, IL-10, IL-17A, IFN- γ (interferon- γ), and TNF- α (tumor necrosis factor- α). The fluorescence intensity of each complex was used to determine cytokine concentrations (pg/mL). An Accuri C6 BD® flow cytometer facilitated the measurements, and the CBA data analysis was conducted using FCAP 1.0.1 software.

2.8 Blood pressure analysis

BP was assessed using a digital sphygmomanometer (Omron Healthcare® HBP-1100), which has been validated for pediatric use according to the Brazilian Society of Cardiology (32). Following an initial stabilization period of five minutes, three consecutive measurements were taken at one-minute intervals for each child, and the average of these readings was used for analysis (32).

2.9 RR interval records and heart rate variability analysis

An iPhone 12 smartphone was used to record RR intervals via the Elite HRV app (Elite HRV LLC, Asheville, NC, USA, Release 4.0.2, 2018). A comfortable, adjustable elastic belt was positioned around the participant's chest, with a Polar H10 transmission device (Polar Electro, Finland; 5 kHz transmission system coded with

Owncodes, Polar Electro) attached at the front, aligned with the xiphoid process. RR intervals were recorded for 10 minutes (6, 33). The raw data were exported as a text file and analyzed using Kubios HRV Standard software (version 3.2.0; Biosignal Analysis and Medical Image Group, Department of Physics, University of Kuopio, Kuopio, Finland). Series interpolation was performed via cubic spline with frequency values set at 4 Hz. R-wave time instants were automatically detected using Kubios' built-in QRS detection algorithm, and if ectopic beats exceeded 5% of the total analyzed, the measurement was repeated.

Previous studies have shown that smartphone apps and chest straps (such as the Polar model) provide excellent electrocardiogram compliance across time domain, frequency domain, and nonlinear indexes (34, 35). The following heart rate variability (HRV) measures were assessed: the root mean square of successive differences between adjacent normal-to-normal beats (RMSSD), standard deviation of NN interval durations (SDNN), low-frequency band (LF), high-frequency band (HF), LF/HF ratio, standard deviation of instantaneous variability of continuous NN intervals in the Poincaré plot (SD1), standard deviation of long-term continuous NN intervals (SD2), and detrended fluctuation analysis (DFA).

2.10 Statistical analysis

Data normality was assessed by the Kolmogorov-Smirnov test. Descriptive statistics were presented as mean (standard deviation) or median (min-max). The comparison of most pre- and post-intervention non-omics variables was performed using the paired Student's t-test or its nonparametric equivalent, and statistical analysis was performed in GraphPad Prism® (version 8), with statistical significance when $p < 0.05$. Metabolomics analysis was performed on the MetaboAnalyst website, considering data pairing. For metabolomics and intestinal microbiota analyses, values were also presented corrected by the Benjamini-Hochberg equation (FDR). Using the R software, in addition to the intestinal microbiota analyses described above, correlations between bacterial genera, anthropometric variables, metabolites, and HRV indices were analyzed using the Spearman correlation test with appropriate packages.

3. Results

The study was carried out between June 2021 and December 2022. Twenty-seven (52.9%) children were female and 24 (47.1%) were male, with a mean age of 9.13 ± 1.05 years. The multicomponent intervention protocol combining physical exercise and FNE improved anthropometric indices such as weight, BMI-Z, waist-hip ratio (WHR) and BFP in children. Multicomponent intervention increased biochemical variables such as albumin, total cholesterol and HDL-c serum levels and reduced triglycerides and urea (Table 4). Glycaemia, HbA1C, ALT, AST, GGT, LDL-c, creatinine, and hs-CRP were not changed by the intervention protocol.

The intervention decreased the relative abundance of Proteobacteria phylum ($p=0.01$; FDR=0.19). In addition, it reduced the relative abundance of the families *Rikenellaceae* ($p=0.01$; FDR=0.64), *Sutturellaceae* ($p=0.02$; FDR=0.64), and *Marinifilaceae* ($p=0.04$; FDR=0.69). There was also a reduction in bacterial genera *Alistipes* ($p=0.02$; FDR=0.54), *Faecalibacterium* ($p=0.02$; FDR=0.54), *Escherichia-Shigella* ($p=0.02$; FDR=0.54) and *Odoribacter* ($p=0.05$; FDR=0.54) and a trend towards a reduction in *Methanobrevibacter* ($p=0.08$; FDR=0.54).

The principal component analysis (PCA) of serum metabolomics showed different distribution of points representing each individual when comparing before and after the intervention (Figure 2a). There were significant changes in several metabolites, including an increase in the sum of phosphatidylethanolamines, phosphatidylglycerols, and a decrease in choline and the sum of branched-chain amino acids (BCAA). Regarding metabolism indicators (sums and ratios of metabolite concentrations), there was a decrease of methionine sulfoxide to methionine ratio potentially reflecting methionine oxidation and an increase in phosphatidylethanolamine/phosphatidylserine ratio and in myasthenia gravis/folic acid ratio that respectively could reflect phosphatidylserine decarboxylase activity and monoacylglycerol lipase activity (Figure 2b and c). The inflammatory profile was improved by the intervention, with a decrease in serum levels of pro-inflammatory cytokines such as IL-17 and TNF- α and an increase in IL-4, an anti-inflammatory interleukin (Figure 3).

Regarding cardiovascular variables, the intervention reduced systolic BP (SBP) and diastolic BP (DBP). Furthermore, HR and HRV-derived indices improved, mainly

in the time domain indices (RMSSD and SDNN) and non-linear indices (SD1, SD2/SD1 ratio and DFA- α 1), with no significant difference in the frequency domain indices, as shown in Table 5.

Correlations between gut microbiota and metabolites, as well as correlations between anthropometric and HRV-related indices with metabolites and gut microbiota are described in Figures 4, 5 and 6. Bacterial genera such as *Roseburia* were negatively correlated with variables indicating cardiovascular health impairment such as BCAA, asymmetric dimethylarginine (ADMA) and trimethylamine N-oxide (TMAO). Other genera, such as *Dialister*, *Odoribacter* and *Monoglobus* were positively correlated with these variables, respectively. In addition, high levels of BCAA were strongly positively correlated with HRV indices representing sympathetic activity and negatively correlated with indices representing parasympathetic activity. The observed correlations foster discussions about metabolites serving as markers of cardiovascular risk through alteration of autonomic function, as well as possible bacterial genera associated with the levels of these metabolites, and also with indices representing autonomic function in obesity.

4. Discussion

In this study, an intervention combining a circuit of physical exercises at school and FNE improved body composition and biochemical parameters in children with obesity. The multicomponent intervention effectively modulated the gut microbiome, with a reduction in the Proteobacteria phylum and some anaerobic genera such as *Alistipes*, *Faecalibacterium*, *Escherichia-Shigella* and *Odoribacter*, and also the serum levels of specific metabolites such as an increase in membrane lipid classes, and a decrease of choline, BCAAs and oxidized methionine. Such changes were accompanied by a reduction in the serum concentrations of pro-inflammatory cytokines such as IL-17A and TNF- α , and by an increase of IL-4, an interleukin with anti-inflammatory properties. Lastly, the study showed that multicomponent intervention reduced blood pressure and improved cardiac autonomic function in childhood obesity. Secondary analysis showed that *Roseburia* genus was negatively correlated with BCAA, ADMA, and TMAO metabolites, while *Dialister*, *Odoribacter* and *Monoglobus* were positively correlated with these metabolites. Serum levels of BCAA were positively correlated with sympathetic indices of HRV and negatively correlated with parasympathetic indices. *Roseburia* genus was positively correlated with parasympathetic indices of HRV and negatively correlated with sympathetic indices.

The assessment of different parameters of body composition in pediatric population is an important tool due to the rapid periods of growth and physical development (36). In the present study, BMI-Z, WHR, and BF were reduced in children with obesity after the multicomponent intervention, indicating that there was an increase in lean mass, and a reduction in fat mass, a classical result of physical exercise interventions (37).

The observed reduction in serum levels of lipid profile parameters such as total cholesterol, HDL-c and triglycerides, is associated with a reduction in body fat and an improvement in metabolism likely related to higher capacity for lipid oxidation for energy production redirecting proteins to muscle building (38). In addition, an increase in serum albumin supports the anthropometric data of greater muscle mass synthesis, since these values may represent a greater protein turnover required for protein synthesis (39). Urea represents a pathway for eliminating the amine part of amino acids,

and its reduction may be related to an increase in protein synthesis for muscle building. Furthermore, the reduction in serum urea levels is an important finding given the association of high serum urea levels with components of the metabolic syndrome and non-communicable diseases, including obesity, insulin resistance, dyslipidemia, arterial hypertension and chronic kidney disease (40).

Intervention studies have already found positive effects on body composition and biochemical variables, especially lipid profile. A multicomponent intervention consisting of dietary, physical activity, and environmental approaches was implemented during the school year in three different regions of China with 1392 children aged 8-10 years in the school environment. The researchers reported a reduction in BMI in the intervention group compared with the control group (41). A 20-week aerobic and resistance exercise intervention improved cardiovascular health in children with overweight and obesity, with beneficial effects mainly on body fat mass and LDL-c (42). In addition, a prospective 5-month crossover randomized clinical trial investigated the effects of mostly high-intensity physical exercise combined with FNE for children with obesity aged 5-10 years and their parents. Among the results, the percentage of fat mass decreased in all participants, with a statistical difference compared to the no intervention group, which was also seen in serum triglycerides levels (43).

It has been suggested that changes in the gut microbiota can contribute to exercise-induced weight loss in obesity, but the taxon-level variation remains to be explored (44) especially in children. The analysis of the gut microbiota provides pioneer information allowing therefore a deeper understanding of this topic. Although alpha and beta diversity has not been changed after the multicomponent intervention, we found important changes in bacterial phyla, and genera. In agreement with a previous study, physical exercise reduced the relative abundance of Proteobacteria in childhood obesity suggesting that Proteobacteria is an exercise-responsive phylum (19).

Concerning bacterial genera, the multicomponent intervention reduced bacterial genera with anaerobic characteristics, such as *Alistipes*, *Faecalibacterium*, *Escherichia shigella* and *Odoribacter*, and *Methanobrevibacter*. The relative abundance of *Odoribacter* and *Escherichia shigella* is higher in individuals with obesity than lean control subjects (45, 46). The reduction in anaerobic bacteria may be associated with increased parasympathetic flow to the gut, resulting in increased blood perfusion and

oxygenation. There is evidence of an association between hemodynamic parameters improved by exercise and diet and beneficial modulation of the gut microbiota in adolescents with obesity (47).

The physical exercise protocol combined with FNE modulated the serum metabolome of children with obesity. The multi-component intervention increased the levels of plasmeyl phosphatidylethanolamines and phosphatidylglycerols. Plasmeyl phosphatidylethanolamines are more abundant in neuronal and cardiac membranes and can be modulated by FNE through dietary modification (48). These substances have been found to contribute to mechano-transduction in muscle, converting the mechanical stimulus of exercise into a chemical stimulus and inducing adaptations to physical exercise (49). In addition, the intervention reduced serum levels of BCAA and choline, the oxidation of methionine and the synthesis of taurine. High serum levels of BCAA are associated with a higher risk of mortality from cardiovascular disease (50). A systematic review and meta-analysis of 17 studies involving 33,009 individuals found that elevated serum choline levels are also positively associated with increased cardiovascular risk and all-cause mortality (51), although the mechanisms are not elucidated. They could involve a role of TMAO, a choline metabolite, which has been extensively described in the literature as increasing cardiovascular risk through vascular inflammation (52). Furthermore, a reduction in circulating choline may be related to increased production of acetylcholine, a very important neurotransmitter in the parasympathetic nervous system (53).

Regarding the metabolism indicators, the reduction in the methionine oxidation pathway could suggest a reduction in oxidative stress and a consequent lower atherosclerotic risk in individuals following the intervention (54). Taurine plays healthy-related roles in relation to obesity (55), so the reduction in the rate of taurine synthesis may indicate that levels are adequate for these functions to occur. The results indicate an increase in the activity of enzymes such as phosphatidylserine decarboxylase, which is involved in membrane biogenesis (56), and monoacylglycerol lipase, which breaks down triglycerides into fatty acids and glycerol, improving lipid metabolism (57). In addition, a depletion of the phosphatidylserine decarboxylase enzyme has been shown to result in a reduction of mitochondrial

phosphatidylethanolamines, which appears to play an important role in uncoupling protein 1-dependent thermogenesis 1 (58).

The multicomponent intervention decreased serum levels of pro-inflammatory cytokines such as IL-17A and TNF- α and increased IL-4, an anti-inflammatory cytokine. Obesity is associated with chronic low-grade inflammation, contributing to neuroinflammation and sympathetic hyperactivity (11). Proinflammatory cytokines produced in peripheral sites such as the gut and adipose tissue reached the systemic circulation and can be recognized by the subfornical organ, a region unprotected by the blood-brain barrier (BBB), which projects to the paraventricular nucleus and induces microglial activation and neuroinflammation (11). In addition, endotoxins such as LPS can cross the BBB and interact with toll-like receptors 4 (TLR4), increasing the synthesis of proinflammatory cytokines and microglial activation (11). In obesity and in situation of gut dysbiosis, there is also a reduction in the vagal reflex, which leads to a reduced immune response to inflammation, resulting in a reduction in the hypothalamic-pituitary-adrenal (HPA) axis activity and consequently reduced acetylcholine secretion (11, 59). One study showed that the consumption of a high-fat diet and consequent obesity led to the withdrawal of vagal afferents from the intestine to the nucleus of the solitary tract, with significant microglial activation in the nodose ganglia, which contain the vagal afferent neuron cell bodies (59).

Regarding the cardiovascular variables, an improvement in HR, SBP and DBP was observed after intervention, giving an indication of a better cardiac autonomic control. In addition to these positive changes in HR and BP, we observed an improvement in indices derived from HRV in the time domain and in non-variable indices, reinforcing the improvement in cardiac autonomic control and indicating a greater efficiency of the mechanisms involved in this process.

Other studies carried in children with obesity have shown that interventions focused on improving physical activity levels and dietary choices have obtained good results on cardiovascular variables. A 20-week randomized clinical trial demonstrated the beneficial effects of exercise combined with lifestyle guidelines on brain function in children (60). Since 1997, there has been evidence of the beneficial effects of exercise on HRV in children with obesity (61). An aerobic exercise protocol had positive effects in children with obesity, reducing LF/HF and increasing HF, RMSSD and SDNN

compared to baseline (62). It was demonstrated that 12 weeks of 2 sessions of 60 minutes per week of judo reduces cardiac autonomic dysfunction in obese children by reducing LF/HF and increasing HF (63).

Multicomponent interventions combining physical exercise and dietetic and educational interventions can improve cardiac autonomic function (64, 65). For example, physical exercise combined with a low-calorie diet improved HR recovery one minute after exercise and cardiac autonomic function in children with obesity (64). In addition, a study conducted in a school setting that included physical activity and FNE showed that although there was an improvement in autonomic function in children with obesity by an increase in relative amplitude and constriction velocity, which represents parasympathetic function (65). The findings of the present study showed that the protocol combining different types of physical exercise and dietary and FNE protocol organized in a scholar environment increased parasympathetic components and short-term and long-term variability of HR in children with obesity.

To our knowledge, this is the first study to demonstrate that the improvement of cardiac autonomic function promoted by multicomponent intervention protocol was accompanied for improvement in the gut microbiome in children with obesity. This relationship was further explored through correlation analyses between gut microbiome parameters and indices related to HRV.

Serum BCAA levels were positively correlated with increased HRV indices that indicate sympathetic activity and negatively correlated with parasympathetic indices. In addition, there was a positive correlation with anthropometric indices, such as WHR, BMI-Z, weight and BFP. Meta-analysis has related high BCAA levels to increased cardiovascular risk, but there is still a gap in the scientific literature regarding the mechanisms by which this occurs (50, 66). This leads us to hypothesize that bacteria associated with BCAA production may contribute to increased sympathetic activity in children with obesity. In this sense, several bacterial genera were strongly positively correlated with serum BCAA levels, such as *Akkermansia*, *Flavonifractor*, *Bacteroides* and *Alistipes*, and others, such as *Intestinebacter* and *Coproccoccus*, were negatively correlated. *Copropoccus*, a genus characterized by short chain fatty acids (SCFA)-producing bacteria, was also negatively correlated with BMI-Z, WHR, and SBP. Because the correlation showed before, we think that the reduction in the genus

Alistipes caused by the intervention may have contributed to the reduction in serum BCAA levels. ADMA, an important marker of cardiovascular risk related to inflammation and atherosclerosis (67, 68), was also correlated with anthropometric parameters and HR, and negatively with indices of parasympathetic activity. Regarding ADMA, *Odoribacter* was positively correlated and *Roseburia* was negatively correlated.

Some bacterial genera seem to have a general effect on the main variables studied in this research. *Roseburia*, in addition to being negatively correlated with serum BCAA levels, was also negatively correlated with anthropometric indices characteristic of obesity, SBP, and indices related to HRV of sympathetic activity (LF), and positively correlated with indices of HRV related to sympathetic activity (RMSSD). In addition, *Roseburia* was negatively correlated with cardiovascular risk markers such as ADMA and TMAO. These data reinforce evidence that bacteria of the genus *Roseburia*, producers of SCFA, are important health markers and the reduction in the relative abundance of this genus may be detrimental to health (69).

The genus *Monoglobus* was associated with increased BCAA levels and BP. In addition, increased *Blautia* was also positively correlated with increased DBP and decreased HF. These two genera have been associated with cardiovascular disease, such as coronary heart disease (70). In addition, elevated TMAO was positively correlated with *Dialister* and negatively correlated with *Parabacteroides*. The genus *Dialister* has previously been reported in the literature to be positively correlated with elevated TMAO levels (71), and the genus *Parabacteroides* has been associated with reduced inflammation, primarily through the production of SCFA (72).

A potential limitation of the study was that it was an uncontrolled clinical trial due to the need to perform the intervention on all children classified as having a nutritional status of obesity, a decision based on ethical precepts due to the socio-economic characteristics of the place where the study was conducted. In addition, the sample size of the study is not large, which may affect the FDR values. However, we believe that the set of variables analyzed and recorded represents a good diagnosis of the effects of the multicomponent intervention on children with obesity in their school environment.

5. Conclusion

This study demonstrated that a multicomponent intervention consisting of physical exercise and FNE modulates the gut microbiome, reduces systemic inflammation, and improves the cardiac autonomic function in children with obesity. Furthermore, correlations between gut microbiota, metabolites and autonomic function provide new perspectives in the study of this topic and provide novel information about this interaction, especially regarding the relationships of bacterial genera with metabolites and autonomic function. However, mechanistic studies targeting the gut-brain axis should be performed in the future to elucidate these relationships. Finally, this study provides relevant information for improving lifestyle modification strategies in the treatment of childhood obesity in a school environment.

