

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

YOHANNA DE OLIVEIRA SANTOS DE ARAÚJO

**DETERMINAÇÃO DO EFEITO DE CEPAS DE *Limosilactobacillus fermentum* SOBRE
A PRESSÃO ARTERIAL E FUNÇÃO AUTONÔMICA NA PROLE DE RATOS
ORIUNDOS DE MÃES DISLIPIDÊMICAS**

João Pessoa

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Ciências da Nutrição, Departamento de Nutrição, Centro de Ciências da Saúde, Universidade Federal da Paraíba, em cumprimento aos requisitos para obtenção do título de Doutora em Ciências da Nutrição.

Linha de pesquisa: Clínica e Epidemiologia Aplicada à Nutrição.

Orientador: Prof. Dr. José Luiz de Brito Alves

Co-orientador: Prof. Dr. Marinaldo Pacifico Cavalcanti Neto

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Ata da 43ª (quadragésima terceira) Sessão Pública de Defesa de Tese para obtenção do grau de Doutor em Ciências da Nutrição, da discente Yohanna de Oliveira Santos de Araújo.

Aos vinte e dois dias do mês de março de dois mil e vinte e dois (22/03/2022) às 14:00 h, reuniram-se em cerimônia pública on line por meio da ferramenta Google meet, <https://meet.google.com/fad-morm-fab>, os membros da Banca Examinadora e a doutoranda Yohanna de Oliveira Santos de Araújo, candidata ao grau de Doutor em Ciências da Nutrição. A Banca Examinadora foi constituída pelos seguintes Professores: Dr. José Luiz de Brito Alves (Presidente/Orientador), Dr. Marinaldo Pacifico Cavalcanti Neto (Co-orientador), Dr. Vinicius José Baccin Martins (Examinador Interno), Drª Naiara Naiana Dejani (Examinadora Interna), Dr. Alexandre Sérgio Silva (Examinador Interno Suplente), Dr. Helioswilton Sales de Campos (Examinador Externo), Dr. João Henrique da Costa Silva (Examinador Externo), e Drª Monique Assis de Vasconcelos Barros (Examinadora Externa Suplente). Dando início a sessão pública, o Prof. José Luiz de Brito Alves, convidou a mim, Carlos Fernando da Silva para secretariá-los comunicando o fim específico da reunião. A seguir, foi cedida a palavra à examinada, para que, no tempo previsto no Regimento do PPGCN/CCS/UFPB, fizesse sua exposição de motivos e objetivos da sua tese, metodologia adotada, resultados encontrados, discussão e conclusão, trabalho esse sob o título “DETERMINAÇÃO DO EFEITO DE CEPAS DE *Limosilactobacillus fermentum* SOBRE A PRESSÃO ARTERIAL E FUNÇÃO AUTONÔMICA NA PROLE DE RATOS ORIUNDOS DE MÃES DISLIPIDÊMICAS”. Concluída a exposição, a candidata em seguida foi arguida sucessivamente por cada membro da banca examinadora. Dando continuidade, os membros examinadores foram inquiridos pelo Sr. Presidente se estavam aptos a proferir o julgamento e, recebendo respostas afirmativas, procederam com a deliberação da avaliação. Após certo espaço de tempo, o Sr. Presidente proclamou como “APROVADA” a tese de doutorado intitulada “DETERMINAÇÃO DO EFEITO DE CEPAS DE *Limosilactobacillus fermentum* SOBRE A PRESSÃO ARTERIAL E FUNÇÃO AUTONÔMICA NA PROLE DE RATOS ORIUNDOS DE MÃES DISLIPIDÊMICAS”. A seguir, preenchendo devidamente os mapas concernentes aos valores e conceitos, declarou que sua autora estava em condições de receber o grau de Doutor em Ciências da Nutrição, devendo a Universidade Federal da Paraíba, providenciar na forma da lei a expedição do respectivo Diploma. Nada mais havendo a ser tratado, eu, Carlos Fernando da Silva Secretário do Programa de Pós-Graduação em Ciências da Nutrição, lavrei a presente Ata que vai datada e assinada pelos membros da Banca Examinadora. João Pessoa, 22 de março de 2022.

José Luiz de Brito Alves

João Henrique da Costa Silva

Naiara N. Dejani



Universidade Federal da Paraíba

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EXAME DE DEFESA DE: () DISSERTAÇÃO (X) TESE	
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TÍTULO DO TRABALHO FINAL DETERMINAÇÃO DO EFEITO DE CEPAS DE <i>Limosilactobacillus fermentum</i> SOBRE A PRESSÃO ARTERIAL E FUNÇÃO AUTÔNOMICA NA PROLE DE RATOS ORIUNDOS DE MÃES DISLIPIDÊMICAS NOME DA DISCENTE: Yohanna de Oliveira Santos de Araújo	
NOME(S) DO(S) ORIENTADOR(ES) José Luiz de Brito Alves Marinaldo Pacifico Cavalcanti Neto	
OBSERVAÇÕES DA BANCA EXAMINADORA	
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LOCAL: Sessão pública não presencial por meio da utilização da plataforma virtual <i>Google Meet</i> , com divulgação pública do link de acesso à sala virtual.	
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Centro de Ciências da Saúde/UFPB - Campus I - Cidade Universitária
CEP: 58051-970, João Pessoa - PB, E-mail: ppgcen@ccs.ufpb.br, Tel: (83) 3216-7417



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PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COORDENAÇÃO GERAL DE PÓS-GRADUAÇÃO**

**RELATÓRIO FINAL DO (A) ORIENTADOR (A)
DOUTORADO**

Eu, José Luiz de Brito Alves, orientador do trabalho final da aluna Yohanna de Oliveira Santos de Araújo, matrícula nº 20181017024, do Programa de Pós-graduação em Ciências da Nutrição e área de concentração em Ciências da Nutrição, da Universidade Federal da Paraíba, após exame de vida acadêmica da mencionada aluna, tenho a relatar:

A integralização de curso foi feita em 48 meses, portando no prazo estabelecido pela Legislação vigente da UFPB.

Quanto ao desempenho acadêmico, constata-se que a doutoranda cursou 44 créditos da estrutura curricular a que está submetida e foi aprovada no exame de verificação da capacidade de leitura em Inglês e Espanhol no período letivo 2018.1.

Já na apresentação da Tese, intitulada “DETERMINAÇÃO DO EFEITO DE CEPAS DE *Limosilactobacillus fermentum* SOBRE A PRESSÃO ARTERIAL E FUNÇÃO AUTÔNOMICA NA PROLE DE RATOS ORIUNDOS DE MÃES DISLIPIDÊMICAS”, realizada no dia 22 de março de 2022, às 14h00 horas, por meio do uso plataforma *Google Meetings*), a discente obteve conceito APROVADA.

A Banca examinadora foi composta pelos (as) seguintes especialistas:

Prof. Dr. José Luiz de Brito Alves (UFPB)
Prof. Dr. Vinicius José Baccin Martins (UFPB)
Profª Drª Naiara Naiana Dejani (UFPB)
Prof. Dr. Helioswilton Sales de Campos (UFG)
Prof. Dr. João Henrique da Costa Silva (UFPE)

Diante do exposto, considerando que YOHANNA DE OLIVEIRA SANTOS DE ARAÚJO, dentro do prazo regimental, satisfaz todas as exigências do Regimento Geral da UFPB, do Regulamento Geral dos Cursos e Programas de Pós-Graduação Stricto Sensu da UFPB e do Regulamento do Programa de Pós-graduação em Ciências da Nutrição/CCS/UFPB, está APTA a obter o grau de DOUTOR EM CIÊNCIAS DA NUTRIÇÃO a ser conferido pela Universidade Federal da Paraíba.

João Pessoa, 22 de março de 2022.

José Luiz de Brito Alves

Orientador do Trabalho Final

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A *Deus* por tudo que me concede em minha jornada, por iluminar todo o meu percurso e me guiar sempre no caminho da verdade, da luz e da justiça. Por ser minha fortaleza em qualquer lugar. Por me fazer companhia nos livros, nos desenhos, nas partituras, na poesia, na serenidade e na coragem.

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Muito obrigada!

*“A loba, a velha, aquela que sabe está dentro de nós.
Floresce na mais profunda psique da alma das mulheres,
a antiga e vital Mulher Selvagem.
Ela descreve seu lar como um lugar no tempo em que
o espírito das mulheres e o espírito dos lobos entram em contato.
É o ponto em que o Eu e o Você se beijam,
o lugar em que as mulheres correm com os lobos (...).”*

Clarissa Pinkola Estés

RESUMO

A dislipidemia materna contribui para o desenvolvimento de hipertensão arterial (HA), associada com hiperatividade simpática, prejuízo no controle barorreflexo, maior sensibilidade de quimiorreceptores periféricos e desequilíbrio na composição da microbiota intestinal na vida adulta da prole. Estudos indicam que a administração de probióticos pode ser efetiva na melhoria de distúrbios no metabolismo lipídico, atenuando o estresse oxidativo e reduzindo a pressão arterial (PA). Sendo assim, o objetivo geral desse estudo foi investigar os efeitos da intervenção com uma formulação probiótica de *L. fermentum* sobre os parâmetros cardiovasculares e metabólicos na prole de ratos expostos à dislipidemia materna. Os protocolos foram aprovados pelo CEUA/UFPA (nº 9492260418/2018). Ratas Wistar prenhes (n=14) foram alimentadas com dieta controle (CTL=7) ou dieta dislipidêmica (DLP=7) durante a gestação e a lactação. Após o desmame, as proles de machos e fêmeas receberam dieta comercial até os 90 dias de vida. Os ratos foram divididos em três grupos: grupo CTL + solução salina (n = 14; 7 machos e 7 fêmeas), grupo DLP + solução salina (n = 14; 7 machos e 7 fêmeas) e grupo DLP + formulação probiótica de *L. fermentum* (n = 14; 7 machos e 7 fêmeas). A intervenção com a formulação probiótica de *L. fermentum* contendo as cepas 139, 263 e 296 (10^9 UFC/mL) foi realizada diariamente por gavagem desde o pós-desmame à vida adulta (30º ao 90º dia de vida). Após 8 semanas de tratamento, o sangue foi coletado para testes bioquímicos, teste oral de tolerância à glicose (TOTG) e tolerância à insulina (TTI). Amostras de fezes foram coletadas para contagem de micro-organismos viáveis. Em seguida, os ratos foram anestesiados com cetamina (80 mg/kg, i.p.) e xilazina (10mg/kg, i.p.) para inserção de cateteres de polietileno na artéria e veia femoral para posterior aferição da PA e frequência cardíaca (FC) basais, análise espectral da pressão arterial sistólica e intervalo de pulso e balanço simpático-vagal. Em sequência, KCN 0,04 % (100 µl/rato, i.v.) foi utilizado para avaliar a sensibilidade de quimiorreceptores periféricos. Para avaliação do controle barorreflexo, administrou-se nitroprussiato de sódio (25 µg/kg) e fenilefrina (8 µg/kg). A contribuição do tônus simpático sobre a manutenção da PA foi avaliada através da administração de hexametônio (25 mg/kg, i.v.). A intervenção com formulação probiótica de *L. fermentum* na prole de machos e fêmeas DLP atenuou os níveis séricos de colesterol total (CT) ($p<0,05$) e malondialdeído (MDA) ($p<0,05$) e exibiu maiores níveis séricos de lipoproteína de alta densidade (HDL) ($p<0,05$), mas não atenuou a resistência à insulina (RI) ($p>0,05$) induzida pela dislipidemia materna. Além disso, os grupos DLP de ambos os sexos que receberam a formulação probiótica de *L. fermentum* também demonstraram melhora nas contagens fecais de *Lactobacillus* sp. ($p<0,05$), na resposta pressórica ($p<0,05$) e no tônus simpático ($p<0,05$) quando comparados aos grupos DLP não tratados. Estes resultados indicam que a formulação probiótica de *L. fermentum* pode efetivamente atenuar a resposta simpato-excitatória induzida pelo quimiorreflexo periférico, bem como reduzir o tônus simpático e o estresse oxidativo, sendo uma estratégia promissora para prevenir a hipercolesterolemia e consequentemente, o desenvolvimento de HA na prole adulta.

Palavras-chave: Dislipidemia materna. Intervenção. Formulação de probióticos. Hipertensão arterial.

ABSTRACT

Maternal dyslipidemia contributes to the development of arterial hypertension (AH) associated with sympathetic hyperactivity, impaired baroreflex control, augmented peripheral chemoreceptor sensitivity and unbalance in the gut microbiota composition in adulthood. Studies indicate that the administration of probiotics can be effective in the improvement of disorders in lipid metabolism, attenuating oxidative stress and reducing blood pressure (BP). Thus, the general objective of this study was to investigate the effects of the intervention with a probiotic formulation of *L. fermentum* on cardiovascular and metabolic parameters in rats offspring exposed to maternal dyslipidemia. The protocols were approved by CEUA / UFPB (n° 9492260418/2018). Pregnant Wistar rats (n = 14) were fed a control diet (CTL = 7) or a dyslipidemic diet (DLP = 7) during pregnancy and lactation. After weaning, the male and female offspring received a commercial diet until the 90 days of age. The rats were divided into three groups: CTL group + saline solution (n = 14; 7 males and 7 females), DLP group + saline solution (n = 14; 7 males and 7 females) and DLP group + probiotic formulation of *L. fermentum* (n = 14; 7 males and 7 females). The intervention with the probiotic formulation of *L. fermentum* containing the strains 139, 263 and 296 (10^9 CFU / mL) was performed daily by gavage from post-weaning to adulthood (30th to 90th day of age). After 8 weeks of treatment, blood was collected for biochemical tests, oral glucose tolerance (OGTT) and insulin tolerance (ITT) tests, fecal samples were collected to microbial viable counts, and finally, rats were anaesthetised with ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) to insert polyethylene catheters in the femoral artery and vein for later measurement of baseline BP and heart rate (HR), spectral analysis of systolic blood pressure and pulse interval and sympathetic-vagal balance. Then, 0.04% KCN (100 μ l/rat, i.v.) was used to evaluate the sensitivity of peripheral chemoreceptors. To evaluate baroreflex control, sodium nitroprusside (25 μ g/kg) and phenylephrine (8 μ g/kg) were administered. The contribution of sympathetic tone to BP maintenance was evaluated by administration of hexamethonium (25 mg/kg, i.v.). The intervention with probiotic formulation of *L. fermentum* in DLP male and female offspring attenuated the serum levels of total cholesterol (TC) ($p < 0.05$) and malondialdehyde (MDA) ($p < 0.05$) and exhibited higher serum levels of high-density lipoprotein (HDL) ($p < 0.05$), but did not attenuate insulin resistance (IR) ($p > 0.05$) induced by maternal dyslipidemia. In addition, the DLP groups of both sexes that received the probiotic formulation of *L. fermentum* also showed improvement in fecal *Lactobacillus* sp. counts ($p < 0.05$), pressor response ($p < 0.05$) and sympathetic tone ($p < 0.05$) when compared to the non-treated DLP groups. These results indicate that the probiotic formulation of *L. fermentum* may effectively attenuate the sympathoexcitatory response induced by the peripheral chemoreflex, as well as reduce the sympathetic tone and oxidative stress, being a promising strategy to prevent hypercholesterolemia and, consequently, the development of AH in adult offspring.

Keywords: Maternal dyslipidemia. Intervention. Probiotic formulation. Arterial hypertension.