634

635 **Abbreviations**

636 ADMA - Asymmetric dimethylarginine

637 ALT - Alanine transaminase

638 AST - Aspartate aminotransferase

639 BBB - Blood-brain barrier

640 BCAA - Branched-chain amino acids

641 BFP – Body fat percentage

642 BP – Blood pressure

643 BMI-Z - Body mass index-for-age (z score)

644 DFA - Detrended fluctuation analysis

645 DBP – Diastolic blood pressure

646 FDR - Benjamini-Hochberg equation

647 FNE – Food and nutrition education

648 GGT - Gamma-glutamyl transferase

649 HbA1c - Glycated hemoglobin

650 HDL-c - High-density lipoprotein cholesterol

651 HF - High frequency band

652 HRV – Heart rate variability

653 HPA - Hypothalamic-pituitary-adrenal

654 IFN- γ - Interferon- γ

655 IL - Interleukin

656 hs-CRP - High sensitivity c-reactive protein

657 LDL-c - Low-density lipoprotein cholesterol

658 LF - Low frequency band

659 LPS - Lipopolysaccharides

660 NCBI - National center for biotechnology information

661 PCA - Principal component analysis

662 qPCR – Quantitative real-time PCR

663 QIIME - Quantitative insights into microbial ecology

664 RMSSD - Squares of the successive differences between adjacent normal-to-normal
665 beats

666 SBP - Systolic blood pressure
 667 SDNN - Standard deviation between the duration of NN intervals
 668 SD1 - Standard deviation of the instantaneous variability of continuous NN intervals in
 669 the Poincaré graph
 670 SD2 - Standard deviation of long-term continuous NN intervals
 671 TLR4 - Tool-like receptors 4
 672 TNF- α - Tumor necrosis factor- α
 673 TMAO - Trimethylamine N-oxide
 674 WHR - Waist-hip ratio
 675

676 **CRedit authorship contribution statement**

677 **Paulo César Trindade da Costa:** Writing – original draft & editing, Validation,
 678 Software, Investigation, Conceptualization; **Marciane Magnani:** Writing – review &
 679 editing; **Vinicius José Baccin Martins:** Writing – review & editing, Supervision;
 680 **Rúbia Cartaxo Squizato de Moraes:** Investigation; **Cristiane Cosmo Silva-Luis:**
 681 Investigation; **José Milton de Araújo Rodrigues:** Investigation; **Lucélia Cabral:**
 682 Software; **Melline Fontes Noronha:** Software; **Oriane Vitalis:** Investigation; **Karim**
 683 **Chikh:** Investigation; **Murielle Godet:** Writing – review & editing; **Hubert Vidal:**
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694 **Ethics approval and consent to participate**

695 This study was conducted in agreement with the declaration of Helsinki. The study was
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Declaration of competing interest

There is no conflict of interest related to the manuscript.

Data availability

The original data presented in this study are included in the article/Supplementary Material, and further inquiries can be directed to the corresponding authors. In addition, FastQ sequency may be accessed in NCBI (PRJNA1255541).

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922 **Table 4.** Anthropometric and biochemistry variables before and after the intervention in
 923 children with obesity (n=51)

Variables	Before intervention	After intervention	p-value
Weight (Kg) †	48.50 (35.50 – 90.30)	49.00 (36.90 – 99.00)	<0.0001
BMI-Z†	2.62 (1.92 – 5.66)	2.54 (1.01 – 5.57)	<0.0001
WHR (cm)	0.867 ± 0.06	0.847 ± 0.06	0.0016
BFP (%)†	35.00 (22.70 – 58.80)	33.00 (23.30 – 51.74)	0.0005
Albumin (g / dL)	4.58 ± 0.28	4.76 ± 0.30	<0.0001
FG (mg / dL)	83.90 ± 10.47	80.56 ± 6.80	0.214
HbA1C (%) †	4.60 (3.20 – 7.80)	4.10 (4.70 – 8.80)	0.147
ALT (U / L) †	25.00 (2.00 - 96.00)	16.00 (2.00 – 77.00)	0.055
AST (U / L) †	38.00 (19.00 - 62.00)	32.00 (14 – 105.00)	0.818
GGT (U / L) †	18.00 (4.00 – 162.00)	16.00 (2.00 – 155.00)	0.563
TC (mg / dL) †	122.00 (127.00 – 249.00)	141.00 (129.00 – 270.00)	0.0003
LDL-c (mg / dL) †	84.50 (65.00 – 143.00)	92.00 (58.00 – 160.00)	0.148
HDL-c (mg / dL) †	46.50 (27.00 – 82.00)	52.00 (30.00 – 82.00)	<0.0001
TG (mg / dL)†	95.00 (38.00 – 280.00)	71.00 (25.00 – 273.00)	0.027
Urea (mg / dL)	21.88 ± 5.97	18.38 ± 5.26	0.004
Creatinine (mg / dL) †	0.57 (0.36 – 0.91)	0.52 (0.38 – 0.77)	0.129
c-RP (mg / dL) †	1.45 (0.20 – 13.40)	1.10 (0.10 – 15.50)	0.849

924 BMI-Z: body mass index-for-age (z-score); WHR: waist-hip ratio; BFP: body fat
 925 percent; FG: fasting glucose; AST: aspartate aminotransferase; ALT: alanine
 926 aminotransferase; GGT: gamma-glutamyl transferase; TC: total-cholesterol; LDL-c:
 927 low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; TG:
 928 triglycerides; CRP: C reactive protein. †: non-parametric data.

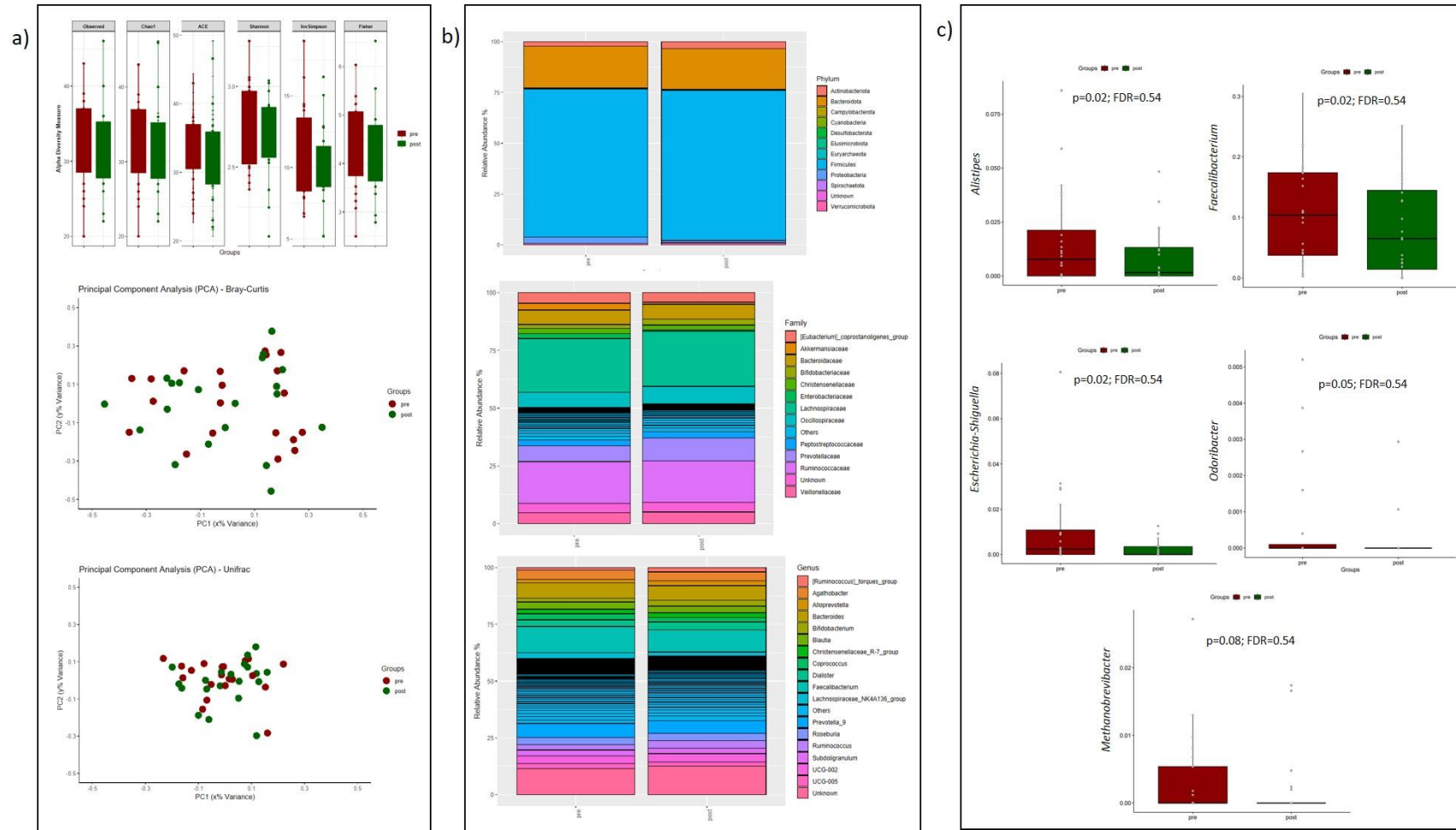
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Table 5. Blood pressure and heart rate variability indices before and after the intervention in children with obesity (n=51)

HRV Indices	Before intervention	After intervention	<i>p</i> -value
HR (bpm)	96.88 ± 11.60	93.16 ± 8.95	0.044
DBP (mmHg)	63.00 (45.00 – 94.00)	61.00 (39.00 – 90.00)	0.026
SBP (mmHg)	109.70 ± 11.89	104.90 ± 9.44	0.005
RMSSD (ms) †	26.50 (7.20 – 92.50)	31.40 (10.80 – 78.50)	0.018
SDNN (ms) †	33.20 (11.00 – 84.90)	35.40 (19.30 – 77.70)	0.043
LF (nu)	63.51 ± 13.95	60.72 ± 15.14	0.198
HF (nu)	36.43 ± 13.92	39.19 ± 15.11	0.202
LF/HF†	1.83 (0.43 – 5.91)	1.64 (0.37 – 7.20)	0.246
SD1 (ms) †	18.70 (5.10 – 65.50)	22.20 (7.70 – 55.50)	0.021
SD2 (ms) †	41.90 (14.50 – 100.20)	46.50 (24.60 – 94.90)	0.062
SD2/SD1 (ms) †	2.37 (1.39 – 3.65)	1.94 (1.20 – 4.05)	0.014
DFA- α1	1.185 ± 0.200	1.111 ± 0.211	0.025
DFA- α2	0.586 ± 0.121	0.551 ± 0.121	0.083

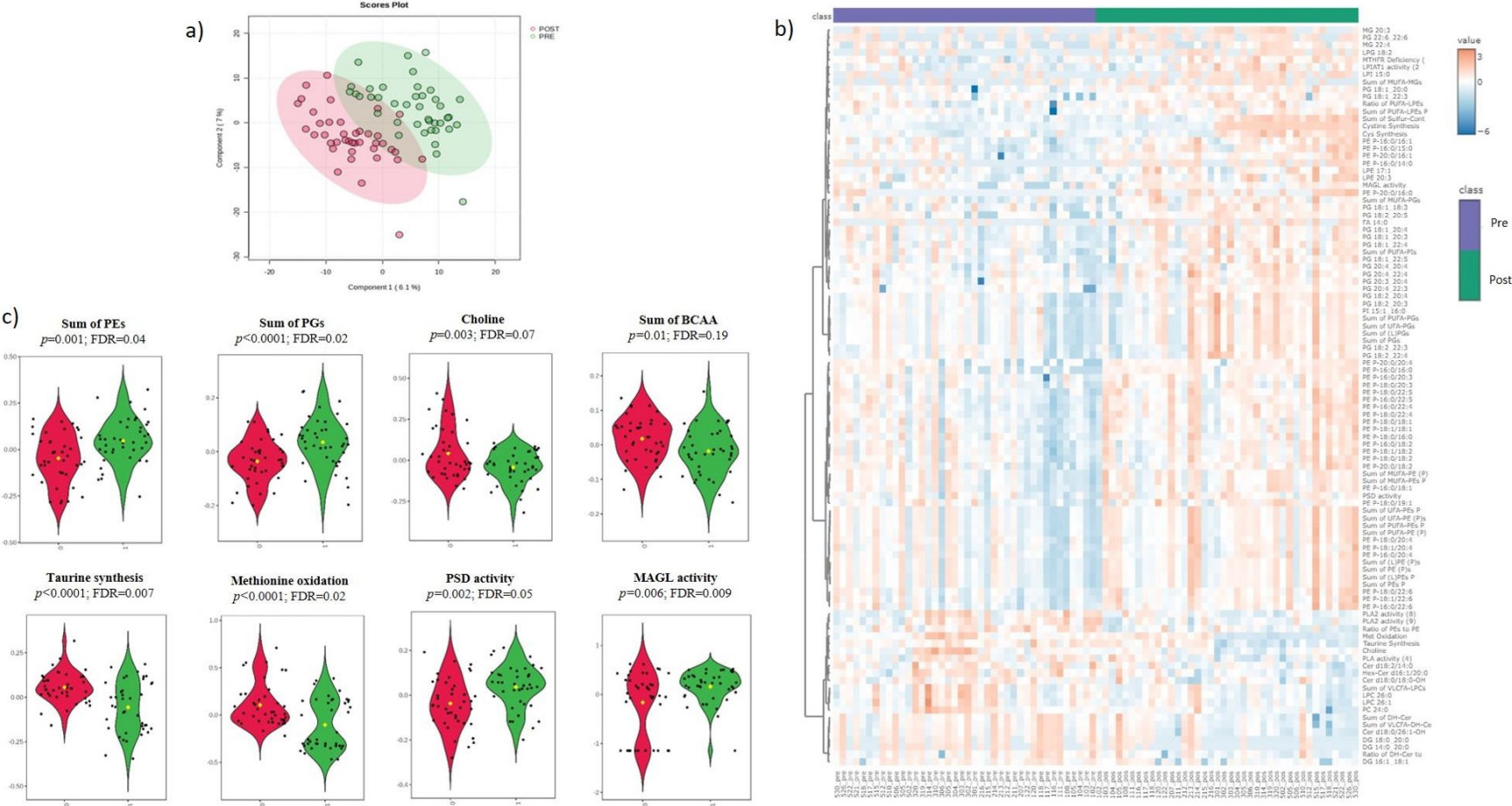
Paired T-test to analyze of difference between HRV indices during training before and after intervention. HRV= heart rate variability; RMSSD: square root of the mean of the square of the differences between consecutive NN intervals; SDNN: standard deviation between NN intervals; LF: low frequency; HF: high frequency; SD1: standard deviation associated with instantaneous R-R variability; SD2: continuous long-term R-R variability; DFA: detrended fluctuation analysis; ms: milliseconds; nu: normalized units. †: non-parametric data

942 **Figure 1.** Gut microbiota analysis before and after the intervention in children with obesity (n=20)



943 a: Alpha diversity and Beta diversity (Bray-Curtis and Unifrac); b: Phylum, Family and Genus relative abundance; c: Individual presentation of
944 important Genus
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946 **Figure 2.** Metabolomics analysis before and after the intervention in children with obesity (n=40)

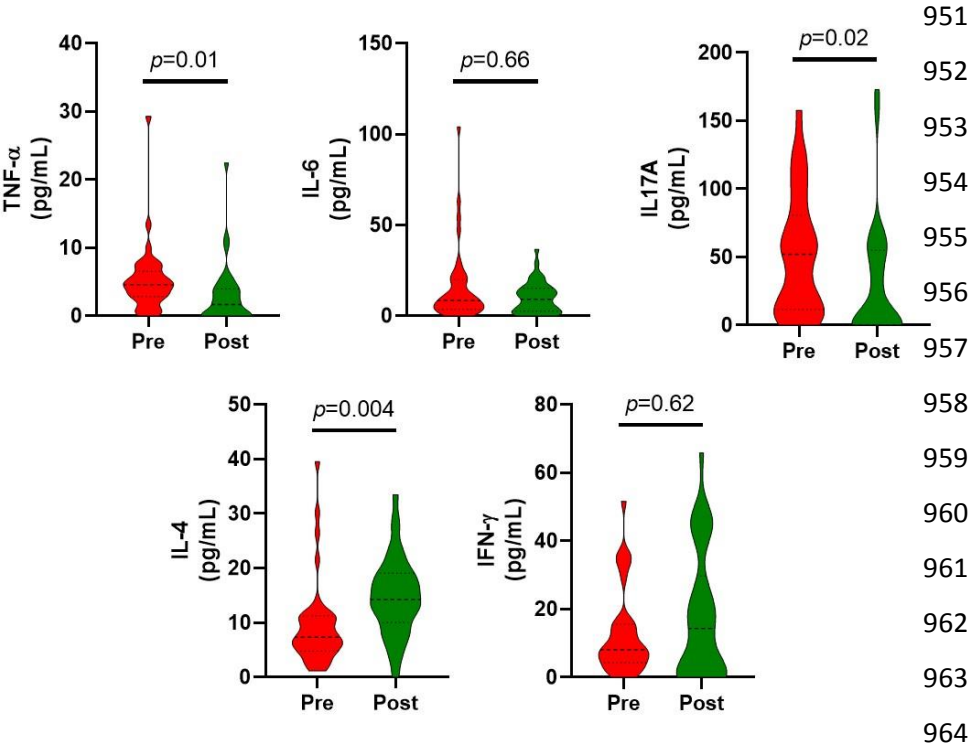


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948 PEs: Plasmeryl phosphatidylethanolamines; PGs: Phosphatidylglycerol; BCAA: Branched-chain amino acids; PSD: phosphatidylserine decarboxylase; MAGL:

949 monoacylglycerol lipase

950 **Figure 3.** Cytokines analysis before and after intervention in children with obesity (n=51)



965 TNF-α: tumor necrosis factor-α; IL-6: interleukin 6; IL-17A: interleukin 17A; IL-4: interleukin 4; IFN-γ: interferon-γ.