LISTA DE FIGURAS

FIGURAS DA TESE

Figura 1.	Protocolo experimental do estudo.....	40
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FIGURAS DO ARTIGO I

Figure 1.	Schematic drawing showing that maternal cardiometabolic disorders, such as gestational diabetes mellitus (GDM), pre-eclampsia, obesity and dyslipidemias, are associated with disrupted microbiota and that probiotic intervention could improve glycemic control (GC), insulin resistance (IR) and lipid profiles in pregnant women.....	67
Figure 2.	Schematic drawing proposing “The Transgenerational Microbiome Project” as a future direction for investigations: i) the transgenerational impact of maternal cardiometabolic disorders on the microbiota, immune function and cardiometabolic parameters in offspring; and ii) evaluate if probiotic therapy in the course of cardiometabolic disorders in pregnancy could exert beneficial effects on microbiota, immune function and cardiometabolic parameters in offspring.....	67

FIGURAS DO ARTIGO II

Figure 1.	Effects of the probiotic formulation of <i>L. fermentum</i> on resting blood pressure and heart rate in male and female offspring from dams exposed to maternal dyslipidemia. Evaluation of resting systolic blood pressure (SAP), diastolic blood pressure (DAP), mean arterial pressure (MAP), and heart rate (HR) in male (A, B, C, D) and female (E, F, G, H) offspring at 90 days of age that received or did not receive intervention with <i>L. fermentum</i> after both were exposed to maternal dyslipidemia during pregnancy and lactation. Groups: control group (CTL, n = 14; 7 females and 7 males), dyslipidemic group (DLP, n = 14; 7 females and 7 males) and the DLP group that received the probiotic formulation of <i>L. fermentum</i> (DLP + Lf, n = 14; 7 females and 7 males). Data are shown as mean ± SEM, analyzed by	
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one-way ANOVA, and followed by Tukey's post-test. * $p < 0.05$ indicates significant difference in mean values of the variable between the DLP and CTL groups. # $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and DLP groups. $\Psi p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and CTL groups..... 76

Figure 2. Effects of the probiotic formulation of *L. fermentum* on the spectral analysis of systolic arterial pressure (SAP), cardiac interval and spontaneous baroreflex sensitivity (SBRS) in male and female offspring from dams exposed to maternal dyslipidemia. Evaluation of low frequency (LF) power, high frequency (HF) power, LF/HF ratio and spontaneous baroreflex sensitivity (SBRS) in male (A, B, C, D) and female (E, F, G, H) offspring at 90 days of age that received or did not receive intervention with *L. fermentum* after both were exposed to maternal dyslipidemia during pregnancy and lactation. Groups: control group (CTL, n = 14; 7 females and 7 males), dyslipidemic group (DLP, n = 14; 7 females and 7 males) and the DLP group that received the probiotic formulation of *L. fermentum* (DLP + Lf, n = 14; 7 females and 7 males). Data are shown as mean $\pm$ SEM, analyzed by one-way ANOVA, and followed by Tukey's post-test. * $p < 0.05$ indicates significant difference in mean values of the variable between the DLP and CTL groups. # $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and DLP groups. $\Psi p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and CTL groups..... 77

Figure 3. Effects of the probiotic formulation of *L. fermentum* on peripheral chemoreflex evaluation in male and female offspring from dams exposed to maternal dyslipidemia. Evaluation of the variation in mean arterial pressure (MAP) and heart rate (HR) during peripheral chemoreflex activation (KCN, 0.04%) in male (A, B) and female (C, D) offspring at 90 days of age that received or did not receive intervention with *L. fermentum* after both were exposed to maternal dyslipidemia during pregnancy and lactation. Groups: control group (CTL, n = 14; 7 females and 7 males), dyslipidemic group (DLP, n = 14; 7 females and 7 males) and the DLP group that received the probiotic formulation of *L. fermentum* (DLP + Lf, n = 14; 7 females and 7

males). Data are shown as mean $\pm$ SEM, analyzed by one-way ANOVA, and followed by Tukey's post-test. * $p < 0.05$ indicates significant difference in mean values of the variable between the DLP and CTL groups. # $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and DLP groups. $\Psi p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and CTL groups..... 78

Figure 4. Effects of the probiotic formulation of *L. fermentum* on the sympathetic tone in male and female offspring from dams exposed to maternal dyslipidemia. Assessment of delta change of the mean arterial pressure (MAP) after blockade with hexamethonium (30 $\mu\text{g kg}^{-1}$, i.v.) in male (A) and female (B) offspring at 90 days of age that received or did not receive intervention with *L. fermentum* after both were exposed to maternal dyslipidemia during pregnancy and lactation. Groups: control group (CTL, n = 14; 7 females and 7 males), dyslipidemic group (DLP, n = 14; 7 females and 7 males) and the DLP group that received the probiotic formulation of *L. fermentum* (DLP + Lf, n = 14; 7 females and 7 males). Data are shown as mean $\pm$ SEM, analyzed by one-way ANOVA, and followed by Tukey's post-test. * $p < 0.05$ indicates significant difference in mean values of the variable between the DLP and CTL groups. # $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and DLP groups. $\Psi p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and CTL groups..... 79

Figure 5. Schematic drawing showing the impact of the probiotic formulation with *L. fermentum* on the fecal microbiota, biochemical variables, blood pressure and autonomic function in male and female rats exposed to maternal dyslipidemia..... 80

LISTA DE TABELAS

TABELAS DA TESE

Tabela 1.	Composição nutricional dietética (g/100g).....	39
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TABELAS DO ARTIGO I

Table 1.	Summary of clinical trials investigating the relationship between probiotic supplementation in pregnant women and clinical responses.....	62
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TABELAS DO ARTIGO II

Table 1.	Composition of the control and dyslipidemic diets offered to dams during pregnancy and lactation.....	72
Table 2.	Body weight and biochemical variables in dams that received a control (CTL) or dyslipidemic (DLP) diet during pregnancy and lactation.....	74
Table 3.	Body weight, biochemical parameters and microbial counts in feces.....	75

LISTA DE ABREVIações E SIGLAS

AGCT	Ácidos graxos de cadeia curta
AGT	Ácidos graxos trans
AHA	<i>American Heart Association</i>
AIN	<i>American Institute of Nutrition</i> (Instituto Americano de Nutrição)
BAL	Bactérias ácido-láticas
CAT	Catalase
CC	Corpúsculo carotídeo
CEUA	Comissão de Ética no Uso de Animais
CONCEA	Conselho Nacional para Controle de Experimentação Animal
CT	Colesterol total
CTL	Controle
DCNT	Doenças crônicas não transmissíveis
DCV	Doenças cardiovasculares
DHGNA	Doença hepática gordurosa não alcoólica
DLP	Dislipidêmico
DMG	Diabetes mellitus gestacional
DMT2	Diabetes mellitus tipo 2
ECA	Enzima conversora da angiotensina
EFSA	<i>European Food Safety Authority</i> (Autoridade Europeia para a Segurança dos Alimentos)
EROs	Espécies reativas de oxigênio
FAO	<i>Food and Agriculture Organization</i> (Organização das Nações Unidas para Alimentação e Agricultura)
FC	Frequência cardíaca
GPx	Glutathione Peroxidase
GRAS	<i>Generally recognized as safe</i> (Geralmente reconhecido com seguro)
HA	Hipertensão Arterial
HDACs	Histonas desacetilases
HDL	<i>High density lipoprotein</i> (Lipoproteína da alta densidade)
HFD	<i>High-fat diet</i> (Dieta rica em gordura)
IMC	Índice de massa corporal

IpeFarm	Instituto de Pesquisa em Fármacos e Medicamentos
KCN	Cianeto de potássio
LANEX	Laboratório de Nutrição Experimental
LDL	<i>Low density lipoprotein</i> (Lipoproteína de baixa densidade)
LPS	Lipopolissacarídeos
MDA	Malondialdeído
NTS	Núcleo do trato solitário
OMS	Organização Mundial de Saúde
PA	Pressão arterial
PAD	Pressão arterial diastólica
PAS	Pressão arterial sistólica
PBS	<i>Phosphate buffered saline</i> (Solução tampão fosfato salina)
QRP	Quimiorreceptores periféricos
RCQ	Relação cintura-quadril
SM	Síndrome metabólica
SNC	Sistema nervoso central
SNCA	Sistema nervoso central autônomo
SOD	Superóxido Dismutase
TG	Triglicerídeos
TGI	Trato gastrointestinal
TOTG	Teste oral de tolerância à glicose
TTI	Teste tolerância à insulina
UFC	Unidades formadoras de colônias
VLDL	<i>Very Low Density Lipoprotein</i> (Lipoproteína de muito baixa densidade)

SUMÁRIO

1 INTRODUÇÃO.....	18
2 REFERENCIAL TEÓRICO.....	20
2.1 DISLIPIDEMIA MATERNA E O IMPACTO SOBRE A PROLE.....	20
2.2 CONTROLE NEURAL DO SISTEMA CARDIOVASCULAR E HIPERTENSÃO ARTERIAL.....	23
2.3 MICROBIOTA INTESTINAL.....	25
2.4 O EIXO MICROBIOTA-INTESTINO-CÉREBRO E HIPERTENSÃO ARTERIAL.....	29
2.5 PROBIÓTICOS.....	31
2.5.1 Efeitos da intervenção com probióticos na hipertensão arterial: achados experimentais e clínicos.....	34
3 ABORDAGEM METODOLÓGICA.....	37
3.1 CARACTERIZAÇÃO DO ESTUDO.....	37
3.2 ANIMAIS E ASPECTOS ÉTICOS.....	37
3.3 CEPAS PROBIÓTICAS.....	37
3.4 PREPARAÇÃO DA SUSPENSÃO DE CÉLULAS PROBIÓTICAS.....	38
3.5 GRUPOS EXPERIMENTAIS E INTERVENÇÃO COM FORMULAÇÃO DE <i>L.</i> <i>fermentum</i>	38
3.6 AVALIAÇÃO DO PESO CORPORAL E DO CONSUMO ALIMENTAR.....	40
3.7 TESTE ORAL DE TOLERÂNCIA À GLICOSE E TESTE DE TOLERÂNCIA À INSULINA.....	40
3.8 ANÁLISES BIOQUÍMICAS.....	41
3.9 PROCEDIMENTO CIRÚRGICO PARA REGISTRO DA PRESSÃO ARTERIAL.....	41
3.10 ANÁLISE ESPECTRAL.....	42
3.11 ATIVAÇÃO DOS QUIMIORRECEPTORES PERIFÉRICOS.....	42
3.12 AVALIAÇÃO DO CONTROLE BARORREFLEXO.....	42
3.13 AVALIAÇÃO DO TÔNUS SIMPÁTICO SOBRE O SISTEMA CARDIOVASCULAR.....	43
3.14 CONTAGEM DE MICRO-ORGANISMOS VIÁVEIS NAS FEZES.....	43
3.15 ANÁLISES ESTATÍSTICAS.....	44
4 RESULTADOS.....	45

5 CONCLUSÃO.....	47
REFERÊNCIAS BIBLIOGRÁFICAS.....	48
APÊNDICE A – ARTIGO I.....	60
APÊNDICE B – ARTIGO II.....	71
APÊNDICE C – CAPÍTULO DE LIVRO.....	85
ANEXO A – CERTIFICADO DE APROVAÇÃO DO PROJETO PELA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DE LABORATÓRIO (CEUA)	98

1 INTRODUÇÃO

A hipertensão arterial (HA) é uma condição clínica de etiologia multifatorial caracterizada por níveis elevados e persistentes de pressão arterial (PA) e que está associada ao desenvolvimento de distúrbios cardiovasculares e metabólicos, além de maior risco de mortalidade em todo o mundo (OPARIL *et al.*, 2018).

Tem sido descrito que o consumo materno excessivo de uma dieta rica em gordura durante os estágios críticos do desenvolvimento como gravidez, lactação e primeira infância, podem alterar a fisiologia de sistemas do controle cardiovascular e aumentar a predisposição para o desenvolvimento de HA na vida adulta (JIN *et al.*, 2016; SHEEN *et al.*, 2018).

Estudos experimentais têm demonstrado que a prole adulta de machos e fêmeas de mães expostas a uma dieta dislipidêmica durante a gestação e lactação desenvolveram HA associada com hiperatividade simpática, maior sensibilidade de quimiorreceptores periféricos e prejuízo do barorreflexo, além de apresentarem resistência à insulina e níveis séricos elevados de malondialdeído (MDA), um indicador de peroxidação lipídica (GUIMARÃES *et al.*, 2017; DE ARAÚJO *et al.*, 2019; DE ARAÚJO *et al.*, 2020). Esses resultados demonstram que a dislipidemia materna durante os períodos de gestação e lactação pode contribuir diretamente para o desenvolvimento de HA e distúrbios metabólicos na vida adulta da prole.

A ingestão de uma dieta rica em gordura durante a gestação e lactação altera a composição da microbiota intestinal materno-fetal, com prejuízo da integridade epitelial e aumento da permeabilidade intestinal, favorecendo a translocação de lipopolissacarídeos (LPS) e diminuição das bactérias produtoras de ácidos graxos de cadeia curta (AGCC), contribuindo para a inflamação sistêmica de baixo grau e causando danos em órgãos-chave do controle da PA (XIE *et al.*, 2018; HSU *et al.*, 2019). Em parte, a disbiose intestinal pode influenciar a neuroinflamação do sistema nervoso central (SNC), levando ao aumento da atividade simpática (TORAL *et al.*, 2019; WANG *et al.*, 2021), atuando como um mecanismo de sinalização ascendente através do eixo microbiota-intestino-cérebro, contribuindo para o desenvolvimento da HA (MURALITHARAN *et al.*, 2020).

Neste sentido, é possível sugerir que intervenções que tenham como alvo potencial a microbiota intestinal poderiam ser uma estratégia coadjuvante para prevenir o desenvolvimento de hipertensão e distúrbios metabólicos na prole. Nessa perspectiva, a modulação terapêutica da microbiota intestinal, através da intervenção com probióticos, pode representar uma estratégia profilática promissora e inovadora durante o desenvolvimento da

HA, assim como de distúrbios metabólicos na prole de mães alimentadas com dieta dislipidêmica.

Probióticos são definidos como “microrganismos vivos que, quando administrados em quantidades adequadas, conferem efeitos benéficos à saúde do hospedeiro” (HILL *et al.*, 2014). Recentemente, foi proposta uma nova classificação taxonômica da espécie *Lactobacillus fermentum*, sendo renomeado como *Limosilactobacillus fermentum*. As cepas deste gênero têm sido amplamente utilizadas como probióticos, demonstrando várias propriedades favoráveis *in vitro* e *in vivo*, além de conferir uma melhor adaptação às condições do trato-gastrointestinal (TGI) humano (ZHENG *et al.*, 2020).

Cepas de *Limosilactobacillus* foram isoladas de subprodutos do processamento de frutas e caracterizadas por um conjunto de propriedades probióticas *in vitro* (GARCIA *et al.*, 2016). Entre as cepas previamente isoladas, *L. fermentum* 139, *L. fermentum* 263 e *L. fermentum* 296 apresentaram desempenho satisfatório para funcionalidades fisiológicas de adesão, agregação, coagregação, antagonismo e sobrevivência à exposição a condições gastrointestinais simuladas, além de não apresentar atividades hemolíticas e mucinolíticas e resistência a antibióticos (DE ALBUQUERQUE *et al.*, 2018). No entanto, o potencial cardiovascular e metabólico dessas cepas ainda não foi elucidado em modelos experimentais *in vivo*.

Neste sentido, o objetivo geral deste trabalho foi investigar os efeitos da intervenção com uma formulação probiótica composta por *L. fermentum* 139, *L. fermentum* 263 e *L. fermentum* 296 sobre os parâmetros cardiovasculares e metabólicos na prole de ratos machos e fêmeas de mães expostas à dislipidemia materna durante a gestação e lactação. Os objetivos específicos incluem: avaliação dos níveis pressóricos e da frequência cardíaca, investigação da sensibilidade de quimiorreceptores periféricos, exame do controle barorreflexo e da resposta do tônus simpático, análise dos parâmetros glicolipídicos e contagem de micro-organismos viáveis nas fezes.