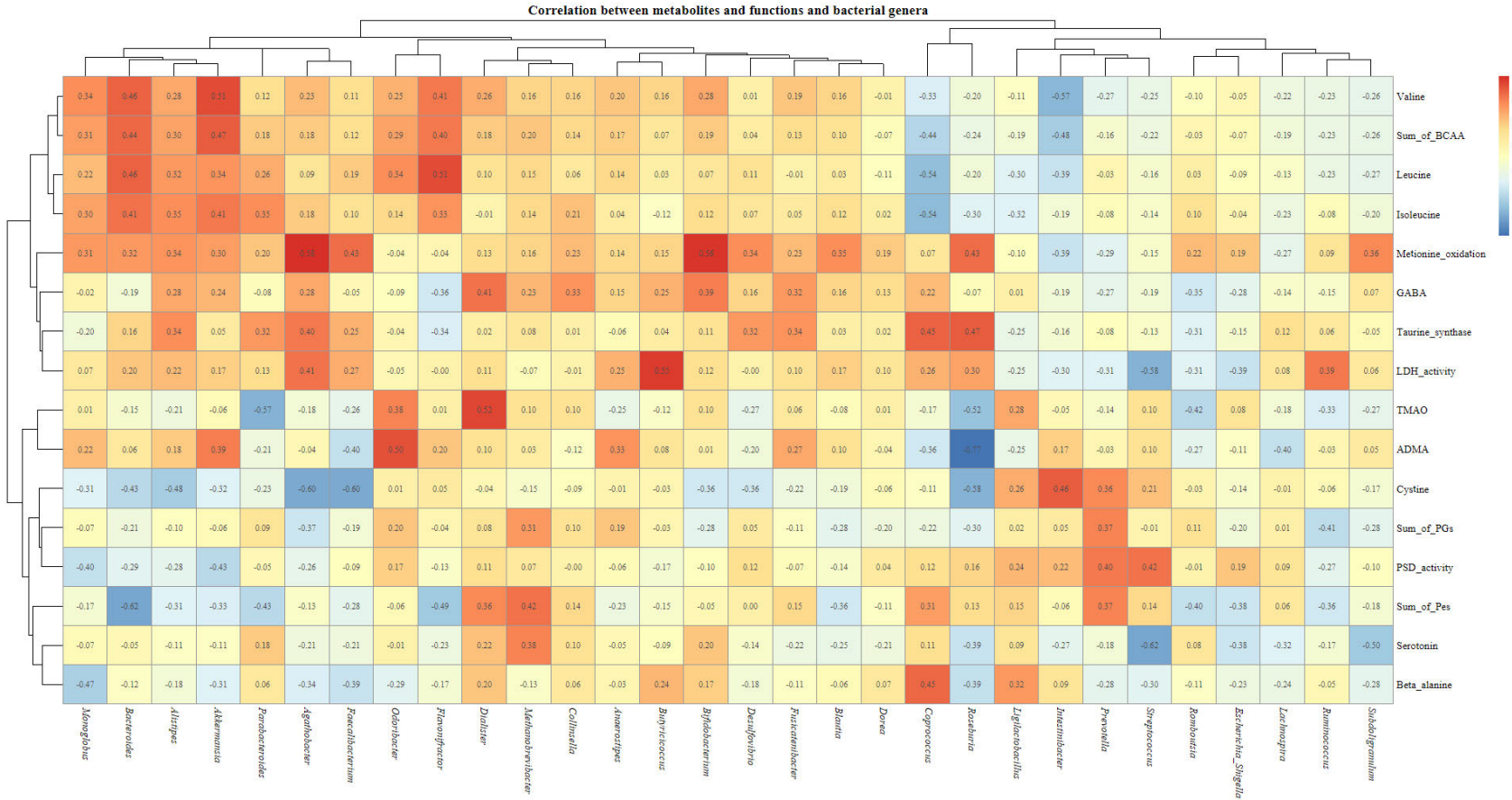
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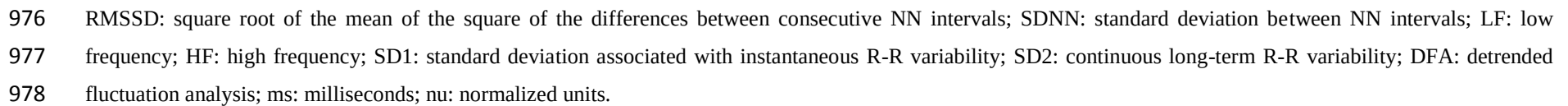
970 **Figure 4.** Correlation between metabolites and functions and bacterial genera



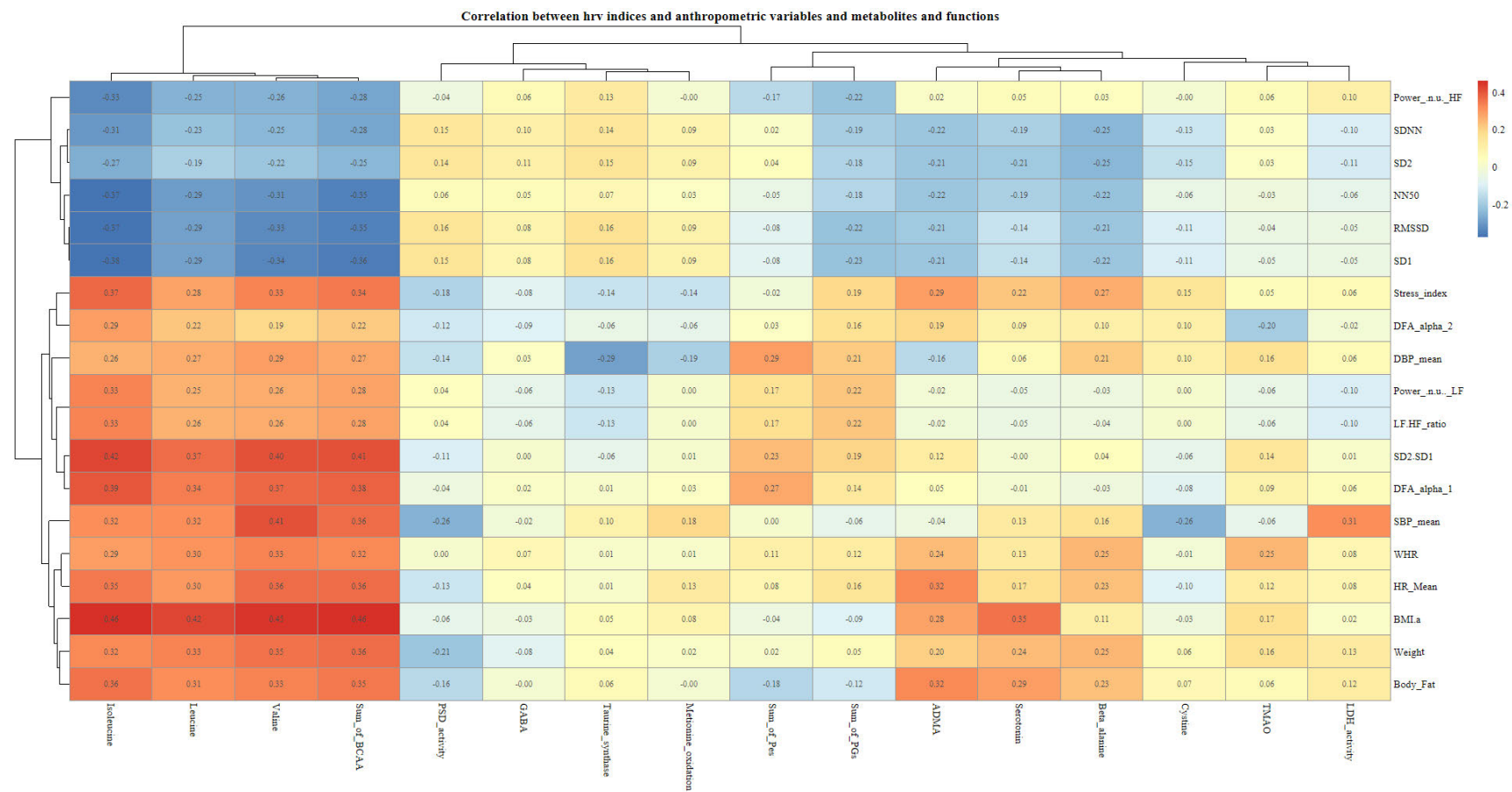
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972 BCAA: Branched-chain amino acids; GABA: Gamma-AminoButyric Acid; LDH: Lactate dehydrogenase; TMAO: trimethylamine N-oxide; ADMA: Asymmetric

973 Dimethylarginine; PSD: phosphatidylserine decarboxylase; PEs: Plasmeyl phosphatidylethanolamines; PGs: Phosphatidylglycerol



979 **Figure 6.** Correlation between HRV-related indices and anthropometric indices and metabolites and functions



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982 RMSSD: square root of the mean of the square of the differences between consecutive NN intervals; SDNN: standard deviation between NN intervals; LF: low
983 frequency; HF: high frequency; SD1: standard deviation associated with instantaneous R-R variability; SD2: continuous long-term R-R variability; DFA: detrended
984 fluctuation analysis; ms: milliseconds; nu: normalized units. BCAA: Branched-chain amino acids; GABA: Gamma-AminoButyric Acid; LDH: Lactate
985 dehydrogenase; TMAO: trimethylamine N-oxide; ADMA: Asymmetric Dimethylarginine; PSD: phosphatidylserine decarboxylase; PEs: Plasmeyl
986 phosphatidylethanolamines; PGs: Phosphatidylglycerol

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APÊNDICE 6 – Artigo que será submetido à Microbiome (ARTIGO 6)

Aerobic physical exercise decreases cardiac autonomic dysfunction by modulating gut microbiota and gut-brain axis in rats fed a high-fat diet

Abstract

Obesity is a complex condition influenced by multiple factors, especially when exposure to nutritional stressors begins in childhood. Evidence shows that the pathogenesis of obesity may be related to impairments in the gut-brain axis. Therefore, approaches that modulate the gut microbiota, such as physical exercise, may improve compromised gut integrity, peripheral inflammation, neuroinflammation, and autonomic dysfunction. This study investigated the effects of aerobic exercise on the gut microbiota composition, intestinal barrier markers, intestinal and nervous tissue inflammatory biomarkers, and cardiac autonomic function in rats fed a high-fat diet (HFD).. This study showed that the Verrucomicrobiota decreased in the HFD+PE group compared to the HFD group. At the family level, PE increased the relative abundance of UCG.10, Monoglobaceae, and Rumonococcaceae while decreasing Atopobiaceae, Chlamydiaceae, and Erysipelotrichaceae. At the genus level, PE decreased the relative abundance of *Adlercreutzia*, *Frisingicoccus*, *Fusicatenibacter*, and *Dubosiella*, while increasing *Butyricimonas* and *Roseburia*. Furthermore, PE increased the expression of the occludin gene in the colon, enhancing intestinal barrier integrity, and decreased inflammatory markers, including TNF- α , MCP1, NLRP3, caspase 1, and IL-1 β . Additionally, PE increased the expression of VIP (vasoactive intestinal peptide), which is associated with vagal activity... PE was able to reduce SBP and MAP, as well as improve autonomic activity. Consistent with the physiological data, PE reduced the expression levels of genes related to sympathetic activity in the brainstem, such as BDNF and NOS1. Additionally, secondary analyses revealed correlations between colonic and brainstem genes related to inflammation, barrier activity, and autonomic function and bacterial genera. Notably, there was a negative correlation between *Roseburia* and colonic genes related to inflammation, as well as with BDNF and NOS1 in the brainstem, which influence autonomic activity. These results suggest that PE enhances the communication between the gut and the brainstem and that reducing inflammation is essential for improving this interaction in rats with obesity.

1. Introduction

Obesity is a complex condition influenced by multiple factors, including genetics, socioeconomic status, and environmental influences, and is widely recognized as a major contributor to various health complications (1).

High-fat diet-induced obesity is associated with a chronic low-grade inflammatory state, hypothalamic inflammation, and a range of cardiometabolic disturbances, including insulin resistance, elevated blood pressure, and impaired autonomic control of heart rate (2). While the mechanisms linking obesity, inflammation, and autonomic dysfunction are not fully understood, evidence suggests a key role of gut microbiota dysbiosis (3). This condition is marked by an imbalance in the composition and diversity of intestinal bacteria, compromised gut barrier integrity, and increased translocation of lipopolysaccharides (LPS), free fatty acids, and pro-inflammatory cytokines into systemic circulation (4).

A pro-inflammatory environment has been implicated in the development of neuroinflammation and cardiac autonomic dysfunction, characterized by increased sympathetic tone and withdrawal of parasympathetic activity, contributing to increased blood pressure, and reduced heart rate variability (HRV) (5, 6).

Lifestyle interventions, particularly those involving regular physical activity, are foundational in managing metabolic complications related to obesity. It is recognized that physical exercise mitigates high-fat induced-obesity (7) and improves autonomic function through neural mechanisms involving the modulation of arterial and cardiopulmonary baroreceptors and peripheral chemoreceptors. Additionally, recent studies suggest that physical exercise may alter gut microbiota composition and reduce systemic inflammation in obesity condition (8).

Although exercise is known to influence the microbiota-gut-brain axis via vagal pathways (8), direct evidence linking physical exercise-induced changes in gut microbiome to improvements in cardiac autonomic function in obesity condition remains to be investigated. Therefore, the present study aimed to evaluate the effects of six weeks of aerobic physical exercise on the modulation of gut microbiome, gut-brain inflammation and cardiac autonomic function of rats fed a high-fat diet. We

hypothesized that aerobic physical exercise modulate the gut microbiome attenuate inflammation and improve autonomic function in rats fed a high-fat diet.

Methods

Animals and Ethical aspects

Thirty male *Wistar* rats (*Rattus norvegicus*, 21 days old) were used in the study. The animals were housed in polypropylene cages (3 animals/cage) under controlled temperature (21 ± 1 °C), humidity between 50-55% and a 12-hour light/dark cycle, with free access to water and food. All experimental procedures were submitted to and approved by the Institutional Animal Care and Use Committee of the Federal University of Paraíba (CEUA-UFPB Protocol No. 4724190723) and followed the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) and the International Principles for Biomedical Research Involving Animals.

Experimental design

The rats were allocated into 3 groups: Control group (CTL, n=5) fed with a control diet; HFD group (n=5), fed with a high-fat diet; and HFD and Physical Exercise (HFD+PE, n=5). Additionally, the HFD and HFD+PE animals began consuming diet and sugary drink at 21 days of age and continued until the end of the experiment. At 80 days, the animals in the HFD+PE group began performing aerobic exercise five days a week for six weeks.

Anthropometric measurements were assessed throughout the experimental period. After six weeks of training, the animals underwent experimental protocols divided into two stages. The first stage included metabolic, biomolecular, and gut microbiota analyses (n = 15). The second stage investigated blood pressure and autonomic function (n = 15).

Aerobic physical exercise

The moderate aerobic exercise protocol was designed according to previous studies (9-11). The duration and intensity of the exercise was progressively increased each week as described in Table 1.

Table 1. Progression of duration and intensity of aerobic physical exercise

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
15 min at 5 m/s	35 min at 13 m/s	45 min at 13 m/s	50 min at 14 m/s	55 min at 14 m/s	60 min at 15 m/s
20 min at 6 m/s	35 min at 13 m/s	45 min at 13 m/s	50 min at 14 m/s	55 min at 14 m/s	60 min at 15 m/s
25 min at 7 m/s	35 min at 13 m/s	45 min at 13 m/s	50 min at 14 m/s	55 min at 14 m/s	60 min at 15 m/s
27.5 min at 8 m/s	35 min at 13 m/s	45 min at 13 m/s	50 min at 14 m/s	55 min at 14 m/s	60 min at 15 m/s
30 min at 9 m/s	35 min at 13 m/s	45 min at 13 m/s	50 min at 14 m/s	55 min at 14 m/s	60 min at 15 m/s

Min: minutes; m/s: meter/second

Anthropometry and Diet

The animals were also weighed weekly throughout the experiment using a balance (model AS-1000; Marte, Santa Rita, Minas Gerais). The control diet was prepared according to the American Institute of Nutrition - AIN-93M and the HFD was purchased from the company Rhooster® (Araçoiaba da Serra, São Paulo, Brazil). Regarding the dietary composition, there is a difference between both in the caloric percentages of proteins (12.85 kcal % HFD vs. 20.27 kcal % CTL) and carbohydrates (40.16 kcal % HFD vs. 63.76 kcal % CTL), while the percentage of lipids is high only in the HFD diet (46.98 kcal % HFD vs. 15.96 kcal % CTL). In terms of lipid sources, the CTL diet contains 15.9% soybean oil, while the HFD diet is composed of 3.61% soybean oil to which 27.10% animal fat and 16.29% non-hydrogenated vegetable fat are added. The composition of the CTL and HFD diets is shown in Table S1.

RNA extraction and real-time quantitative PCR (RT-qPCR)

Total RNA from several tissues (colon and brainstem) were extracted using TRI-Reagent (Sigma Aldrich, ref #T9424), in accordance with the manufacturer's instructions. First-strand cDNAs were synthesized from 1 µg of total RNAs using TAKARA Prime Script™ RT Reagent (TAKARA Bio, ref #RR037A). Purity and

concentration of RNAs were determined using NanodropOne (Ozyme, Saint-Cyr-L'Ecole, France), and the quality was checked by using a Bioanalyser (Agilent Technologies, Santa Clara, United-States). Real-time PCR assays were performed with Rotor-Gene Q (Qiagen, Hilden, Germany) using SYBR® Premix Ex-Taq™ (TAKARA Bio, ref #RR420L). TATA-box binding protein (TBP) was used as a reference gene to normalize the results, as previously reported (12). Primers are listed in Table 1.

Metataxonomic analysis and data processing

Analysis of the gut microbiota was carried out by sequencing bacterial 16S rRNA in feces by the GENEPII (Genomique Epidémiologique des Maladies Infectieuses) platform (Lyon, France). Purified DNA was extracted from 15 mg of feces with the Zymobiomics DNA microprep kit (Zymo Research, ref #D4301). 40 ng of DNA were used for the generation of libraries targeting the V3-V4 regions (Quick-16S NGS Library Prep Kit, Ozyme) and then sequenced on Illumina MiSeq standard v3. The sequences were “demultiplexed” using Bcl2fastq software (v2.17.1.14) then cleaned with cutadapt (v1.9.1). Amplicon sequence variants (ASVs) were identified using Qiime2 DADA2 with a Zero noise OTU approach. Finally, a taxonomic assignment was made with Greengenes-13.8-nr99 base.

Cardiovascular analysis

Determination of cardiovascular parameters

Rats were anesthetized with ketamine hydrochloride (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) for insertion of polyethylene catheters into the femoral artery and vein (cannulation). The catheters were tunneled through the dorsal region, and then ketoprofen (5 mg/kg) was administered subcutaneously (13). BP and HR were recorded in conscious animals 24 h after surgery. BP and HR recordings were made through the arterial cannula connected to a pressure transducer (ML866/Pro, AD Instruments, Power Lab, Bella Vista, NSW, Australia) as previously described (14). Pulsatile BP (PAP) and HR were recorded for 50-60 min under basal conditions, and systolic BP (SBP),

diastolic BP (DBP), mean BP (MAP) and HR values were calculated for a selection of 10 min for each animal (LabChartTM Pro, ADInstruments, Bella Vista, NSW, Australia) (14).

Autonomic function assessment

After 60 min of baseline BP and HR recordings, vasoactive drugs were administered to assess the autonomic function of the animals. To assess parasympathetic function, methylatropine (2 mg/kg iv; Sigma-Aldrich, St Louis, MO, USA), a muscarinic receptor antagonist, was administered to promote changes in basal HR ($\Delta\text{HR} = \text{HR post-methylatropine} - \text{HR pre-methylatropine}$) (13). After 15 min, propranolol hydrochloride (4 mg/kg, iv; Sigma-Aldrich, St. Louis, MO, USA) was administered to assess sympathetic tone as assessed by changes in HR after drug administration ($\Delta\text{HR} = \text{post-propranolol} - \text{HR pre-propranolol}$) (13).

Statistical analysis

Statistical analyses and graphical representations were performed using RStudio (V2021.09.1 Build 372) and GraphPad Prism 9. For each parameter, normality (Shapiro test), homogeneity of variances (Levene test), and independence of residuals (Durbin Watson test) were tested. Depending on the results, either a Kruskal–Wallis test followed by Benjamini–Hochberg (BH) multiple correction and a Dunn posthoc test or a one-way ANOVA with BH multiple correction followed by a posthoc Tukey test were used to compare all groups with HFD group. Nonparametric Spearman rank correlations between markers of qPCR analysis and gut microbiota analysis were conducted using a Kruskal wallis test. Alpha and beta diversities were calculated under RStudio (version 4.3.3) using the phyloseq and metagenomeseq libraries. Data are presented as the mean $\pm$ SEM. The results were considered significant when $p < 0.05$. Stars were used to illustrate significant differences between all groups compared to HFD group (* $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$).