2 REFERENCIAL TEÓRICO

2.1 DISLIPIDEMIA MATERNA E O IMPACTO SOBRE A PROLE

A gestação é um processo biológico que consiste em um período vital de crescimento e desenvolvimento fetal, que pode ser significativamente influenciado por fatores ambientais, hormonais, genéticos, nutricionais, assim como pela composição da microbiota intestinal materna. Neste período, o feto passa por um período crítico de adaptabilidade, no qual a nutrição materna adequada exerce um papel determinante para o desenvolvimento fetal ideal e a manutenção do metabolismo materno saudável (KOLETZKO *et al.*, 2019).

Durante uma gravidez, ocorrem mudanças no metabolismo lipídico devido a alterações das concentrações hormonais. Por exemplo, níveis elevados de estrogênio e resistência à insulina podem contribuir para o aumento dos níveis séricos de lipídios durante a gravidez. Na 12ª semana de gestação, os níveis de colesterol de lipoproteína de alta densidade (HDL) aumentam de 15 a 30%, enquanto os níveis de triglicerídeos (TG) aumentam a partir da 14ª semana, podendo aumentar até quatro vezes mais, e permanece elevado durante toda a gravidez, sendo esses valores elevados atribuídos ao aumento das concentrações circulantes de estrogênio. Os níveis de colesterol total (CT) aumentam gradualmente, podendo aumentar em 25 a 50% no último trimestre, e os níveis de colesterol de lipoproteína de baixa densidade (LDL) aumentam de 40 a 50%, observando que, à medida que os níveis de LDL aumentam, há um aumento progressivo das concentrações de progesterona. Esses valores de perfil lipídico materno tendem a diminuir em até três meses após o parto, retornando aos valores basais (ARMISTEAD *et al.*, 2020; THOBANI *et al.*, 2021).

Essas mudanças fisiológicas são consideradas benéficas, já que o colesterol materno possui um papel fundamental no crescimento fetal e no desenvolvimento do cérebro no início da gravidez (JI *et al.*, 2018). No entanto, o aumento contínuo e excessivo dos níveis séricos de CT, TG e LDL e uma diminuição dos níveis de HDL, caracterizando um quadro de dislipidemia materna, representaram um risco elevado para desenvolver condições fisiopatológicas na gravidez e efeitos deletérios na prole (LU *et al.*, 2021).

A dislipidemia compreende quatro categorias bem definidas: I) Hipercolesterolemia isolada: aumento isolado do LDL-c ($\text{LDL-c} \geq 160 \text{ mg/dL}$); II) Hipertrigliceridemia isolada: aumento isolado de TG ($\text{TG} \geq 150 \text{ mg/dL}$ ou $\geq 175 \text{ mg/dL}$); III) Hiperlipidemia mista: aumento do LDL-c ($\text{LDL-c} \geq 160 \text{ mg/dL}$) e de TG ($\text{TG} \geq 150 \text{ mg/dL}$ ou $\geq 175 \text{ mg/dL}$ e IV)

Diminuição do HDL-c: redução isolada do HDL-c (homens < 40 mg/dL e mulheres < 50 mg/dL) ou em associação com LDL-c e/ou TG aumentados (FALUDI *et al.*, 2017).

Na gestação, a dislipidemia pode ser avaliada por dois critérios: pelo critério dos percentis, no qual a dislipidemia é caracterizada por concentrações de CT, TG e LDL superiores ao percentil 95 e concentrações de HDL inferiores ao percentil 5 para a idade gestacional, ou pelo critério da V Diretriz Brasileira de Dislipidemia, que estabelece os valores de CT, LDL e TG, respectivamente, superiores a 200 mg/dl, 160 mg/dl e 150 mg/dl, e o HDL inferior a 50 mg/dl, sendo diagnosticada com dislipidemia a gestante que apresentar alteração em pelo menos uma fração lipídica (FEITOSA *et al.*, 2017).

Há evidências de que a exposição fetal a condições nutricionais desequilibradas durante a gravidez e lactação pode contribuir para a maturação de órgãos de menor tamanho e com capacidade funcional reduzida, resultando em implicações para a saúde em longo prazo na prole (HSU; TAIN, 2019). Neste tocante, surge a hipótese denominada “Origens desenvolvimentistas da saúde e da doença” (DOHaD), a qual correlaciona os efeitos adversos experimentados durante períodos críticos do desenvolvimento embrionário com o processo de programação fetal, a qual predispõe o indivíduo, na idade adulta, ao desenvolvimento de doenças crônicas não transmissíveis (DCNT) (GLUCKMAN; HANSON, 2007; HOFFMAN *et al.*, 2021).

Este conceito é bem estabelecido e propõe que fatores nutricionais maternos desfavoráveis podem modular os processos de desenvolvimento no útero durante os períodos críticos do desenvolvimento, que incluem a pré-concepção e gestação, e são caracterizados pela rápida diferenciação e maturação celular, com efeitos persistentes na saúde da prole, desempenhando um papel crucial na etiologia de DCNT, incluindo diabetes mellitus tipo 2 (DMT2), doenças cardiovasculares (DCV), doenças respiratórias, obesidade, alergias, câncer, transtornos psicológicos e prejuízos cognitivos (FLEMING *et al.*, 2018).

Neste sentido, o consumo crônico e contínuo de uma dieta rica em colesterol, ácidos graxos saturados e trans durante os períodos de gestação e lactação tem sido identificado como um importante fator de risco na indução da dislipidemia materna (GUIMARÃES *et al.*, 2017; GAWLINSKA *et al.*, 2021). Uma meta-análise avaliou modelos experimentais de exposição à dieta dislipidêmica materna e o impacto sobre o metabolismo da prole, e constatou que a exposição materna à dieta dislipidêmica, embora não tenha afetado o peso ao nascer, resultou em um fenótipo metabólico perturbado na prole, com aumento do peso corporal ao desmame e na vida adulta, aumento da adiposidade, hipertrigliceridemia, hipercolesterolemia e hiperinsulinemia na prole de machos e fêmeas, e hiperglicemia na prole

de fêmeas, demonstrando o impacto duradouro da dieta materna rica em gordura sobre o crescimento e a saúde da prole até a vida adulta (RIBAROFF *et al.*, 2017).

Evidências têm demonstrado que o aumento dos níveis de TG excedendo 300mg/dL no primeiro trimestre de gravidez podem exercer potenciais efeitos adversos na saúde materno-fetal, como por exemplo, desordens hipertensivas na gravidez, diabetes mellitus gestacional (DMG), pré-eclâmpsia, obesidade e parto prematuro (SHEN *et al.*, 2016; WANG *et al.*, 2017). Em adição, a exposição fetal a níveis elevados de TG e CT maternos podem afetar o metabolismo lipídico placentário, gerando o acúmulo de TG na circulação fetal e níveis elevados de leptina e insulina, contribuindo para o aumento do tamanho fetal e depósito de massa gorda, aumentando a predisposição para o desenvolvimento de obesidade, doença hepática gordurosa não alcoólica (DHGNA) e síndrome metabólica (SM) em longo prazo na prole (DELHAES *et al.*, 2018).

Estudos de coorte prospectivos têm demonstrado que o aumento dos níveis séricos de TG durante o terceiro trimestre de gravidez foi associado com um risco aumentado para DMG, pré-eclâmpsia, colestase intra-hepática gestacional, recém-nascido grande para a idade gestacional, e macrossomia, enquanto que a diminuição dos níveis séricos de HDL foi associada com um risco aumentado para DMG e macrossomia (JIN *et al.*, 2016; LIANG *et al.*, 2018; WANG *et al.*, 2018).

Resultados do *Framingham Heart Study*, um renomado estudo multigeracional, de coorte de base populacional iniciado em 1948, demonstrou que adultos que tinham sido expostos a níveis elevados de LDL-c pré-gestacionais maternos, possuíam um risco de 3,8 vezes mais chances de apresentar níveis elevados de LDL-c na vida adulta, e essas associações persistiram após o ajuste para o índice de massa corporal (IMC) materno e tabagismo, bem como para o ajuste de fatores de estilo de vida, IMC e variantes genéticas herdadas nos descendentes (MENDELSON *et al.*, 2016).

Nosso grupo de pesquisa demonstrou que descendentes machos e fêmeas de ratas alimentadas com uma dieta dislipidêmica durante a gestação e lactação desenvolveram HA em longo prazo, associada com hiperatividade simpática, barorreflexo prejudicado e maior sensibilidade de quimiorreceptores periféricos, além de resistência à insulina e níveis séricos elevados de MDA (GUIMARÃES *et al.*, 2017; DE ARAÚJO *et al.*, 2019; DE ARAÚJO *et al.*, 2020). Em adição, foi demonstrado que o consumo de uma dieta dislipidêmica durante os períodos de gestação e lactação influenciaram negativamente o metabolismo lipídico, parênquima intestinal e microbiota fecal das mães e da prole, apresentando um aumento nas

contagens de *Enterobacteriaceae* em detrimento a contagens reduzidas de *Lactobacillus* e *Bacteroides* (PINHEIRO *et al.*, 2019).

Tomados em conjunto, esses achados demonstram que o consumo de uma dieta com alta densidade calórica e rica em ácidos graxos trans (AGT), saturado e colesterol durante a gestação e lactação é capaz de alterar a composição da microbiota materno-fetal, com prejuízo na integridade da barreira intestinal, contribuindo diretamente para o desenvolvimento de hipertensão e desordens metabólicas na vida adulta da prole, sugerindo que intervenções com alvo na microbiota intestinal podem representar uma estratégia para reduzir os efeitos deletérios da dislipidemia materna na prole.

2.2 CONTROLE NEURAL DO SISTEMA CARDIOVASCULAR E HIPERTENSÃO ARTERIAL

De acordo com as diretrizes recentemente publicadas pelo *American College of Cardiology* (ACC) e *American Heart Association* (AHA), novos valores inferiores foram estabelecidos para a hipertensão arterial, sendo definida por níveis de pressão arterial sistólica (PAS) ≥ 130 mmHg ou níveis de pressão arterial diastólica (PAD) ≥ 80 mmHg (WHELTON *et al.*, 2018), em contraste com valores de PAS ≥ 140 mmHg ou uma PAD ≥ 90 mmHg, que foram utilizados para definir a hipertensão em todas as diretrizes anteriores (JAMES *et al.*, 2014).

Em 2019, o número de indivíduos hipertensos em todo mundo era superior a 1 bilhão, e esse número duplicou desde 1990. Em adultos com idades entre 30 e 79 anos, a prevalência mundial de hipertensão era de 32% em mulheres e 34% em homens, com um aumento relevante em países de baixa e média renda, e continua sendo o principal fator de risco para o desenvolvimento de DCV (NCD-RisC, 2021).

A etiologia da HAS é multifatorial, envolvendo um conjunto de influências genéticas e a integração fisiológica do sistema cardiovascular, renal, neural e endócrino (OPARIL *et al.*, 2018). Assim, alguns mecanismos têm sido descritos como relevantes no desenvolvimento de hipertensão arterial, como por exemplo, prejuízos no controle barorreflexo (KANBAR *et al.*, 2008), maior sensibilidade de quimiorreceptores periféricos (SIMMS *et al.*, 2009) e hiperatividade simpática (BRIANT *et al.*, 2015).

O barorreflexo é descrito como um mecanismo de controle em curto prazo da pressão arterial através da modulação da atividade simpática. Os barorreceptores localizados nos seios carotídeos e arco aórtico detectam alterações na pressão arterial, gerando ajustes autonômicos

reflexos para alterações tampão na pressão arterial. Na hipertensão arterial, há prejuízo do controle autonômico da pressão arterial e da frequência cardíaca, resultando na redução da sensibilidade barorreflexa (JULIEN, 2008).

A estimulação aguda dos quimiorreceptores periféricos (QRP) ativados principalmente por hipóxia, e os quimiorreceptores centrais induzidos por hipercapnia, geram respostas reflexas cardiorrespiratórias, caracterizadas por hiperventilação, hiperatividade simpática, taquicardia e aumento da pressão arterial (BARNETT *et al.*, 2017).

A hiperatividade simpática é caracterizada por um aumento na intensidade e na frequência das despolarizações elétricas do nervo simpático e também por um aumento nos níveis plasmáticos de catecolaminas, promovendo constrição dos vasos sanguíneos periféricos, aumento na resistência vascular periférica e consequentemente aumento nos níveis basais da PA (MALPAS, 2010).

O acoplamento cardiorrespiratório possui uma interação complexa com o sistema nervoso central autônomo (SNCA), que controla a pressão arterial e frequência cardíaca através de seus ramos simpático e parassimpático, exercendo uma influência recíproca entre a modulação da pressão arterial e o controle respiratório. A regulação desse sistema integrado é realizada pelos quimiorreceptores periféricos ou centrais (quimiossensores que detectam principalmente variações nos níveis de O_2) e barorreceptores (mecanorreceptores relacionados à detecção de variações de pressão arterial) (MACHADO; ZOCCAL; MORAES, 2017).

O corpúsculo carotídeo (CC), localizado perifericamente na bifurcação carotídea e arco carotídeo (região do pescoço), é o principal órgão quimiorreceptor constituído por terminações nervosas quimiorreceptoras especializadas, responsáveis pela detecção de variações na pressão parcial arterial nas concentrações de oxigênio (O_2), dióxido de carbono (CO_2) e íons H^+ e atuam no controle do reflexo cardiorrespiratório induzido por hipóxia (ITURRIAGA, 2018).

O CC é composto principalmente por aglomerados de células do tipo I, denominadas também como células glomus, organizadas em torno dos capilares e circundadas por células do tipo II, conhecidas como sustentaculares. As células glomus são inervadas por terminações nervosas sensoriais, sendo as principais unidades sensibilizadas pela queda de PO_2 , elevação de PCO_2 ou diminuição do pH, enviando sinapses do núcleo do trato solitário (NTS) via nervo glossofaríngeo e vago ao sistema nervoso central (SNC), na região do bulbo, originando respostas para os órgãos efetores, enquanto que as células sustentaculares exercem função de suporte da estrutura física do CC (ORTEGA-SÁENZ; LÓPEZ-BARNEO, 2020).

Assim, o CC desempenha um papel crucial na homeostase cardiovascular, respiratória e neuro-humoral, e evidências demonstram que o aumento da sensibilidade dos quimiorreceptores no CC pode favorecer o desenvolvimento de alterações autonômicas, provocando hiperatividade simpática, e consequentemente hipertensão arterial, insuficiência cardíaca, apnéia obstrutiva do sono, resistência à insulina e outras doenças metabólicas (PRABHAKAR, 2016).

A hiperatividade simpática desencadeada pelo aumento da descarga de quimiorreceptores, juntamente com uma redução na eficiência do barorreflexo, prejudica a regulação do tônus vasomotor dos vasos sanguíneos, resultando em disfunção endotelial e hipertensão. Embora os mecanismos subjacentes à potenciação dos quimiorreceptores do CC não estejam completamente elucidados, é provável que esta alteração seja devido ao aumento do estresse oxidativo e inflamação (GUYENET *et al.*, 2020).

2.3 MICROBIOTA INTESTINAL

Primeiramente, é necessária uma diferenciação dos termos específicos. A microbiota é geralmente definida como a comunidade dos micro-organismos vivos pertencentes a diferentes reinos, classificados em células procariontes (englobam os reinos *Archaea* e *Bacteria*) e eucariontes (englobam os reinos *Protista*, *Fungi* e *Alga*), que estão presentes em um determinado ambiente (BERG *et al.*, 2020).