Results

At weaning, the rats were similar in weight between groups; however, after 60 days on the HFD, it was observed that the HFD group had a significant increase in weight compared to the control group ($p=0.01$). In addition, the average weight gain was lower in the exercise rats, although these results were not statistically significant (Figure 3a).

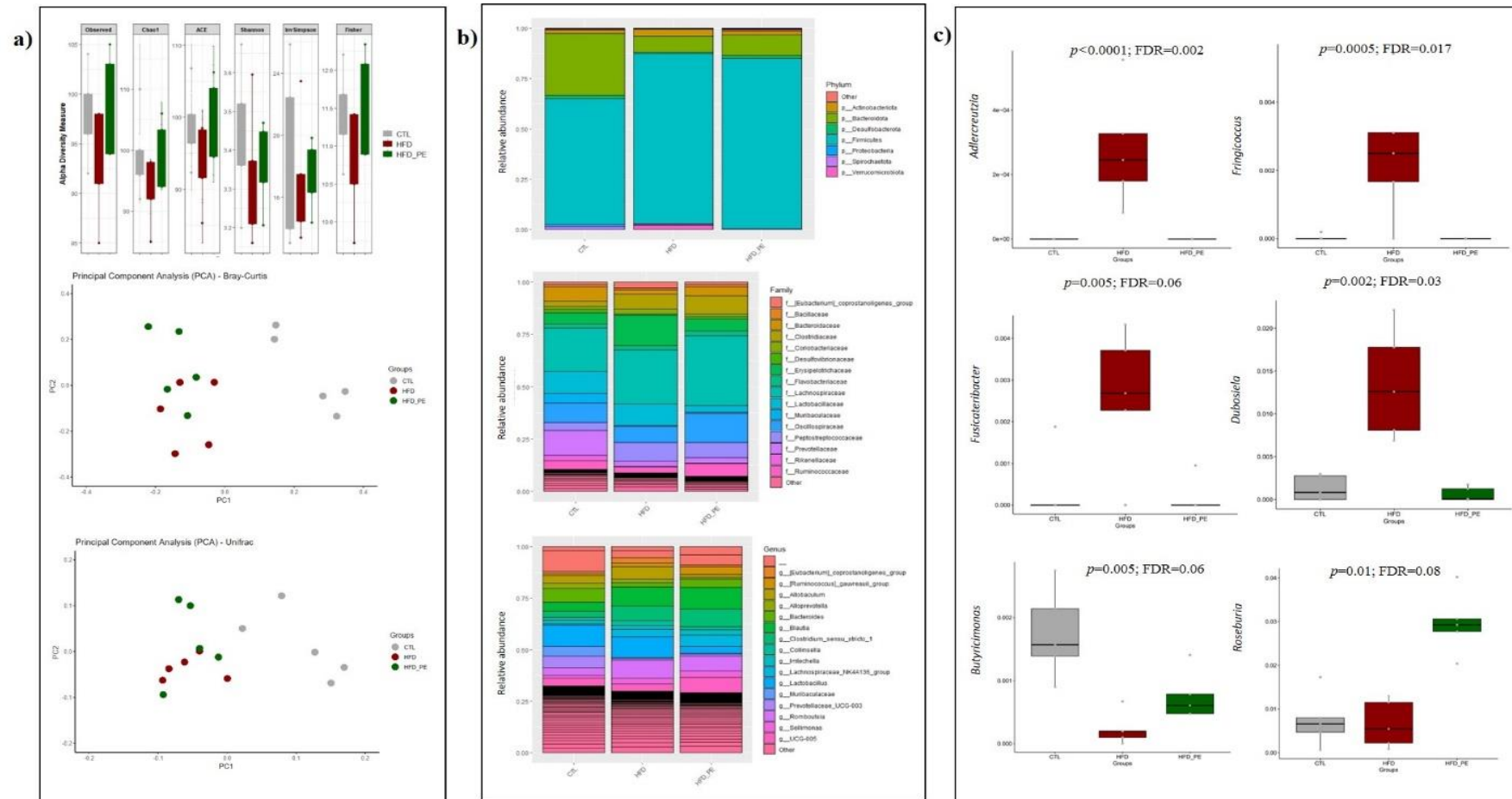
The results of the gut microbiota analysis demonstrate significant differences in beta diversity using both the Unifrac ($p < 0.001$) and Bray-Curtis ($p < 0.001$) methods. As can be seen in Figure 1a, the points in the CTL group are farther apart than those in the HFD-fed animals in the PCA plot. However, there was no statistical difference in alpha diversity indices. Figure 1b shows the graphical distribution of the relative abundance of the main bacterial phyla, families, and genera. The results expressed in Supplementary Table 3 demonstrate that the HFD group had a lower relative abundance of Bacteroidota, Proteobacteria, and Desulfobacterota, as well as a higher abundance of Firmicutes and Verrucomicrobiota, compared to the control group. Furthermore, the Verrucomicrobiota relative abundance decreased in the HFD+PE group compared to the HFD group. At the family level, Table S4 shows that physical exercise increased the relative abundance of UCG.10, Monoglobaceae, and Rumonicoccaceae while decreasing the relative abundance of Atopobiaceae, Chlamydiaceae, and Erysipelotrichaceae compared to the HFD group.

Analysis of the gut microbiota at the genus level revealed that PE decreased the abundance of *Adlercreutzia*, *Frisingicoccus*, *Fusicatenibacter*, and *Dubosiella* while increasing the abundance of *Butyricimonas* and *Roseburia*. Additionally, significant differences in bacterial genera were observed when comparing rats fed a HFD with those fed a standard diet. These differences are described in Supplemental Table 5 and presented graphically in Figure 1c.

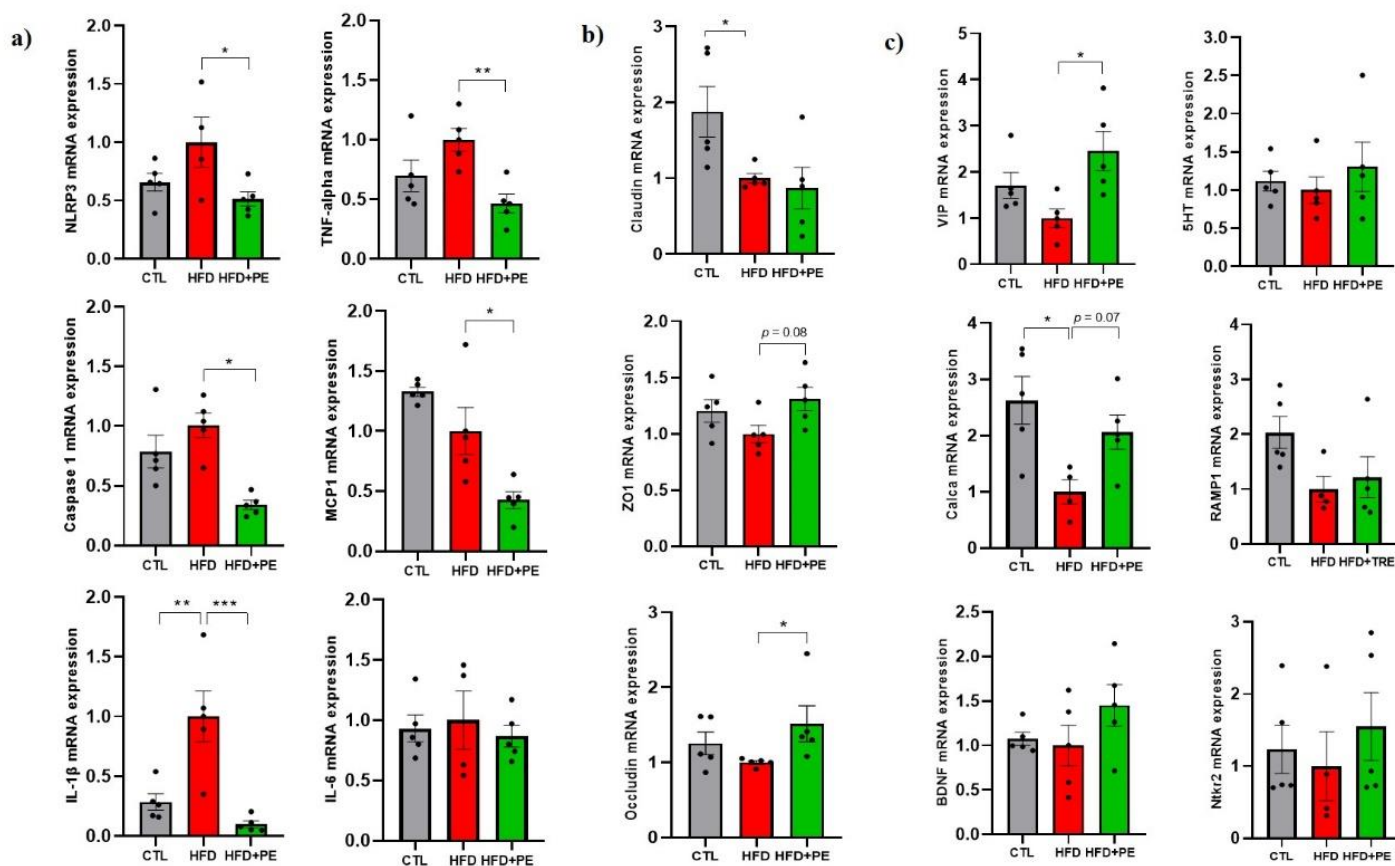
This study identified positive effects of physical exercise on inflammation-related gene expression in the colon. This resulted in reduced expression of genes such as TNF- α and MCP1, as well as others comprising the inflammasome pathway, including NLRP3, caspase-1, and IL-1 β . These positive effects also extended to the

upregulation of junction proteins, such as occludin, related to improving the integrity of the intestinal barrier. Additionally, there was an increase in the expression of genes associated with vagal activity, including vasoactive intestinal peptide (VIP).

Figure 1 - Gut microbiota analysis of rats with obesity



a) Alpha diversity and Beta diversity (Bray-Curtis and Unifrac); b) Phylum, Family and Genus relative abundance; c) Individual presentation of Genus

Figure 2 - Gene expression in the colon of rats with obesity

CTL: control; HFD: high fat diet; HFD+PE: high fat diet + physical exercise; NLRP3: NLR family pyrin domain containing 3; TNF- α : Tumor necrosis factor alpha; IL-1 β : Interleukin 1 β ; MCP1: Monocyte Chemoattractant Protein 1; ZO-1: Zonulin; VIP: Vasoactive intestinal peptide; 5-HT: Serotonin; Calca: calcitonin gene-related peptide; RAMP1: Receptor Activity Modifying Protein 1; BDNF: Brain-Derived Neurotrophic Factor; Ntrk2: Neurotrophic tyrosine receptor kinase 2. a) Genes related with inflammation; b) Genes related intestinal barrier function; c) Genes related with parasympathetic activity.

Regarding cardiovascular parameters, levels of SBP ($p=0.0008$), DBP ($p=0.01$) and MAP ($p=0.0003$) showed a significant increase in the HFD group compared to the CTL group. However, training prevented the increase in SBP ($p=0.01$) and MAP ($p=0.03$) compared to the HFD group. HR results were similar between groups. Autonomic function was also assessed by administration of methylatropine (vagal tone) and propranolol (sympathetic tone). The results showed that the training group had a significant increase in vagal tone compared to the HFD group ($p=0.04$). The propranolol

infusion showed that the cardiac sympathetic impulse of the HFD group was increased compared to the CTL group ($p=0.005$), whereas training was able to attenuate this increase as observed in the HFD+PE group ($p=0.03$).

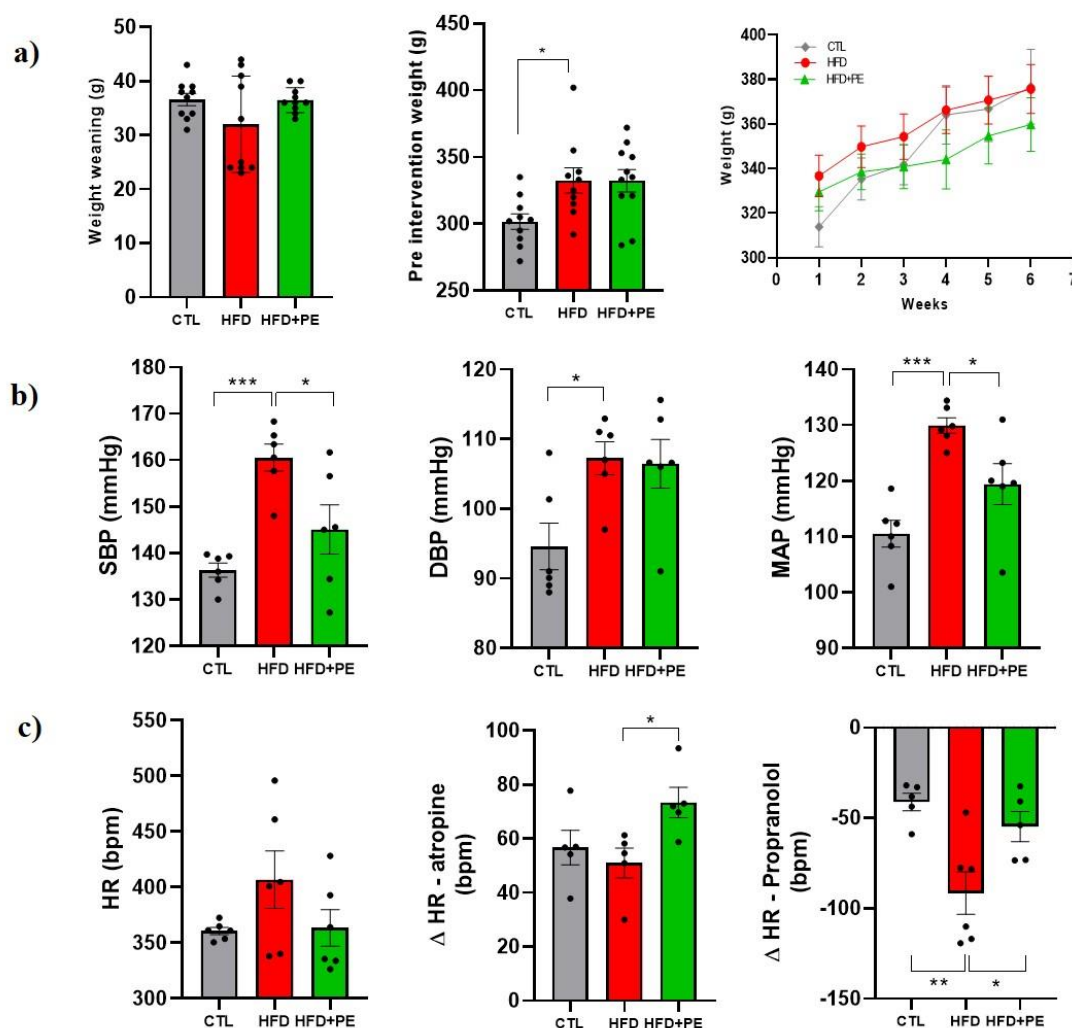


Figure 3 - Anthropometric and cardiovascular variables in rats with obesity

CTL: control; HFD: high fat diet; HFD+PE: high fat diet + physical exercise; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean blood pressure; HR: heart rate. ANOVA test was used.

The analysis of brainstem gene expression demonstrated greater expression of inflammation-related genes, such as TNF- α ($p = 0.03$) and IL-1 β ($p = 0.02$), in the HFD

group compared to the CTL group. Additionally, it was observed that the HFD+PE group exhibited a decrease in genes associated with sympathetic activity including BDNF ($p = 0.01$) and NOS1 ($p = 0.03$).