O conceito de microbioma é muito mais abrangente do que o de microbiota. A definição do termo microbioma inclui a informação genética coletiva contida na microbiota (conjunto de micro-organismos), bem como o seu “teatro de atividades”, que contempla seus elementos estruturais microbianos (ácidos nucleicos, proteínas, lipídios, polissacarídeos), metabólitos produzidos (moléculas sinalizadoras, toxinas, moléculas orgânicas e inorgânicas) e condições ambientais circundantes (BERG *et al.*, 2020). Deste modo, elementos como bacteriófagos, vírus, plasmídeos, príons, viróides, RNA e DNA livre, devem ser incluídos no termo microbioma, porém, não fazem parte da microbiota (BERG *et al.*, 2020).

O trato gastrointestinal (TGI) humano é um complexo ecossistema, densamente colonizado por milhares de espécies bacterianas (LLOYD-PRICE *et al.* 2017). Em humanos saudáveis, a microbiota intestinal é constituída principalmente pelos filos bacterianos *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria* e *Tenericutes*, enquanto os filos *Fusobacteria*, *Saccharibacteria*, *Spirochaetes*, *Synergistetes* e *Verrucomicrobia* estão presentes em menor abundância (ALMEIDA *et al.*, 2019).

A composição da microbiota intestinal é variável entre os indivíduos e ao longo do desenvolvimento, depende do genótipo do hospedeiro e de fatores ambientais, além de ser influenciada por diversos fatores como, mudanças na dieta, idade, gênero e uso de medicamentos e antibióticos (DIXIT *et al.*, 2021). Além de sua colonização inicial ser marcada pela influência do tipo de parto e aleitamento materno nos primeiros dias de vida (REYMAN *et al.*, 2019).

Consequentemente, a diversidade e estabilidade da microbiota intestinal, bem como o conjunto de interações microbianas e a simbiose micro-organismos-hospedeiro, contribuem substancialmente na regulação de diversas funções fisiológicas no hospedeiro, como sinalização do sistema imunológico, resposta inflamatória, incluindo o metabolismo de nutrientes e a regulação da barreira intestinal, que conduzem para o estabelecimento de uma ecologia saudável (DAS; NAIR, 2019).

Estabilidade, resiliência e resistência são características ecológicas essenciais do microbioma intestinal saudável. A estrutura da comunidade microbiana de um indivíduo é estabelecida durante os primeiros meses de vida, e durante este período, o indivíduo está vulnerável a diversas perturbações externas. Em humanos saudáveis, o estado de equilíbrio geralmente é atingido na adolescência e prevalece estável ao longo da vida adulta. Embora a estabilidade esteja representada na ausência de perturbações, na realidade, também está submetida a pequenas variações constantemente (FASSARELLA *et al.*, 2020).

O conceito de estabilidade refere-se à característica de um determinado ecossistema para manter um estado de equilíbrio e resistir a perturbações, ou para mostrar resiliência após alguma perturbação (LOZUPONE *et al.*, 2012). Mesmo após uma perturbação externa, como infecção por patógenos, diarreia ou tratamento antibiótico, a microbiota intestinal tem a capacidade de restaurar o seu equilíbrio, devido à capacidade de autorregeneração, também conhecida como fenômeno da resiliência, enquanto a resistência pode ser entendida como a capacidade de um ecossistema de permanecer inalterado diante de uma perturbação, pois descreve a facilidade com que um sistema pode sair de seu estado estável (SOMMER *et al.*, 2017).

Tomados em conjunto, a resiliência da microbiota é capaz de determinar se uma perturbação em específico poderá conduzir o ecossistema microbiano estável para um estado instável e transitório, sendo possível restaurar o seu estado fisiológico normal posteriormente, ou promover mudanças duradouras na estrutura do ecossistema microbiano, aumentando a predisposição para o desenvolvimento de doenças crônicas (SOMMER *et al.*, 2017).

A disbiose representa a perda da homeostase microbiana nas características de composição (ou seja, diversidade microbiana e abundância relativa) e capacidade funcional alterada (ou seja, aptidão microbiana, capacidade metabólica e influência no hospedeiro) que excede suas competências de resiliência e resistência (DAS; NAIR, 2019). A diversidade microbiana é categorizada em diversidade α e β , em que a diversidade α explica a riqueza da comunidade microbiana (número de táxons) e a uniformidade significa a abundância relativa de espécies microbianas em uma amostra. Por outro lado, a diversidade β explica a variabilidade na composição da comunidade entre as amostras dentro de um habitat. Enquanto a diversidade microbiana corresponde à presença ou ausência e distribuição de bactérias, a funcionalidade refere-se às atividades das bactérias, como estabelecimento de nichos e biofilmes, resposta a estímulos ambientais, produção de metabólitos e impacto na saúde do hospedeiro (WAGNER *et al.*, 2018).

No geral, a disbiose intestinal é caracterizada pela diminuição da diversidade e estabilidade da população microbiana, diminuição da riqueza de micro-organismos e desenvolvimento e expansão de patobiontes (PETERSEN; ROUND, 2014). Na hipertensão, a disbiose é caracterizada pela diminuição na diversidade e riqueza microbiana, apresentando mudanças na abundância relativa dos principais filos, como aumento de *Firmicutes* e diminuição de *Bacteroidetes*, além da diminuição nas bactérias produtoras de acetato e butirato e um aumento nas populações bacterianas produtoras de lactato (YANG *et al.*, 2015).

Em adição, estudos de análises metagenômicas identificaram que pacientes com hipertensão apresentaram várias alterações taxonômicas na composição da microbiota intestinal, com aumento dos gêneros *Prevotella*, *Klebsiella*, *Parabacteroides*, *Actinomyces*, *Porphyromonas*, *Streptococcus*, *Clostridium* e *Salmonella* que foram associados à disbiose intestinal, e ao mesmo tempo, apresentaram diminuição dos gêneros *Bacteroidetes*, *Bifidobacterium*, *Synergistetes*, *Faecalibacterium* e *Roseburia*, sendo esses dois últimos conhecidos como os principais produtores de butirato no intestino, o qual está diretamente associado a uma menor PAS (LI *et al.*, 2017; YAN *et al.*, 2017; KIM *et al.*, 2018).

A microbiota intestinal pode potencialmente influenciar a PA através de múltiplos mecanismos (ROBLES-VERA *et al.*, 2017). Evidências indicam que a modulação do sistema imunológico pela microbiota intestinal pode ocorrer através de moléculas indesejáveis que entram na circulação. Por exemplo, uma alteração na homeostase intestino-microbiota, incluindo uma baixa sobrevivência de bactérias ácido-láticas (BAL) e disbiose intestinal, tem sido associada à passagem facilitada de lipopolissacarídeos (LPS) e EROs da barreira intestinal para a circulação sistêmica, estimulando a produção de citocinas pró-inflamatórias e aumento

do estresse oxidativo, causando danos em órgãos-chaves do controle da PA e favorecendo o desenvolvimento da hipertensão (CAVALCANTI NETO *et al.*, 2018).

Além disso, a microbiota intestinal e os seus metabólitos produzidos, como os ácidos graxos de cadeia curta (AGCC) desempenham um papel importante na modulação do sistema imune, como inibição da proliferação microbiana patogênica através da acidificação do pH, modulação da ativação de células dendríticas, macrófagos, células T e influenciando principalmente na indução de células Treg pela inibição das histonas desacetilases (HDACs) (HAASE *et al.*, 2018).