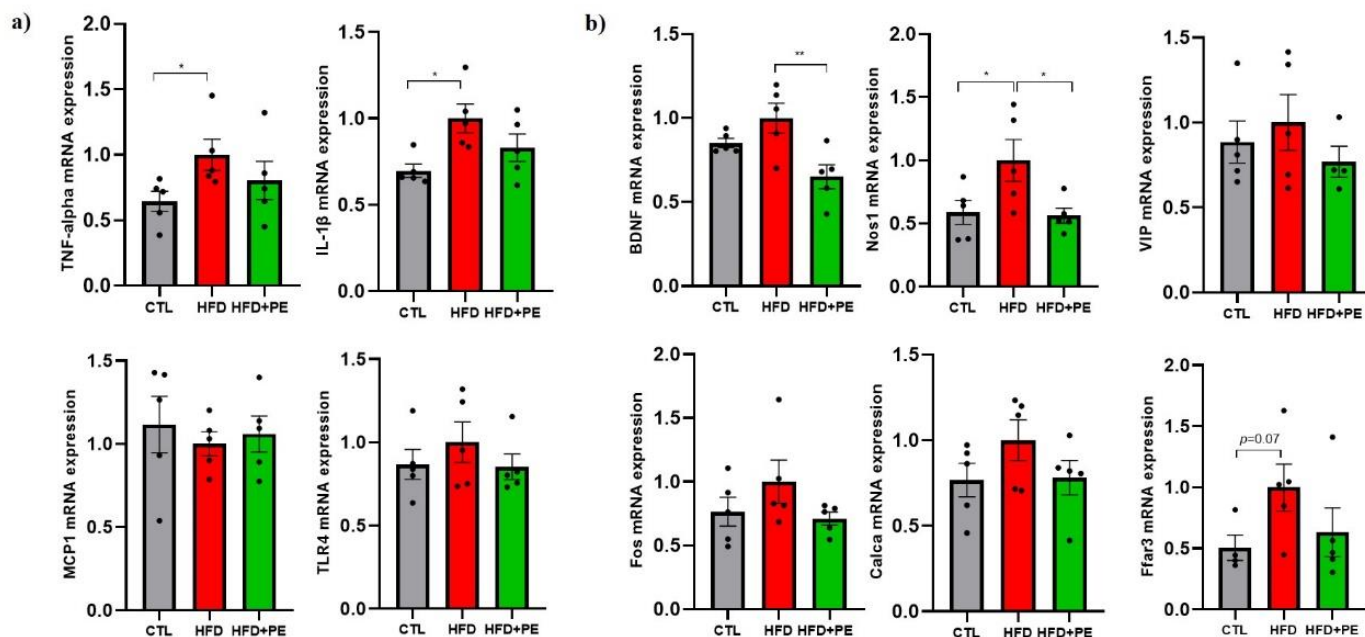


Figure 4 - Gene expression in the brainstem of rats with obesity

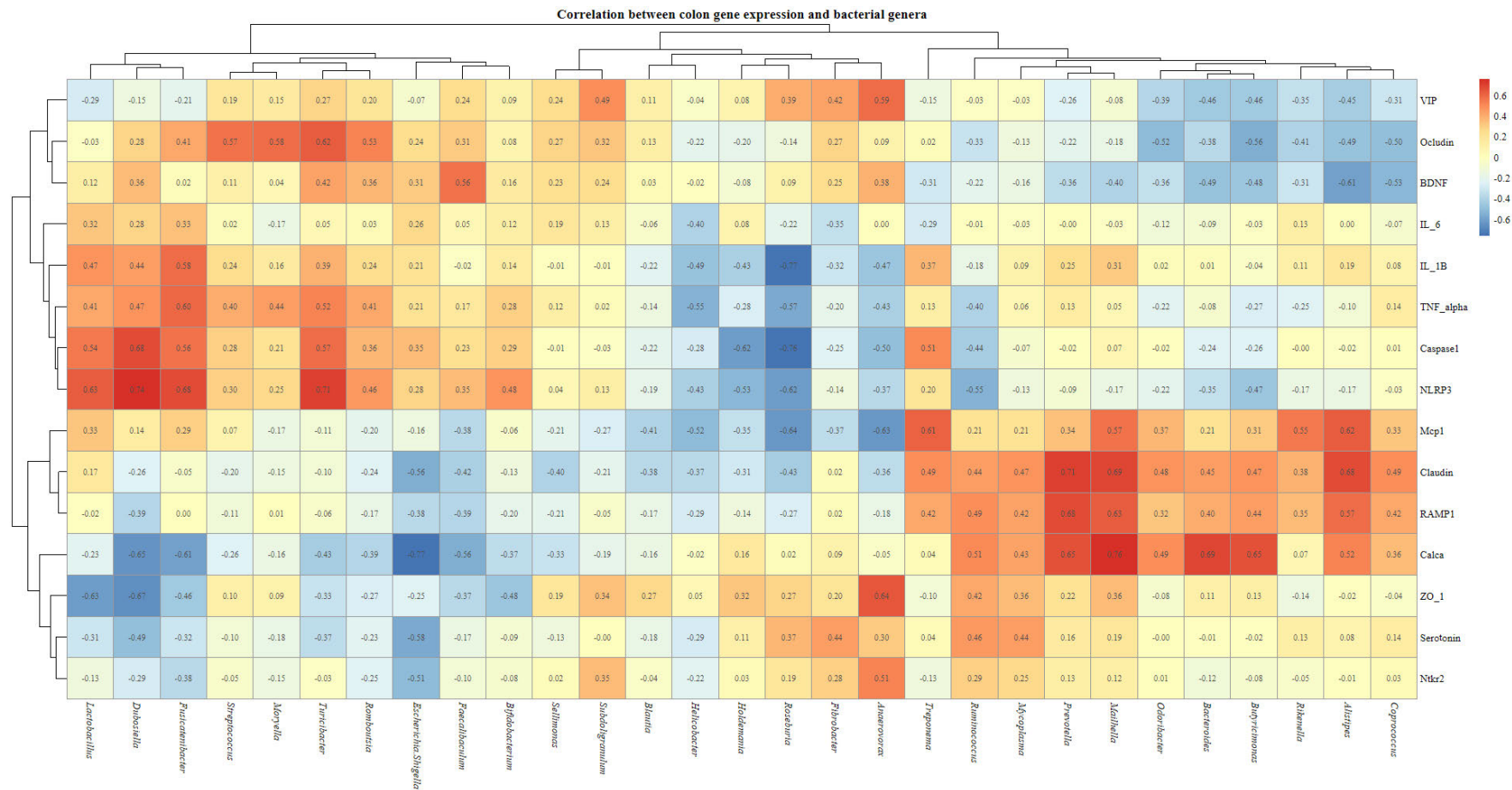
CTL: control; HFD: high fat diet; HFD+PE: high fat diet + physical exercise; TNF- α : Tumor necrosis factor alpha; IL-1 β : Interleukin 1 β ; BDNF: Brain-Derived Neurotrophic Factor; NOS1: Nitric oxide synthase 1; VIP: Vasoactive intestinal peptide; MCP1: Monocyte Chemoattractant Protein 1; TLR4: Toll-like receptor 4; Calca: calcitonin gene-related peptide; Ffar3: Free Fatty Acid Receptor. a) Genes related with inflammation; b) Genes related with sympathetic activity. Kruskal-Wallis test was used.

Correlations between bacterial genera and genes in the colon and brainstem revealed key characteristics of different bacterial genera. In the colon, for example, there was a positive correlation between the *Roseburia* genus and inflammation-related genes such as NLRP3, IL-1 β , caspase-1, and TNF- α . Other bacterial genera, such as *Lactobacillus* and *Dubosiella*, were also positively correlated with these genes. Another noteworthy finding is that the expression of junction proteins differs among bacterial

genera. Additionally, an increased abundance of *Escherichia/Shigella* is negatively correlated with genes important for intestinal vagal activity, such as serotonin and calca (Figure 5).

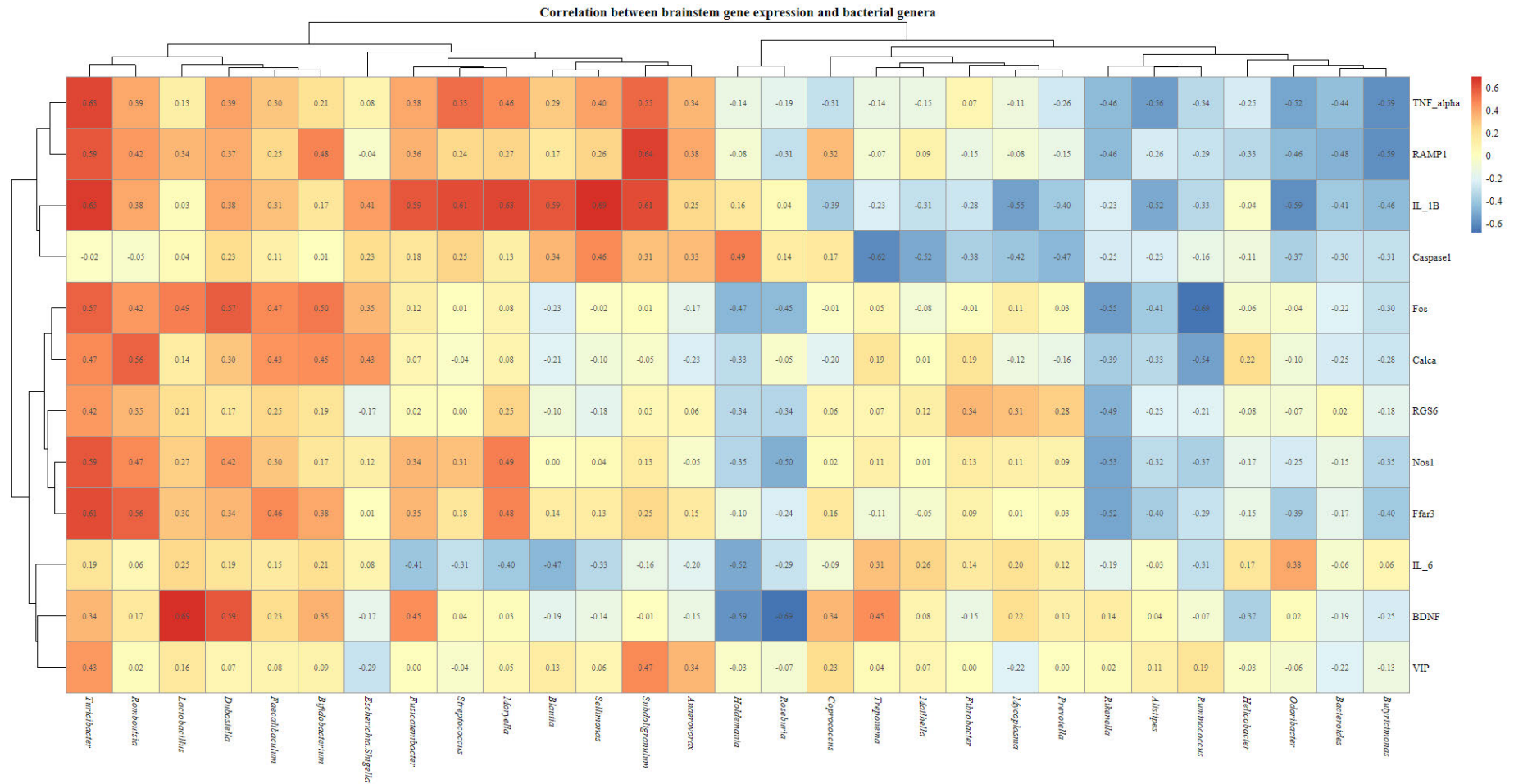
Upon analyzing the correlation between bacterial genera and gene expression in the brainstem, we found that *Roseburia* was negatively correlated with genes associated with sympathetic activity, including BDNF, NOS1, and FOS. Additionally, we found that *Lactobacillus* and *Dubosiella* were positively correlated with these genes, particularly with BDNF and FOS. The *Escherichia/Shigella* genus was positively correlated with IL-1 β expression. Additionally, greater abundance of *Subdoligranulum* was associated with increased inflammation due to a positive correlation with IL-1 β and TNF- α (Figure 6).

Figure 5. Correlation between colon gene expression and bacterial genera



RMSSD: square root of the mean of the square of the differences between consecutive NN intervals; SDNN: standard deviation between NN intervals; LF: low frequency; HF: high frequency; SD1: standard deviation associated with instantaneous R-R variability; SD2: continuous long-term R-R variability; DFA

Figure 6. Correlation between brainstem gene expression and bacterial genera



RMSSD: square root of the mean of the square of the differences between consecutive NN intervals; SDNN: standard deviation between NN intervals; LF: low frequency; HF: high frequency; SD1: standard deviation associated with instantaneous R-R variability; SD2: continuous long-term R-R variability; DFA

Discussion

This study demonstrated that six weeks of physical exercise in high-fat diet (HFD)-fed rats modulated the gut microbiota, resulting in positive changes at the bacterial phylum, family, and genus levels. Additionally, exercise reduced the expression of genes associated with inflammatory cytokines and the inflammasome pathway in the colon and increased genes related to the junction proteins and vagal activity. Physical exercise also promoted antihypertensive effects by reducing SBP and MAP and attenuating autonomic dysfunction. In line with these results, exercise reduced the expression of genes associated with sympathetic activity, including BDNF and NOS1, in the brainstem. Together, these findings suggest the beneficial effects of aerobic exercise on parameters related to the gut-brain axis.

The reduction in the Verrucomicrobia phylum observed in this study contrasts with the results of a study on mice that ran on a treadmill for eight weeks, in which the relative abundance of the Verrucomicrobia phylum increased compared to the control group (15). This discrepancy underscores the need for a more detailed analysis at the family and genus levels.

At the family level, physical exercise increased the relative abundance of Monoglobaceae and Ruminococcaceae while decreasing the relative abundance of Chlamydiaceae and Erysipelotrichaceae. A low relative abundance of Monoglobaceae has been associated with immune senescence, inflammation, and increased intestinal permeability (16), factors that are improved by physical exercise, as demonstrated in our study. Exercise increased the abundance of Ruminococcaceae in obese mice (17). Additionally, Chlamydiaceae and Erysipelotrichaceae families are associated with diseases such as trachoma (18) and gastrointestinal diseases related to inflammation (19).

Exercise reduced the relative abundance of *Adlercreutzia*, *Frisingicoccus*, *Fusicatenibacter*, and *Dubosiella* and increased the abundance of *Butyricimonas* and *Roseburia*. The reduction in anaerobic bacteria is believed to be due to the vasodilatory effect mediated by increased parasympathetic activity, which leads to greater blood perfusion and oxygen availability in the colon. It is also important to note that *Frisingicoccus* is a genus associated with intestinal dysfunctions (20) and that *Dubosiella* was found more abundantly in mice fed an HFD without exercise than in those fed an HFD

and undergoing 13 weeks of aerobic exercise (21). After four weeks of vigorous exercise training, individuals experienced an increase in *Butyricimonas*, a butyrate-producing microorganism (22). Additionally, *Roseburia* has been discussed as an important health marker associated with reduced inflammation (23).

One of the problems caused by a HFD is compromised intestinal barrier function, which is characterized by a reduction in junction proteins such as occludin and zonulin (24). In the present study, exercise increased occludin gene expression. Consistent with this finding, a previous study showed that six months of treadmill training increased occludin gene expression in Sprague-Dawley rats (25). Another study found that 10 days of treadmill training at 30 m/min with a 0° incline for 60 minutes per day increased zonulin in female rats (26). These results suggest that exercise improves intestinal barrier integrity.

The impact of physical exercise on the gut microbiota and intestinal barrier gene was accompanied by a decrease in the expression of inflammatory cytokines, including TNF- α and MCP1. Evidence shows that physical exercise has a positive effect on intestinal inflammation by reducing TNF- α (27). Exercise promotes the reduction of inflammasome components, including NLRP3, caspase 1, and IL-1 β . The inflammasome pathway is a signaling system that triggers inflammatory responses against damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) by cleaving and releasing active IL-1 β cytokines through NLRP3 and caspase 1. Dysregulated inflammasome activity has been linked to various inflammatory diseases (28). Furthermore, evidence shows that strength and endurance exercises have systemic effects on inflammation, reducing serum concentrations of IL-1 β and TNF- α (29).

Although vagal activity in the intestine was not directly measured, increased VIP gene expression indicates physical exercise improves this parameter. Stimulation of the vagus nerve triggers the release of VIP through nicotinic synapses, which causes relaxation of the gastrointestinal organs (30). VIP can also be released by inflammation-sensitive intrinsic primary afferent neurons (IPANS), which activate immune cells that express receptors for VIP and other neurotransmitters (31). Thus, improvements in intestinal inflammation and intestinal barrier integrity may be closely related to vagus nerve activity. Furthermore, these benefits may improve other obesity-related parameters, such as high blood pressure and autonomic dysfunction.

Hypertension and autonomic dysfunction are clinical disorders associated with obesity and gut dysbiosis. In the present study, we demonstrated that a HFD promoted an increase in SBP, DBP, and MAP levels, accompanied by autonomic dysfunction. However, physical exercise was able to improve these parameters. Aerobic exercise effectively reduces blood pressure in both normotensive and hypertensive individuals, and this reduction is closely linked to autonomic nervous system (ANS) modulation (32). Physical exercise has long been shown to improve autonomic activity in obese individuals, extending beyond its cardiovascular benefits and the reduction of cardiovascular risk factors (33). Regular physical training improves HRV by increasing the compliance of large arteries, such as the carotid sinus and aortic arch. This increase favors baroreceptor activity and intensifies parasympathetic tone. Furthermore, physical exercise can promote remodeling of the cardiorespiratory center in the brainstem. This results in a cardioprotective response, which is characterized by reduced sympathetic outflow and increased parasympathetic modulation of the heart (34).

Impaired autonomic function in obesity has been associated with neuroinflammation, a key feature observed in obese individuals. This study found increased gene expression of TNF- α and IL-1 β in the HFD group. Furthermore, corroborating the physiological data found in this study, physical exercise reduced gene expression levels of genes related to sympathetic activity in the brainstem, such as BDNF and NOS1. BDNF acts as both a neurotrophin and a neurotransmitter. It has been associated with glutamatergic signaling in the brainstem through its actions on sympathetic nerve endings (35). This may explain why BDNF levels decrease in response to physical exercise, as sympathetic excitation at this site is reduced (36). NOS1 is a gene that encodes the enzyme nNOS (neuronal nitric oxide synthase), which is found in higher quantities in the brainstem of hypertensive rats but not in the diencephalon or cerebellum (37). This finding seems to contradict the function of this enzyme, which induces the synthesis of nitric oxide (a potent vasodilator) and inhibits sympathetic activity. However, we believe this is an attempt to compensate for the increased sympathetic activity demonstrated physiologically in this study.

Gut inflammation likely improved due to positive modulation of the gut microbiota, as evidenced by the negative correlation between *Roseburia* genera and inflammasome pathway genes. This system mediates communication between the host and its gut

microbiota. The environmental state of the intestinal lumen continuously influences the host response, leading to the generation of specific signals through IL-1 β production, which modulates the gut microbiota (38). *Anaerovorax* was negatively correlated with inflammation-related genes and positively correlated with ZO-1 and VIP. Conversely, *Lactobacillus* and *Dubosiella* were positively correlated with inflammation and negatively correlated with ZO-1. Furthermore, *Escherichia-Shigella* was negatively correlated with Claudin and Calca. In summary, these results demonstrate the potential effects of bacterial genera on improving or impairing intestinal inflammation, neural signaling, and barrier function.

Butyricimonas was negatively correlated with genes associated with brainstem inflammation, such as TNF- α and IL-1 β . This finding corroborates previous evidence of *Butyricimonas*'s anti-inflammatory properties and explains its reduced relative abundance in hypertension (39). Additionally, genera such as *Streptococcus*, *Turicibacter*, and *Subdoligranum* were positively associated with TNF- α and IL-1 β . Additionally, *Lactobacillus* was positively correlated with BDNF, while *Roseburia* was negatively correlated with BDNF and NOS1. This indicates that these two genera have opposing effects on brainstem efferent autonomic activity.

In relation to the above-described findings, *Roseburia* has been identified as a health marker (23). Additionally, there is evidence suggesting that higher levels of physical activity lead to greater abundance of *Anaerovorax* in children (40). However, there is some contradiction regarding the role of the *Lactobacillus* and *Dubosiella* genera in obesity. We believe this discrepancy is due to the specific species involved. For instance, increased *Lactobacillus johnsonii* abundance contributes to reduced inflammation (21). The literature corroborates that *Escherichia/Shigella* are pathogenic bacteria associated with inflammation, as is *Streptococcus* (41). Regarding *Turicibacter* and *Subdoligranum*, however, the results contradict previous studies on their relationship with inflammation (42, 43).

Conclusion

This study demonstrated that aerobic exercise positively modulates the gut microbiota. Specifically, it increases the expression of genes related to vagal activity and

barrier function while reducing the expression of genes related to inflammation in the colon. In the brainstem, the expression of genes related to inflammation and sympathetic activity also decreased, resulting in improved autonomic function, characterized by reduced sympathetic activity and increased parasympathetic activity at rest. These results suggest that exercise enhances the communication between the gut and the brainstem and that reducing inflammation is essential for improving this cyclical interaction in obese rats. Furthermore, to our knowledge, this is the first study to postulate that aerobic exercise can modulate the gut microbiota and act directly in the brainstem to induce beneficial effects in these feedback systems.

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APÊNDICE 7 – RESUMOS COM DADOS DERIVADOS DA TESE

Três resumos com resultados preliminares da tese foram apresentados no XX Congresso Brasileiro de Obesidade e Síndrome Metabólica, realizado no Rio de Janeiro.

PT.105 EVALUATION OF HEART RATE AS A PREDICTOR OF CHANGES IN HEART RATE VARIABILITY INDICES

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Introduction: Heart rate (HR) is an accessible parameter and can be widely used in the clinic. High HR has been positively associated with autonomic dysfunction. Thus, HR screening may be essential to predict changes in autonomic function. This study aimed to investigate resting HR as a predictor of changes in heart rate variability (HRV) in children. **Patients and methods:** In this cross-sectional study, 84 children with obesity and 41 normal weight were included in the study. Body weight was measured using an electronic scale (Omron[®], HBF-514C), and height was assessed using a stadiometer (alturacxata[®]). A Polar H10 heart rate monitor was used to record the R-R interval. R-R recordings were processed by the Kubios HRV analysis software. The predictive power of HR for changes in HRV was assessed using receiver operating characteristic (ROC) curves. In this study, HR was considered a good predictor when its confidence interval was greater than 0.50 and the area under the ROC curve was greater than 0.70. In addition, a 95% confidence interval (95%CI) was used. **Results:** HR can be a good predictor of changes in the indices RMSSD and LF/HF indices in schoolchildren according to the ROC curve. For RMSSD, children with obesity (ROC = 0.90; 95%CI: 0.83-0.97) and normal weight (ROC = 0.89; 95%CI: 0.77-1.00) showed good predictive values, and for the LF/HF both children with obesity (ROC = 0.77; IC95%: 0.65-0.89) and normal weight (ROC = 0.88; IC95%: 0.74-1.00) showed good predictive values. Regarding the HR cut-off point, a general cut-off point of 93 bpm (sensitivity 84.62% and specificity 77.78%) for changes in RMSSD and 92 bpm (sensitivity 82.69% and specificity 87.50%) for changes in LF/HF was established, independent of nutritional status, and sex. **Discussion:** The values of the area under the ROC curve for HR as a predictor of changes in the HRV indices RMSSD and LF/HF, as well as the cut-off points, sensitivity, and specificity, suggest that resting heart rate may be an important cardiovascular risk screening tool in children. **Conclusion:** HR may be useful as a screening tool for the risk of autonomic nervous system dysfunction using the cutoff points established in this study. **License number of ethics committee:** 37352720.6.1001.5188.

PT.106 ALTERATIONS IN HEART RATE VARIABILITY AND INFLAMMATORY BIOMARKERS IN CHILDREN WITH OBESITY

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Introduction: Childhood obesity is an increasing problem worldwide and is associated with adverse health outcomes. The aim of this study was to evaluate the autonomic function and inflammatory biomarkers in children with obesity. **Patients and methods:** In this cross-sectional study, 125 children were allocated into two groups: 84 children in the obesity group and 41 normal weight group. Body weight was measured using an electronic scale (Omron[®], HBF-514C), and height was assessed using a stadiometer (alturacxata[®]). Children were classified according to the body mass index for age and sex. A Polar H10 heart rate monitor was used to record R-R interval for 10 minutes. Heart rate variability (HRV) was analyzed using the Kubios software. Cytokines were analyzed using the cytometric bead array assay and the cytokines IL-2, IL-4, IL-6, IL-10, IL-17a, IFN- γ , and TNF- α were quantified. **Results:** Children with obesity had increased serum levels of IL-2 (9.14 ± 5.24 vs. 6.44 ± 3.21 pg/mL, $p = 0.009$) and decreased serum levels of IL-10 (4.29 ± 3.93 vs. 5.88 ± 3.70 pg/mL, $p = 0.039$) when compared to the normal weight group. Serum levels of cytokines IL-4, IL-6, IL-17a, IFN- γ , and TNF- α were similar between groups ($p > 0.05$). Regarding the HRV analysis, children with obesity showed reduced RMSSD (30.1 ± 7.2 - 92.5 vs. 42.5 ± 15.3 - 140.6 , $p = 0.001$) and SDNN (37.9 ± 12.7 - 84.9 vs. 47.9 ± 20.8 - 119.8 , $p = 0.002$) in time-domain when compared to the normal weight group. In addition, children with obesity had an increased LF/HF ratio (1.509 ± 0.295 - 5.908 vs. 1.105 ± 0.417 - 7.762 , $p = 0.003$) in frequency-domain, and increased SD2/SD1 ratio (2.15 ± 1.22 - 3.57 vs. 1.77 ± 1.03 - 3.55 , $p = 0.001$) in non-linear indices when compared with normal weight group. **Discussion:** A higher concentration of IL-2 and a lower concentration of IL-10 were observed in children with obesity, which may indicate dysfunction caused by the greater amount of body fat. These children also had autonomic dysfunction, observed in linear and non-linear indices. These findings contribute to a better understanding of dysautonomia and low-grade chronic inflammation in children. **Conclusion:** Children with obesity have decreased HRV and signals of low-grade chronic inflammation, as reported by increased proinflammatory cytokine IL2 and decreased anti-inflammatory cytokine IL-10. **License number of ethics committee:** 37352720.6.1001.5188.

PT.107 PLYOMETRIC TRAINING AND NUTRITIONAL EDUCATION IMPROVE ANTHROPOMETRIC VARIABLES, BLOOD PRESSURE, AND HEART RATE VARIABILITY IN CHILDREN WITH OBESITY

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Introduction: Childhood obesity is a risk factor for cardiovascular disorders. Health strategies focused on the school environment may be essential to improve related cardiovascular function in children with obesity. This study aimed evaluate the effectiveness of a multicomponent intervention (MI) with plyometric training and nutritional education on body mass index (BMI) for age and sex, body fat percentage (%BF), blood pressure (BP) and heart rate variability (HRV) in children with obesity. **Patients and methods:** Thirty-three children of both sexes, aged between 7 and 10 years participated in the study. Body weight was measured using an electronic scale (Omron[®], HBF-514C), and height was assessed using a stadiometer (Alturacxata[®]). Body fat percentage was measured using an adipometer (Sanny[®]). BP was measured with a digital sphygmomanometer (Omron Healthcare[®] HBP-1100). A Polar H10 heart rate monitor was used to record the R-R interval. The data collected by the heart rate monitor were transferred to a computer using the Elite HRV software and processed using the Kubios HRV analysis software. The analysis was performed before and after a four-month MI. The plyometric training was performed twice a week, and there were 12 sessions of nutritional education spread over the four months. **Results:** The MI was effective in reducing BMI for age and sex (2.63 ± 1.94 - 5.66 vs. 2.57 ± 1.78 - 5.57 , $p < 0.05$), %BF (36.10 ± 28.60 - 58.80 vs. 32.14 ± 26.38 - 51.74 , $p < 0.05$), systolic BP (110.3 ± 12.32 vs. 104.9 ± 9.31 mmHg, $p < 0.05$) and diastolic BP (62.0 ± 52.0 - 94.0 vs. 58.0 ± 49.0 - 79.0 mmHg, $p < 0.05$). Although MI has not improved HRV parameters in the frequency-domain (LF/HF ratio) and non-linear methods (SD2/SD1 ratio), MI effectively improved HRV in time-domain, such as SDNN (36.72 ± 17.88 vs. 44.28 ± 18.41 ms, $p < 0.05$) and RMSSD (28.60 ± 7.20 - 92.50 vs. 36.40 ± 15.20 - 129.8 ms, $p < 0.05$). **Discussion:** The improvement in BMI for age and sex, %BF, SBP, DBP, and HRV in the children with obesity after the MI reinforces the need to implement strategies with a nutritional approach and physical education in the school environment with a focus on the prevention of adverse health risks related to obesity, such as cardiometabolic diseases. **Conclusion:** The four-month in school follow-up strategy, consisting of physical training and nutrition education, was effective in improving cardiovascular parameters. **License number of ethics committee:** 37352720.6.1001.5188.

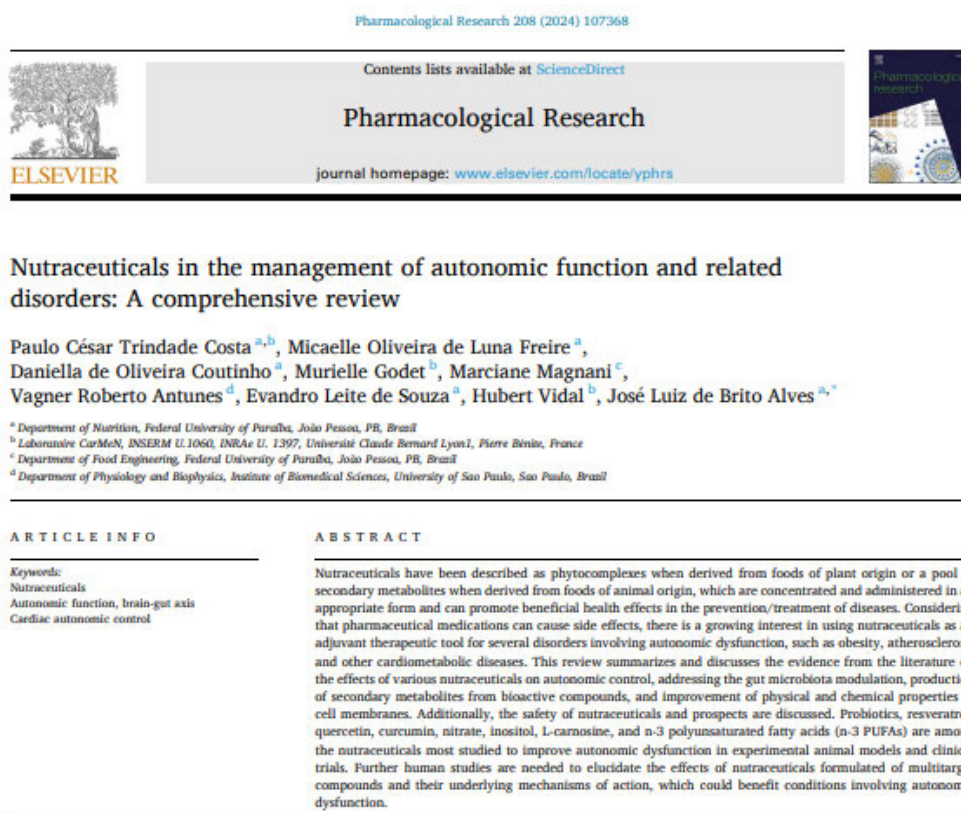
APÊNDICE 8 – RESUMO COM DADOS DERIVADOS DA TESE

Um resumo com resultados preliminares da tese foi apresentado no **XX Congresso Brasileiro de Obesidade e Síndrome Metabólica**, realizado no Rio de Janeiro. Este trabalho nos rendeu o prêmio de segunda melhor apresentação oral deste evento.



APÊNDICE 9 – OUTROS RESULTADOS (ARTIGO 7)

Artigo publicado como autor principal na revista **Pharmacological Research**, classificada como **A1** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 9.1.



APÊNDICE 10 – OUTROS RESULTADOS (ARTIGO 8)

Artigo publicado como segundo autor na revista **Critical Reviews in Food Science and Nutrition**, classificada como **A1** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 7.3.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2024.2323112



REVIEW ARTICLE



Shedding light on the impacts of *Spirulina platensis* on gut microbiota and related health benefits

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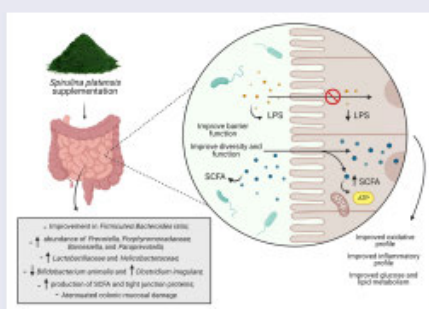
ABSTRACT

Spirulina (*S.*) *platensis* is a blue-green algae with reported nutritional and health-promoting properties, such as immunomodulating, antioxidant, cholesterol-lowering properties, and beneficial effects on inflammatory diseases. *Spirulina platensis* can improve the function and composition of the gut microbiota and exert systemic beneficial effects. Gut dysbiosis is characterized by an imbalance in the composition and function of gut microbiota and is associated with several diseases. Some dietary bioactive compounds can restore the composition, diversity, and function of the gut microbiota and improve health-related parameters. This review proposes to gather relevant information on the effects of *S. platensis* supplementation on the modulation of the function and composition of gut microbiota and local and systemic measures related to gut health, such as inflammation, oxidative stress, and glucose and lipid metabolism. The body of evidence conducted with animals and clinical studies shows that *S. platensis* supplementation increased gut microbiota diversity and improved gut microbiota composition, as reported by a decrease in the *Firmicutes/Bacteroides* ratio, increase in the relative abundance of *Prevotella* and *Lactobacillaceae*, increase in short-chain fatty acid production and decrease of gut permeability. Improvements in gut microbiota have been associated with host health benefits such as anti-obesity, anti-diabetic, anti-hypertensive, anti-lipemic, anti-inflammatory, and antioxidant effects.

KEYWORDS

Spirulina platensis; gut microbiota; inflammation; oxidative stress; chronic diseases

GRAPHICAL ABSTRACT



APÊNDICE 11 – OUTROS RESULTADOS (ARTIGO 9)

Artigo publicado como coautor na revista **Critical Reviews in Food Science and Nutrition**, classificada como **A1** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 7.3.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
<https://doi.org/10.1080/10408398.2022.2136618>



REVIEW



Lactobacillus group and arterial hypertension: A broad review on effects and proposed mechanisms

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ABSTRACT

Hypertension is the leading risk factor for cardiovascular diseases and is associated with intestinal dysbiosis with a decrease in beneficial microbiota. Probiotics can positively modulate the impaired microbiota and impart benefits to the cardiovascular system. Among them, the emended *Lactobacillus* has stood out as a microorganism capable of reducing blood pressure, being the target of several studies focused on managing hypertension. This review aimed to present the potential of *Lactobacillus* as an antihypertensive non-pharmacological strategy. We will address preclinical and clinical studies that support this proposal and the mechanisms of action by which these microorganisms reduce blood pressure or prevent its elevation.

KEYWORDS

ACE inhibition; antihypertensive; blood pressure; probiotic

Introduction

Systemic arterial hypertension (SAH) is a chronic, asymptomatic, silent disease that occurs because of a sustained rise in blood pressure (BP) levels. This disorder is associated with cardiovascular and metabolic disorders and increased mortality rates globally. The complexity of physiological BP control makes hypertension treatment difficult, and adequate control of BP often requires multiple medications (Noubiap et al. 2019). This can increase the number of side effects and decrease patient adherence to treatment, reinforcing the importance of non-pharmacological strategies in SAH management, including a healthy diet and physical activity (Mancia et al. 2014).

Among the potential probiotic strains with antihypertensive properties, those belonging to the *Lactobacillus* genus have been highlighted to be involved in BP decrease. Although several studies have demonstrated the antihypertensive properties of *Lactobacillus*, an in-depth evidence based on preclinical and clinical findings focusing on hypertension therapy using *Lactobacillus* strains has not been found in the available literature.

Recently, the genus *Lactobacillus* has been reclassified owing to its enormous genomic diversity of more than 260 species. A total of 25 genera, including the emended *Lactobacillus*, were defined based on phenotypic, genotypic, and ecological features (Salveti et al. 2018; Zheng et al. 2020). It is worth mentioning that the renaming of most

APÊNDICE 12 – OUTROS RESULTADOS (ARTIGO 10)

Artigo publicado como coautor na revista **Nutrients**, classificada como **A1** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 4.8.



Article

Ultra-Processed Food Consumption and Cardiometabolic Risk Factors in Children Living in Northeastern Brazil

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Abstract: Objective: To analyze the association between ultra-processed food (UPF) consumption and cardiometabolic, biochemical, and inflammatory risk factors in children in a metropolis in Northeast Brazil. Methods: A cross-sectional study using baseline data from a community-based controlled trial was carried out with 151 children from public schools in João Pessoa, Paraíba, Brazil aged 7 to 10 years. Dietary consumption was assessed using 24 h food recall, and UPF consumption was estimated using the NOVA classification system. Anthropometry (BMI for age), blood pressure, biochemical parameters (ALT, AST, GGT, cholesterol, LDL-c, HDL-c, triglycerides, fasting glucose, HbA1c, HOMA-IR, creatinine, urea, hs-CRP), and cytokines (IL-2, IL-4, IL-6, IL-10, IL-17a, IFN- γ , and TNF- α) were also assessed. Results: Children in the third tertile (highest UPF consumption) had higher serum concentrations of LDL-c (p -value = 0.04) and ALT (p -value = 0.01), with a trend towards higher AST (p -value = 0.06). Total energy (p -value = 0.01), trans fatty acid (p -value = 0.02), and sodium (p -value = 0.04) intakes were higher in the highest tertile, whereas protein (p -value < 0.01) and fiber (p -value < 0.01) intakes were lower. Concentrations of IL-17A (p -value = 0.01) and IL-10 (p -value = 0.04) were significantly higher in the second tertile. Multiple linear regression showed that UPF consumption was significantly associated with increased LDL-c, ALT, and AST concentrations. Conclusions: High intake of UPFs was associated with dyslipidemia, elevated liver enzymes, and inflammatory changes in children. Dietary interventions are needed to reduce UPF consumption and prevent cardiometabolic and liver disease in childhood.

Keywords: industrialized foods; dyslipidemias; inflammation; alanine transaminase; childhood

1. Introduction

The global consumption of ultra-processed foods (UPFs) has increased significantly [1].

APÊNDICE 13 – OUTROS RESULTADOS (ARTIGO 11)

Artigo publicado como coautor na revista **Nutrients**, classificada como **A1** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 4.8.




Article

Food Consumption According to the NOVA Food Classification and Its Relationship with Symptoms of Depression, Anxiety, and Stress in Women

Daniel Emiliano de Farias Xavier ¹, Rúbia Cartaxo Squizato de Moraes ¹, Thallyta Alanna Ferreira Viana ¹, Joicy Karla Grangeiro Pereira ¹, Paulo César Trindade da Costa ¹, Davyson Barbosa Duarte ², Melyssa Kellyane Cavalcanti Galdino ³, Adélia da Costa Pereira de Arruda Neta ⁴, José Luiz de Brito Alves ¹ and Vinicius José Baccin Martins ^{3,*}

 **check for updates**

Citation: de Farias Xavier, D.E.; de Moraes, R.C.S.; Viana, T.A.F.; Pereira, J.K.G.; da Costa, P.C.T.; Duarte, D.B.; Galdino, M.K.C.; da Costa Pereira de Arruda Neta, A.; de Brito Alves, J.L.; Martins, V.J.B. Food Consumption According to the NOVA Food Classification and Its Relationship with Symptoms of Depression, Anxiety, and Stress in Women. *Nutrients* **2024**, *16*, 3734. <https://doi.org/10.3390/nu16213734>

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Abstract: Background/Objectives: Depression, anxiety, and stress are highly prevalent mental disorders worldwide, and food consumption can change in individuals with these conditions. We aimed to assess the food consumption of women with depressive symptoms and compare it to a control without symptoms. Methods: A cross-sectional study was conducted with 96 women, aged 18–59, allocated into two groups: control ($n = 62$) or depressive symptoms ($n = 34$). The participants underwent an anthropometric assessment, and food consumption was evaluated using a 24 h food recall and the NOVA classification. Depressive symptoms, anxiety, and stress were measured using the DASS-21 questionnaire. Results: Anthropometric parameters did not differ between the groups. Women with depressive symptoms consumed fewer calories ($p = 0.006$), carbohydrates ($p = 0.014$), proteins ($p = 0.036$), and lipids (0.011) from unprocessed and minimally processed foods (UMPF) compared to the control women. A negative correlation was found between the dietary consumption energy of UMPF and symptoms of depression ($r = -0.337$; $p = 0.001$), anxiety ($r = -0.262$; $p = 0.014$), and stress ($r = -0.260$; $p = 0.014$), as well as a positive correlation between energy intake from ultra-processed foods (UPF) and symptoms of depression ($r = 0.218$; $p = 0.042$) and stress ($r = 0.227$; $p = 0.034$). Regression analysis showed that depressive symptoms accounted for 7.6% of the lower energy consumption from UMPF. Conclusions: Women with depressive symptoms displayed lower UMPF consumption, and this was negatively correlated with symptoms of depression, anxiety, and stress. Professional dietary advice can improve health status in these patients.

Keywords: anxiety; depression; food consumption; NOVA classification; stress; women

APÊNDICE 14 – OUTROS RESULTADOS (ARTIGO 12)

Artigo publicado como coautor na revista **Nutrients**, classificada como **A1** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 4.8.



nutrients



Review

Beneficial Effects of Omega-3 Fatty Acids on Obesity and Related Metabolic and Chronic Inflammatory Diseases

Donia Jerab ^{1,2}, Ferdinand Blangero ¹, Paulo César Trindade da Costa ³, José Luiz de Brito Alves ³, Rym Kefi ², Henda Jamoussi ⁴, Beatrice Morio ¹ and Assia Eljaafari ^{1,5,*}

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Abstract: Omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) are known to help resolve inflammation through generation of anti-inflammatory eicosanoids and specialized pro-resolving mediators, including resolvins, protectins, and maresins. Through binding to the GPR120/FFAR4 receptor, their beneficial effects result from phospholipid membrane remodeling, impairment of inflammatory signaling molecules clustering, subsequent inhibition of NF- κ B and inflammasome activation, and a reduction in oxidative stress. Obesity, a chronic inflammatory disease that contributes to metabolic disorders, is alleviated by *n*-3 PUFAs. In the adipose tissue (AT) of individuals with obesity, *n*-3 PUFAs counteract

APÊNDICE 15 – OUTROS RESULTADOS (ARTIGO 13)

Artigo publicado como coautor na revista **International Journal of Environmental Research and Public Health**, classificada como A1 de acordo com o Qualis CAPES 2017-2020.



Article

Impact of Maternal Nutritional Status and Mental Health on Children with Obesity: Relationship Between Anthropometric Parameters, Food Addiction, and Stress

Joicy Karla Grangeiro Pereira ¹, Rúbia Cartaxo Squizato de Moraes ¹, Thallyta Alanna Ferreira Viana das Neves ¹, Davyson Barbosa Duarte ¹, Cristiane Cosmo Silva-Luis ¹, Paulo César Trindade da Costa ¹, Carla Lúcio Alves ², Melyssa Kellyane Cavalcanti Galdino ², Carla Alexandra da Silva Moita Minervino ², Nassib Bezerra Bueno ³, José Luiz de Brito Alves ¹ and Vinicius José Baccin Martins ^{4,*}

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Abstract

Childhood obesity is a complex and multifactorial disease influenced by various factors including behavioral, physical, and psychological conditions. This cross-sectional study aimed to evaluate the impact of maternal and child stress and food addiction on the nutritional status of children with obesity. Children aged 6 to 12 years were divided into Control Group (n = 42) and Obesity Group (n = 68) according to BMI-Z score. Mothers were allocated to their children's respective groups. Anthropometric measures, body composition, stress levels, and food addiction were evaluated in both children and mothers. Children with obesity exhibited a significantly higher prevalence of food addiction compared to controls (22.1% vs. 3.6%, p = 0.003), while mothers in the control group showed higher



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APÊNDICE 16 – OUTROS RESULTADOS (ARTIGO 14)

Artigo publicado como coautor na revista **International Journal of Mental Health and Addiction**, classificada como **A2** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 2.5.

International Journal of Mental Health and Addiction
<https://doi.org/10.1007/s11469-025-01535-w>

ORIGINAL ARTICLE



Association of Depression with Cardiac Autonomic Function and Food Addiction in Individuals in Psychopharmacological Treatment

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 Vinicius José Baccin Martins² · Alfredo José Minervino³ · José Luiz de Brito Alves¹

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Abstract

Major depressive disorder is a multifactorial condition and may be associated with food addiction and changes in cardiac autonomic dysfunction. This study investigated the occurrence of food addiction and cardiac autonomic function among individuals with depression and undergoing psychopharmacological treatment. The group depression (D) exhibited a higher body mass index (BMI) [mean 29.4 (5.7) vs. 26.4 (3.3); $p = 0.022$] and greater number of food addiction symptoms [3 (0–9) vs. 1.5 (0–9); $p = 0.005$] compared to the group control (C). Group D had lower LF (Low-Frequency band) (46.8 ± 21.0 vs. 61.1 ± 21.9), LF/HF ratio [0.78 (0.17–6.89) vs. 1.77 (0.15–6.67)], SD2/SD1 ratio (1.66 ± 0.67 vs. 2.05 ± 0.70), and DFA1 (0.90 ± 0.35 vs. 1.15 ± 0.25), but higher HF (High-Frequency band) (52.9 ± 20.9 vs. 34.9 ± 19.4). Furthermore, LF/HF ratio was positively correlated with depression severity ($p = 0.002$; Spearman's $\rho = 0.558$; effect size Fisher's $z = 0.629$). Regression models adjusted for potential confounders showed that depressive symptoms were positively associated with low-frequency (LF) components and food addiction symptoms and negatively associated with high-frequency (HF) components ($p < 0.05$). Patients with depression exhibited changes in BMI, addictive eating symptoms, and cardiometabolic parameters, with a greater prevalence of parasympathetic tone.

Keywords Depression · Food addiction · Heart rate variability · Body mass index · Psychopharmacological treatment

APÊNDICE 17 – OUTROS RESULTADOS (ARTIGO 15)

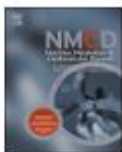
Artigo publicado como coautor na revista **Nutrition, Metabolism and Cardiovascular Diseases**, classificada como **A2** de acordo com o *Qualis CAPES 2017-2020* e Fator de impacto de 3.7.

Nutrition, Metabolism and Cardiovascular Diseases xxx (xxxx) xxx

Contents lists available at [ScienceDirect](#)

Nutrition, Metabolism and Cardiovascular Diseases

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

Is low heart rate variability associated with cardiometabolic dysfunction in children with obesity? A cross-sectional study

Thallyta Alanna Ferreira Viana das Neves^{a,*}, Rúbia Cartaxo Squizzato de Moraes^a,
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 Cristiane Cosmo Silva-Luis^a, Francisco Antônio de Oliveira Júnior^c,
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ARTICLE INFO

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Keywords:
 Autonomic nervous system
 Childhood obesity
 Cortisol
 Food addiction
 Heart rate variability

ABSTRACT

Background and aim: Children with obesity have impaired cardiac function and increased risk of cardiometabolic dysfunction. We investigated children with obesity and low heart rate variability (HRV) in terms of anthropometric parameters, insulin resistance, food addiction (FA), and blood pressure, comparing them with those who have normal heart rate variability.

Methods and results: Children aged 7–11 years, of both sexes, were divided into groups: the control group (CG, n = 34) comprised children with normal weight and normal heart rate variability; obesity plus normal heart rate variability (ONHRV, n = 51); and obesity plus low heart rate variability (OLHRV, n = 39). Anthropometric, biochemical parameters, HRV, blood pressure, and FA were measured. The OLHRV group showed higher weight (p < 0.001), waist circumference (p < 0.001), insulin resistance (p < 0.001), and lower RMSSD (p < 0.001) compared to ONHRV and CG. The prevalence of FA did not differ among the groups. Multiple regression analysis showed that the OLHRV group had a greater impact on insulin resistance (Beta 0.662, p < 0.001) than ONHRV (Beta 0.435, p < 0.001). Both groups explained systolic blood pressure (OLHRV, Beta 0.440, p < 0.001; ONHRV, Beta 0.434, p < 0.001).

Conclusion: Children with obesity plus low heart rate variability showed poor anthropometric indicators, increased insulin resistance, and an imbalance in the autonomic nervous system with low parasympathetic modulation. These findings suggest that the severity of obesity in children is associated with lower HRV and lower HRV plus obesity is an important predictor of insulin resistance and increased blood pressure.

APÊNDICE 18 – OUTROS RESULTADOS (ARTIGO 16)

Artigo publicado como coautor na revista **Food Bioscience**, classificada como **A2** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 4.8.

Food Bioscience 62 (2018) 102004



Contents lists available at ScienceDirect

Food Bioscience

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



The yeast carboxymethyl glucan reduces cardiometabolic risks by improving oxidative stress, endothelial dysfunction and platelet aggregation

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Keywords:
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Antioxidant effect
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Cardiovascular diseases

ABSTRACT

The carboxymethyl glucan (CMG) obtained from *Saccharomyces cerevisiae* has been highlighted for its beneficial potential, especially for its high antioxidant capacity. Cardiovascular diseases are among the leading causes of death worldwide and are often associated with overweight and dietary intake. On the other hand, supplementation with bioactive foods may be important in the prevention and treatment of these diseases. In this sense, this study evaluated the effect of CMG on anthropometric, oxidative and cardiovascular variables. Wistar rats were divided into three groups: NCT - normal caloric control fed a standard diet; HCT - hypercaloric control; H-CMG - hypercaloric + CMG (20 mg/kg/day) fed a hypercaloric diet for 12 weeks. At the end of the experimental period, anthropometric variables, reactive oxygen species (ROS) production, vasorelaxation, platelet aggregation, and systolic blood pressure (SBP) were measured. The results demonstrated that hypercaloric diet induced weight gain, increased oxidative stress, and reduced endothelium-dependent vasorelaxation, as well as increased platelet aggregation levels and SBP. In addition, CMG induced a significant reduction on weight gain (68.3 ± 4.9 vs 111.1 ± 3.8) and tissue (65.3 ± 18.4 vs 176.7 ± 49.7) and platelet ROS production (464.0 ± 99.8 vs 679.0 ± 102.3), leading to improved vasorelaxation, and reduced platelet aggregation. However, CMG treatment did not significantly reduce SBP levels. In this sense, it is reasonable to conclude that CMG was effective in reducing the damage caused by a hypercaloric diet, emerging as a potential compound for the treatment of diseases characterized by these conditions, such as cardiometabolic disorders.

1. Introduction

Yeast β -glucans are glucose polymers largely consumed as functional foods. In the last decades, the β -glucans and its derivatives have been studied because its potential related to improvement of health (Lante, Canazza, & Tessari, 2023). The carboxymethyl glucan (CMG) derives from β -glucans from *Saccharomyces cerevisiae* yeast through carboxymethylation which consists of the exchange of hydroxyl groups for

immune cell receptors with potential improvement in their effects on variables related to cardiovascular risk (Chakka & Zhou, 2020; Yehia, 2022).

Consumption of high energy-density diets is one of the main factors leading to weight gain, which in turn are directly and indirectly strongly associated with higher risk and incidence of cardiovascular disease morbidity and mortality (Auberval et al., 2014; Koskimas et al., 2024; Tenele et al., 2022). Directly, obesity induces structural and functional

APÊNDICE 19 – OUTROS RESULTADOS (ARTIGO 17)

Artigo publicado como coautor na revista **Foods**, classificada como **A2** de acordo com o Qualis CAPES 2017-2020 e Fator de impacto de 4.7.



Article

Impact of a *Limosilactobacillus fermentum*, Quercetin, and Resveratrol Nutraceutical on Fecal Microbiota Composition and Metabolic Activity in Healthy and Hypertensive Subjects

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Abstract: A promising strategy to improve the gut microbiome in hypertension is to target the gut microbiota. This study evaluated the effects of a potential nutraceutical product composed of three strains of *Limosilactobacillus* (*L.*) *fermentum*, quercetin, and resveratrol on the intestinal microbiome of healthy and hypertensive subjects. The nutraceutical product consisting of strains of *L. fermentum* 139, 263 and 296, fructooligosaccharides (200 mg), quercetin (160 mg), and resveratrol (150 mg) (LfQR) was added to the in vitro fecal fermentation process occurring for 48 h. Fecal samples of healthy and hypertensive subjects were allocated into four groups: (i) healthy controls (CTL); (ii) healthy controls with the addition of LfQR (CTL + LfQR); (iii) hypertensive (HTN) subjects; and (iv) hypertensive subjects with the addition of LfQR (HTN + LfQR). The diversity and composition of the fecal microbiota and the production of microbial metabolites were evaluated. CTL and HTN groups exhibited a distinct gut microbiota composition, as shown by the β -diversity assessment. The addition of the potentially nutraceutical-modulated β -diversity was similar between CTL and HTN groups, suggesting a similar gut microbiome composition



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Costa, P.C.T.d.; Duman, H.; Karav, S.;

APÊNDICE 20 – OUTROS RESULTADOS (ARTIGO 18)

Artigo publicado como autor principal na revista **Foods**, classificada como **A2** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 4.7.



Review

Evidence for Quercetin as a Dietary Supplement for the Treatment of Cardio-Metabolic Diseases in Pregnancy: A Review in Rodent Models

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Citation: Costa, P.C.T.d.; de Souza, E.L.; Lacerda, D.C.; Cruz Neto, J.P.R.; Sales, L.C.S.d.; Silva Luis, C.C.; Pontes, P.B.; Cavalcanti Neto, M.P.; de Brito Alves, J.L. Evidence for Quercetin as a Dietary Supplement for the Treatment of Cardio-Metabolic Diseases in Pregnancy: A Review in Rodent Models. *Foods* **2022**, *11*, 2772. <https://doi.org/10.3390/>

Abstract: Quercetin supplementation during pregnancy and lactation has been linked to a lower risk of maternal cardio-metabolic disorders such as gestational diabetes mellitus (GDM), dyslipidemia, preeclampsia, attenuation of malnutrition-related conditions, and gestational obesity in animal studies. Pre-clinical studies have shown that maternal supplementation with quercetin reduces cardio-metabolic diseases in dams and rodents' offspring, emphasizing its role in modifying phenotypic plasticity. In this sense, it could be inferred that quercetin administration during pregnancy and lactation is a viable strategy for changing cardio-metabolic parameters throughout life. Epigenetic mechanisms affecting the AMP-activated protein kinase (AMPK), nuclear factor-kappa B (NF-κB), and phosphoinositide 3-kinase (PI3 K) pathways could be associated with these changes. To highlight these discoveries, this review outlines the understanding from animal studies investigations about quercetin supplementation and its capacity to prevent or decrease maternal and offspring cardio-metabolic illnesses and associated comorbidities.

APÊNDICE 21 – OUTROS RESULTADOS (ARTIGO 19)

Artigo publicado como coautor na revista **Foods**, classificada como **A2** de acordo com o *Qualis CAPES* 2017-2020 e Fator de impacto de 4.7.



Review

Effectiveness of Polyphenols on Perinatal Brain Damage: A Systematic Review of Preclinical Studies

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Abstract: Polyphenol supplementation during early life has been associated with a reduction of oxidative stress and neuroinflammation in diseases caused by oxygen deprivation, including cerebral palsy, hydrocephaly, blindness, and deafness. Evidence has shown that perinatal polyphenols supplementation may alleviate brain injury in embryonic, fetal, neonatal, and offspring subjects, highlighting its role in modulating adaptive responses involving phenotypic plasticity. Therefore, it is reasonable to infer that the administration of polyphenols during the early life period may be considered a potential intervention to modulate the inflammatory and oxidative stress that cause impairments in locomotion, cognitive, and behavioral functions throughout life. The beneficial effects of polyphenols are linked with several mechanisms, including epigenetic alterations, involving the AMP-activated protein kinase (AMPK), nuclear factor kappa B (NF-κB), and phosphoinositide 3-kinase (PI3K) pathways. To highlight these new perspectives, the objective of this systematic review was to summarize the understanding emerging from preclinical studies about polyphenol supplementation, its capacity to minimize brain injury caused by hypoxia-ischemia in terms of morphological, inflammatory, and oxidative parameters and its repercussions for motor and behavioral functions.

Keywords: hypoxia-ischemia; brain; polyphenols; resveratrol; quercetin



Citation: Pontes, P.B.; Toscano, A.E.; Lacerda, D.C.; da Silva Araújo, E.R.; Costa, P.C.T.d.; Alves, S.M.; Brito Alves, J.L.d.; Manhães-de-Castro, R. Effectiveness of Polyphenols on Perinatal Brain Damage: A Systematic Review of Preclinical Studies. *Foods* **2023**, *12*, 2278. <https://doi.org/10.3390/foods12122278>

APÊNDICE 22 – OUTROS RESULTADOS (ARTIGO 20)

Artigo publicado como coautor na revista **Journal of Developmental Origins of Health and Disease**, classificada como **A3** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 1.82.

*Journal of Developmental
Origins of Health and Disease*

www.cambridge.org/doh

Review

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Keywords:

Pregnancy complications; metabolic diseases; polyphenols; resveratrol

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The effect of resveratrol in cardio-metabolic disorders during pregnancy and offspring outcomes: a review

Diego Cabral Lacerda¹ , Paulo César Trindade Costa¹, Yohanna de Oliveira¹ and José Luiz de Brito Alves¹ 

Department of Nutrition, Health Sciences Center, Federal University of Paraíba, João Pessoa, Brazil

Abstract

Resveratrol supplementation during pregnancy and lactation has been associated with a reduced risk of maternal obesity, gestational diabetes mellitus, and preeclampsia. In addition, emerging evidence has shown that maternal resveratrol supplementation diminishes cardio-metabolic disorders in offspring, highlighting its role in modulating adaptive responses involving phenotypic plasticity. Therefore, it is reasonable to infer that administration of resveratrol during pregnancy and lactation periods could be considered an important nutritional intervention to decrease the risk of maternal and offspring cardio-metabolic disorders. To highlight these new insights, this literature review will summarize the understanding emerging from experimental and clinical studies about resveratrol supplementation and its capacity to prevent or minimize maternal and offspring cardio-metabolic disorders.

Introduction

Maternal cardio-metabolic disorders, including gestational diabetes mellitus (GDM) and hypertensive disorders, are reported risk factors for maternal and offspring mortality. In part, inappropriate nutrition (under or overnutrition) has been the leading cause of cardio-metabolic diseases during pregnancy.¹ Epidemiological evidence has demonstrated

APÊNDICE 23 – OUTROS RESULTADOS (ARTIGO 21)

Artigo publicado como coautor na revista **World Journal of Diabetes**, classificada como **A4** de acordo com o *Qualis CAPES* 2017-2020 e Fator de impacto de 4.2.

 World Journal of Diabetes	
Submit a Manuscript: https://www.f6publishing.com	World J Diabetes 2022 September 15; 13(9): 717-728
DOI: 10.4239/wjd.v13.i9.717	ISSN 1948-9358 (online)
MINTREVIEWS	
Potential role of <i>Limosilactobacillus fermentum</i> as a probiotic with anti-diabetic properties: A review	
Diego Cabral Lacerda, Paulo César Trindade da Costa, Paula Brielle Pontes, Lucas Alves Carneiro dos Santos, José Patrocínio Ribeiro Cruz Neto, Cristiane Cosmo Silva Luis, Vanessa Polyana de Sousa Brito, José Luiz de Brito Alves	
Specialty type: Microbiology Provenance and peer review: Invited article; Externally peer reviewed. Peer-review model: Single blind Peer-review report's scientific quality classification Grade A (Excellent): 0 Grade B (Very good): B Grade C (Good): C Grade D (Fair): 0 Grade E (Poor): 0 P-Reviewer: Bala C, Romania; Shi J Received: March 2, 2022 Peer-review started: March 2, 2022 First decision: April 17, 2022 Revised: April 29, 2022 Accepted: May 11, 2022	Diego Cabral Lacerda, Paulo César Trindade da Costa, Lucas Alves Carneiro dos Santos, José Patrocínio Ribeiro Cruz Neto, Cristiane Cosmo Silva Luis, Vanessa Polyana de Sousa Brito, Department of Nutrition, Health Sciences Center, Federal University of Paraíba, João Pessoa, 58051-900, Paraíba, Brazil Paula Brielle Pontes, Department of Neuropsychiatry, Health Sciences Center, Federal University of Pernambuco, Recife, 50670-901, Pernambuco, Brazil José Luiz de Brito Alves, Department of Nutrition, Federal University of Paraíba, João Pessoa, 58045-190, Paraíba, Brazil Corresponding author: José Luiz de Brito Alves, PhD, Adjunct Professor, Department of Nutrition, Health Sciences Center, Federal University of Paraíba, Rue Francisco Carneiro de Araújo, n 55, apt 403, João Pessoa 58045-190, Paraíba, Brazil. jose_luiz_61@hotmail.com Abstract Oxidative stress, inflammation, and gut microbiota impairments have been implicated in the development and maintenance of diabetes mellitus. Strategies capable of recovering the community of commensal gut microbiota and controlling diabetes mellitus have increased in recent years. Some lactobacilli strains have an antioxidant and anti-inflammatory system capable of protecting

APÊNDICE 24 – OUTROS RESULTADOS (ARTIGO 22)

Artigo publicado como coautor na revista **Journal of the Endocrine Society**, classificada como **B1** de acordo com o *Qualis* CAPES 2017-2020 e Fator de impacto de 3.0.

Journal of the Endocrine Society, 2025, 9, bvaf011
<https://doi.org/10.1210/endsso/bvaf011>
 Advance access publication 10 February 2025
 Clinical Research Article



Lower Cortisol and Dehydroepiandrosterone Sulfate and Higher Food Addiction in Childhood Obesity: Associations With Stress and Dietary Parameters

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 Joicy Karla Grangeiro Pereira,¹ Paulo César Trindade da Costa,¹ Davyson Barbosa Duarte,²
 Lydiane de Lima Tavares Toscano,³ Manuel Francisco de Araújo Lima,⁴
 Melyssa Kellyane Cavalcanti Galdino,⁴ Joelma Rodrigues de Souza,⁵
 Francisco Antônio de Oliveira Júnior,⁵ Adélia da Costa Pereira de Arruda Neta,⁶
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Abstract

Context: Obesity has been associated with changes in cortisol and dehydroepiandrosterone (DHEA) sulfate concentrations and increased stress levels and food addiction.

Objectives: We explored changes in morning salivary cortisol and DHEA in childhood obesity and their associations with body composition, metabolic profile, food addiction, food consumption, and stress in a cross-sectional study.

Methods: Children aged 7 to 12 years of both sexes were allocated into 2 groups according to body mass index-for-age: control group ($n = 60$) or obesity group ($n = 98$). Anthropometric, body composition, serum glucose, insulin, lipid profile, and DHEA were measured. Saliva was collected at different times to measure morning salivary cortisol concentrations. Food addiction, food consumption, and stress were assessed using questionnaires.

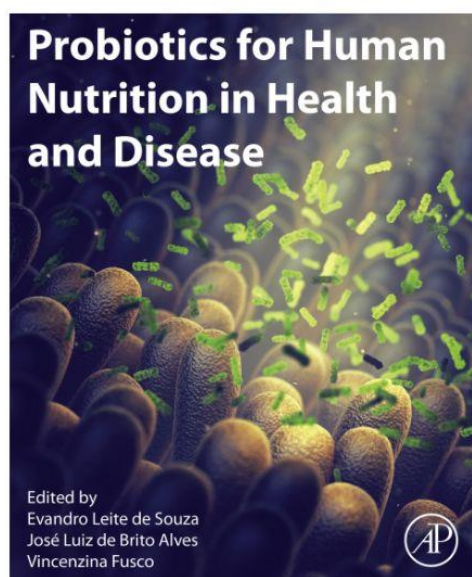
Results: Lower DHEA [1.04 (0.87 – 1.25) ng/mL vs 1.65 (1.30 – 2.07) ng/mL, $P = .002$] and salivary cortisol (6:00 AM: 1.17 ± 0.89 vs 1.45 ± 0.82 nmol/L; 6:30 AM: 1.53 ± 0.68 vs 1.83 ± 0.70 nmol/L; 7:30 AM: 0.72 ± 0.99 vs 1.31 ± 0.94 nmol/L, P -value of time < 0.001 and P -value of group = .002) were observed in children with obesity compared to the control. DHEA correlated negatively with waist circumference ($r = -0.20$, $P < .05$), body mass index-for-age (BMI-Z) ($r = -0.21$, $P < .01$), and weight ($r = -0.25$, $P < .01$). DHEA showed a positive correlation with the cortisol area under the curve ($r = 0.29$, $P = .002$). Food addiction was positively correlated with waist circumference ($r = 0.21$, $P < .01$), BMI-Z ($r = 0.22$, $P < .01$), body weight ($r = 0.20$, $P < .05$), total energy intake ($r = 0.20$, $P < .05$), and lipids ($r = 0.24$, $P < .01$).

Conclusion: Children with obesity showed lower concentrations of salivary cortisol and DHEA and higher food addiction compared to control children. These changes may contribute to the development of chronic diseases over time.

Key Words: childhood obesity, cortisol, food addiction, DHEA, NOVA classification, stress

APÊNDICE 25 – OUTROS RESULTADOS (DOIS CAPÍTULOS DE LIVRO)

Dois capítulos de livro publicados publicado no livro de circulação internacional *Probiotics for human nutrition in health and disease*.



PROBIOTICS FOR HUMAN NUTRITION IN HEALTH AND DISEASE

Edited by

EVANDRO LEITE DE SOUZA

Department of Nutrition, Health Sciences Center, Federal University of Paraíba, João Pessoa, Paraíba, Brazil

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CHAPTER

17

Probiotics for gastrointestinal health and disease treatment

Diego Cabral Lacerda, Paulo César Trindade da Costa, Luciana Caroline Paulino do Nascimento, José Luiz de Brito Alves
Department of Nutrition, Health Sciences Center, Federal University of Paraíba, João Pessoa, Paraíba, Brazil

CHAPTER

21

Probiotic for dyslipidemia prevention and treatment

José Luiz de Brito Alves, Paulo César Trindade da Costa, Luciana Caroline Paulino do Nascimento, Evandro Leite de Souza
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APÊNDICE 26 – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA NUTRIÇÃO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezado (a) Pai, Mãe ou responsável legal, _____

O seu filho (a) _____ está sendo convidado a participar de uma pesquisa intitulada “Treinamento físico e educação nutricional como estratégias para modular a microbiota intestinal e parâmetros metabólicos, inflamatórios e cardiovasculares em escolares com obesidade: uma cooperação para estudo multicêntrico” que está sendo desenvolvido pelo pesquisador José Luiz de Brito Alves, professor do Departamento de Nutrição da Universidade Federal da Paraíba.

Os objetivos do estudo são de analisar se crianças de 7 a 10 anos com obesidade que fazem um tratamento por 6 meses com exercício físico pliométrico e um programa de educação nutricional apresentam melhora na flora intestinal, vício alimentar, na saúde do coração, na inflamação e em hormônios como leptina, cortisol e insulina. Ocorre que tem sido descritas alterações em vários sistemas em crianças com obesidade. Por isso, a finalidade deste trabalho é contribuir para o desenvolvimento de tratamento não medicamentoso para a obesidade.

Caso aceite que seu filho (a) participe do estudo, haverá um sorteio para incluir seu filho (a) no grupo que receberá o tratamento com exercícios e educação nutricional ou somente educação nutricional. Todos os pais/responsáveis também vão receber educação nutricional na escola, uma vez ao mês com uma hora de duração. Caso os pesquisadores detectem que a prática de exercícios pliométricos é eficiente, as crianças que receberam educação nutricional serão estimuladas a realizar. Todas as crianças serão igualmente avaliadas. Primeiramente a criança será pesada e medida e avaliada quanto a sua composição corporal por meio da bioimpedância. Depois será coletada amostra de sangue para analisar insulina, leptina e cortisol, colesterol total e frações, glicose, triglicerídeos e uma série de marcadores inflamatórios como lipopolissacarídeos, TNF-alfa e interleucina 6. Depois será preenchido um questionário socioeconômico, anamnese de criança e questionário de vício alimentar. Será necessário coletar as fezes da criança.

Em relação aos procedimentos realizados, nenhum deles oferece riscos à saúde do seu filho (a). Na coleta sanguínea, existe um pequeno desconforto pela picada da agulha e o risco de ocorrerem fraqueza e tonturas, que são eliminados após a criança tomar café da manhã. Sempre serão utilizados materiais descartáveis. A avaliação por bioimpedância se dá pela passagem de uma corrente elétrica no corpo que é imperceptível e inofensiva. A única recomendação é a de que o participante não tenha objetos metálicos como, adornos, brincos, colares ou relógios, próximos ao corpo no momento da avaliação. O preenchimento dos questionários pode gerar algum desconforto, mas todos os dados são sigilosos.

Quanto aos benefícios da nossa pesquisa, seu filho (a) passará por avaliação laboratorial de hormônios importantes sem que a família tenha nenhum custo, serão orientadas e tratadas quando apresentarem alguma alteração. As crianças também receberão orientação nutricional e/ou tratamento com exercícios físicos. Estas ações podem contribuir para a melhoria da saúde das crianças. Não haverá despesas pessoais para o participante em qualquer fase do estudo, como também não haverá compensação financeira relacionada à participação de seu filho no estudo.

Solicitamos a sua colaboração para que seu filho (a) participe deste projeto juntamente com sua autorização para apresentar os resultados deste estudo em eventos da área de saúde e publicar em revista científica. Por ocasião da publicação dos resultados, seu nome será mantido em sigilo e todos os dados serão analisados em conjunto.

Este documento visa cumprir todas as recomendações da resolução 466/12 que regulamenta a realização de pesquisas em seres humanos no Brasil. Esclarecemos que sua participação no estudo é voluntária e, portanto, o (a) senhor (a) não é obrigado (a) a fornecer as informações e/ou colaborar com as atividades solicitadas pela pesquisadora. Caso decida não participar do estudo, ou resolver a qualquer momento desistir do mesmo, não sofrerá nenhum dano.

Os pesquisadores estarão à sua disposição para qualquer esclarecimento que considere necessário em qualquer etapa da pesquisa. O contato do pesquisador José Luiz é (83) 3216-7499, localizado no Departamento de Nutrição da UFPB. O telefone do comitê de ética é (83) 3216- 7791 e localiza-se no Centro de Ciências da Saúde da Universidade Federal da Paraíba Campus I - Cidade Universitária - 1º Andar – CEP 58051-900 – João Pessoa/PB – E-mail: comitedeetica@ccs.ufpb.br. Diante do exposto, declaro que fui devidamente esclarecido (a) e dou o meu consentimento para meu filho (a) participar da pesquisa e para publicação dos resultados. Estou ciente que receberei uma cópia desse documento.

Assinatura do Pai, mãe ou Responsável Legal

Assinatura do Pesquisador Responsável



Espaço para impressão dactiloscópica

APÊNDICE 27 – TERMO DE ASSENTIMENTO LIVRE E ESCLARECIDO

TERMO DE ASSENTIMENTO LIVRE E ESCLARECIDO - TALE

Você está sendo convidado para participar da pesquisa **Treinamento físico e educação nutricional como estratégias para modular a microbiota intestinal e parâmetros cardiometabólicos em escolares com obesidade**. Seus responsáveis legais permitiram que você participasse. O objetivo desta pesquisa é investigar se uma estratégia multicomponente tem efeitos positivos sobre a saúde de crianças com obesidade.

Você não precisa participar da pesquisa se não quiser. É um direito seu. Não terá nenhum problema se decidir a qualquer momento desistir de participar.

A pesquisa será feita na escola, onde você irá realizar alguns exames, tais como exame de sangue, de fezes, aferição da pressão arterial e da variabilidade da frequência cardíaca. Esta pesquisa é considerada segura, mas é possível ocorrer situações desagradáveis durante a coleta de dados e a intervenção, como dores e quedas. No entanto, a coleta e a intervenção serão feitas por profissionais capacitados e isso minimizará esses riscos. Caso aconteça algo errado, você pode nos procurar pelos telefones 83 993975442 (Paulo César). Mas há coisas boas que podem acontecer como a possibilidade de você entender melhor como seu corpo está funcionando e interagir com os colegas durante as atividades de intervenção.

Ninguém saberá que você está participando da pesquisa. Não falaremos a outras pessoas nem daremos a estranhos as informações que você nos der. Os resultados da pesquisa serão publicados num trabalho científico, mas sem identificar o seu nome.

Eu _____,
aceito participar da pesquisa **Treinamento físico e educação nutricional como estratégias para modular a microbiota intestinal e parâmetros cardiometabólicos em escolares com obesidade**. Entendi as coisas ruins e as coisas boas que podem acontecer. Entendi que posso dizer “sim” e participar. Mas que também, a qualquer momento, posso dizer “não” e desistir que ninguém vai ficar furioso ou me prejudicar. Os pesquisadores tiraram minhas dúvidas e conversaram com os meus responsáveis. Recebi uma cópia deste termo de assentimento, li ou meus responsáveis leram para mim, e concordei em participar da pesquisa.

João Pessoa, ____ de _____ de 20__.

Assinatura do participante



Responsável que assinou TCLE e testemunhou assentimento:

Assinatura: _____

Nome: _____

Pesquisador - assinatura: _____

Nome do pesquisador: _____

ANEXOS

ANEXO 1 – APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA EM SERES HUMANOS DO CENTRO DE EDUCAÇÃO E SAÚDE DA UFPB

**CENTRO DE CIÊNCIAS DA
SAÚDE DA UNIVERSIDADE
FEDERAL DA PARAÍBA -
CCS/UFPB**



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Treinamento físico e educação nutricional como estratégias para modular a microbiota intestinal e parâmetros metabólicos, inflamatórios e cardiovasculares em escolares com obesidade

Pesquisador: José Luiz de Brito Alves

Área Temática:

Versão: 3

CAAE: 37352720.6.1001.5188

Instituição Proponente: Centro de Ciência da Saúde

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.676.103

Apresentação do Projeto:

A pesquisa levanta a hipótese de que o treinamento físico pliométrico em combinação com processo de educação nutricional contribui para o reestabelecimento do equilíbrio da microbiota intestinal e suas funções, reduzem a adição alimentar e melhoram parâmetros metabólicos, posto que o aumento na ingestão de alimentos ultra processados de elevado valor calórico e com alto teor de gordura, associado ao aumento da inatividade física são descritos como os principais fatores de risco para o desenvolvimento da obesidade.

ANEXO 2 – CARTA DE ANUÊNCIA DA SECRETARIA DE EDUCAÇÃO E CULTURA DE JOÃO PESSOA PARA REALIZAÇÃO DO PROJETO

SECRETARIA DE
EDUCAÇÃO E
CULTURA



PREFEITURA MUNICIPAL DE JOÃO PESSOA
SECRETARIA DE EDUCAÇÃO E CULTURA
DIRETORIA DE ENSINO, GESTÃO E ESCOLA DE FORMAÇÃO

CARTA DE ANUÊNCIA

Declaramos, para os devidos fins, que concordamos que o pesquisador **Paulo César Trindade da Costa**, desenvolva o seu projeto de pesquisa, nível de Doutorado, pelo Programa de Pós-Graduação em Ciências da Nutrição, da Universidade Federal da Paraíba – UFPB, intitulado: **Treinamento físico e educação nutricional como estratégias para modular a microbiota intestinal e parâmetros metabólicos, inflamatórios e cardiovasculares em escolares com obesidade: uma cooperação para estudo multicêntrico**, sob a orientação do Prof. Dr. José Luiz de Brito Alves. As escolas que serão contempladas são: EMEF Cônego João de Deus, EMEF Lions de Tambaú, EMEF Olívio Campos Ribeiro, EMEF Rotary Francisco Edwar de Aguiar, EMEF Padre Bartolomeu de Gusmão, EMEF David Trindade, EMEF Índio Piragibe, EMEF Nazinha Barbosa, EMEF Américo Falcão, EMEF Seráfico da Nóbrega e EMEF Rodrigo Otávio. O objetivo geral do trabalho é: Investigar os efeitos de uma intervenção multicomponente com treinamento pliométrico e um programa de educação nutricional sobre a microbiota intestinal e suas funções, adição alimentar e parâmetros cardiovasculares, metabólicos, inflamatórios e hormonais em crianças eutróficas e com obesidade de 7 a 10 anos. E os objetivos específicos se concentram em avaliar antes e após a intervenção multicomponente: indicadores antropométricos e de composição corporal; ingestão alimentar e o estrato socioeconômico; adição alimentar e estresse percebido; ecologia microbiana intestinal; parâmetros bioquímicos, tais como lipidograma, glicose, insulina e HOMA-IR; parâmetros inflamatórios, especificamente os níveis de lipopolissacarídeos, TNF-alfa e IL6; variáveis hormonais, nomeadamente insulina, leptina e cortisol; pressão arterial sistólica e diastólica; frequência cardíaca e variabilidade da frequência cardíaca. A aceitação está condicionada ao comprometimento do pesquisador em utilizar os dados e materiais coletados exclusivamente para os fins da pesquisa.

João pessoa, 25 de maio de 2022

Alcilene da Costa Andry

p/ Clevia Suyene Cunha de Carvalho

Diretora de Ensino, Gestão e Escola de Formação

Alcilene da Costa Andry
Matrícula 62.764-1
Data de Assinatura do Documento: 25/05/2022

ANEXO 3 – APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE FEDERAL DA PARAÍBA



**Universidade
Federal da
Paraíba**



Universidade Federal da Paraíba

*Comissão de Ética no
Uso de Animais*

CERTIFICADO

Certificamos que a proposta intitulada "Formulação nutracêutica e treinamento físico como estratégias para modular parâmetros cardiometabólicos em ratos wistar com obesidade induzida por dieta hiperlipídica", protocolada sob o CEUA nº 4724190723 (ID 002088), sob a responsabilidade de **José Luiz de Brito Alves e equipe; Paulo César Trindade da Costa** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **APROVADA** pela Comissão de Ética no Uso de Animais da Universidade Federal da Paraíba (CEUA/UFPB) na reunião de 15/02/2024.

We certify that the proposal "Nutraceutical formulation and physical training as strategies to modulate cardiometabolic parameters in wistar rats with obesity induced by a high-fat diet", utilizing 55 Heterogenics rats (55 males), protocol number CEUA 4724190723 (ID 002088), under the responsibility of **José Luiz de Brito Alves and team; Paulo César Trindade da Costa** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **APPROVED** by the Ethic Committee on Animal Use of the Federal University of Paraíba (CEUA/UFPB) in the meeting of 02/15/2024.

Finalidade da Proposta: **Pesquisa (Acadêmica)**

Vigência da Proposta: de **08/2023 a 12/2024** Área: **Nutrição**

Origem: **Biotério Convencional de Roedores do Departamento de Fisiologia e Patologia (UFPE)**

Espécie: **Ratos heterogênicos**

sexo: **Machos**

idade: **21 a 30 dias**

Quantidade: **55**

Linhagem: **Rattus Norvegicus - Wistar**

Peso: **20 a 80 g**

João Pessoa, 03 de janeiro de 2025

Profa. Dra. Juliana Késsia Barbosa Soares Moreira
Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal da Paraíba

Profa. Dra. Gláucia Veríssimo Faheina Martins
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal da Paraíba