Um estudo de coorte envolvendo 4.672 participantes demonstrou que os AGCC têm um papel importante na regulação da PA, demonstrando que a ocorrência de hipertensão é normalmente acompanhada pela diminuição da produção desses metabólitos (VERHAAR *et al.*, 2020). O acetato, propionato e butirato são AGCC derivados da fermentação bacteriana de fibras alimentares indigeríveis no cólon, que podem modular a vasodilatação e induzir a hipotensão. O butirato, particularmente, exerce um papel importante na manutenção da integridade da barreira intestinal através da redução da permeabilidade e reparação das *tight junctions*, e de fato, a suplementação com butirato em um modelo de hipertensão induzido por angiotensina II foi capaz de melhorar a disbiose e disfunção da barreira intestinal, além de apresentar uma diminuição da PAS (KIM *et al.*, 2018). A suplementação com propionato em camundongos demonstrou efeitos anti-inflamatórios, através da diferenciação das células Treg e redução das células Th17, atenuando a hipertrofia cardíaca, inflamação vascular e hipertensão (BARTOLOMAEUS *et al.*, 2019). Curiosamente, foi constatado que níveis circulantes mais baixos de acetato, o AGCC mais abundante no plasma, foram associados com níveis mais elevados de PA em indivíduos hipertensos (CALDERÓN-PÉREZ *et al.*, 2020).

Os AGCC produzidos pela microbiota intestinal podem modular diretamente a PA através da ativação dos seus receptores específicos, demonstrando efeitos antagônicos entre os receptores acoplados a proteína G GPR41, GPR43, GPR109a e OLF78. Tem sido sugerido que a ativação dos receptores GPR41, GPR43 e GPR109a induz a vasodilatação e consequentemente reduz a PA, enquanto a ativação do receptor OLF78, expresso em vasos sanguíneos e rins e estimulado por acetato e propionato, é capaz de aumentar a PA (PLUZNICK *et al.*, 2013; WU *et al.*, 2021).

Uma hipótese demonstrou que o aumento da atividade simpática intestinal está associado com o aumento da permeabilidade da barreira intestinal, devido à redução das proteínas constituintes das *tight junctions*, encurtamento das vilosidades intestinais,

diminuição das células caliciformes que produzem mucinas e disbiose intestinal, e essas alterações fisiopatológicas intestinais sugerem que a comunicação disfuncional intestino-cérebro pode desempenhar um papel crítico para o desenvolvimento e estabelecimento de HA (SANTISTEBAN *et al.*, 2017).

2.4 O EIXO MICROBIOTA-INTESTINO-CÉREBRO E HIPERTENSÃO ARTERIAL

A importância da microbiota intestinal tem sido destacada pelo eixo microbiota-intestino-cérebro, que se refere a uma rede complexa de conexões que permite a comunicação bidirecional entre as bactérias intestinais e o sistema nervoso central (SNC), envolvendo diferentes vias, incluindo o nervo vago e sistema nervoso entérico, sistema imunológico, hormônios, neurotransmissores e metabólitos microbianos, que desempenham um papel crítico na regulação de diversos sistemas fisiológicos, tornando o intestino um órgão central para a saúde humana (CRYAN *et al.*, 2019).

A microbiota intestinal e a comunicação intestino-cérebro são cruciais para o início, desenvolvimento e estabelecimento da hipertensão (YANG *et al.*, 2018). Evidências têm demonstrado que a desregulação das vias de sinalização do eixo microbiota-intestino-cérebro pode contribuir para o aumento da PA, disfunção autonômica, disfunção da barreira epitelial intestinal e produção alterada de AGCC, com um aumento na produção de lactato e diminuição na produção de acetato e butirato em modelos de hipertensão arterial (ADNAN *et al.*, 2017; KIM *et al.*, 2018; TORAL *et al.*, 2019).

A microbiota intestinal tem a capacidade de síntese e liberação de neurotransmissores, como noradrenalina, dopamina e serotonina, que conduzem a sinalização intestino-cérebro, atuando nas vias aferentes do nervo vago sinalizando diretamente para o SNC, influenciando no controle da PA (FÜLLING; DINAN; CRYAN, 2019). Por exemplo, bactérias pertencentes às espécies *Lactobacillus*, *Bacteroides*, *Bifidobacterium*, *Parabacteroides* e *Escherichia* podem produzir ácido γ -aminobutírico (GABA), *Escherichia*, *Bacillus* e *Saccharomyces* podem sintetizar noradrenalina, *Streptococcus*, *Escherichia* e *Enterococcus* podem sintetizar serotonina, *Bacillus* podem produzir dopamina e *Lactobacillus* podem sintetizar acetilcolina (STRANDWITZ, 2018).

Por meio do eixo microbiota-intestino-cérebro, os AGCC (acetato, propionato e butirato) gerados no intestino podem influenciar a PA, direta ou indiretamente, pela ativação de receptores acoplados à proteína G (GPRs) (DALILE *et al.*, 2019). Interessantemente, esses AGCC também estão presentes na circulação e são detectados no cérebro. No entanto, a

ausência ou falha nos mecanismos de detecção ou sinalização intracelular dos AGCC e de ligação aos seus receptores GPR41, GPR43 e OLFR78 que são amplamente expressos em diversos órgãos e tecidos, incluindo gânglios simpáticos, células endoteliais, células epiteliais, aparelho justaglomerular e células musculares lisas, podem contribuir para o desenvolvimento de hipertensão (ONYSZKIEWICZ *et al.*, 2019).

Os AGCC podem modular indiretamente a PA via eixo intestino-cérebro através da ligação com os seus receptores GPR43 e GPR41 nos colonócitos, promovendo a sinalização para o cérebro por meio da circulação sistêmica ou das vias vagais, induzindo a secreção de hormônios intestinais, como o peptídeo semelhante ao glucagon 1 (GLP1) e o peptídeo YY (PYY), além de promover a secreção de leptina pelos adipócitos, ativando uma cascata de sinalizações cerebrais envolvidas no aumento da saciedade, melhorando a sensibilidade à insulina e reduzindo a ingestão alimentar (CHRISTIANSEN *et al.*, 2018).

Na hipertensão, a integridade da barreira intestinal é prejudicada devido à diminuição das bactérias produtoras de AGCC, favorecendo a degradação das mucinas e a passagem facilitada de patobiontes e LPS, iniciando uma cascata inflamatória, ativando células Th17 como resultado da disbiose (MIRANDA *et al.*, 2018). Os AGCC exercem um efeito modulador da PA através da regulação da via imunológica no intestino, interagindo com as células epiteliais intestinais e imunes, induzindo a diferenciação das células Treg, com supressão da produção de citocinas pró-inflamatórias e diminuição da PA. Em adição, os AGCC são metabólitos microbianos permeáveis e podem entrar na circulação sistêmica e atravessar a barreira hematoencefálica, desempenhando um papel importante na manutenção da integridade da barreira intestinal e prevenindo a translocação bacteriana, regulando positivamente a expressão das *tight junctions* (JAMA *et al.*, 2019).

A comunicação bidirecional do eixo microbiota-intestino-cérebro exerce mecanismos de controle da PA, modulando a interação entre o sistema nervoso simpático (SNS) e o sistema imunológico (ROBLES-VERA; TORAL; DUARTE, 2020). O aumento da atividade simpática no intestino contribui para a disfunção epitelial, caracterizada pelo aumento da inflamação da mucosa e permeabilidade intestinal, levando a disbiose. Em adição, a redução na disponibilidade de AGCC na circulação e aumento na secreção de serotonina, noreadrenalina e dopamina modulam a resposta aferente via nervo vago para o NTS, contribuindo para o desenvolvimento de hipertensão (ZUBCEVIC *et al.*, 2019). Tomados em conjunto, é possível sugerir que alterações nesse eixo estão implicadas na patogênese da hipertensão, e, portanto, a manipulação da microbiota intestinal através da intervenção com

probióticos representa um potencial alvo terapêutico para prevenção do desenvolvimento de hipertensão arterial (LIU; LIONG; TSAI, 2018).

2.5 PROBIÓTICOS

Probióticos são definidos como “micro-organismos vivos que quando ingeridos em quantidades adequadas conferem benefícios à saúde do hospedeiro” (HILL *et al.*, 2014). *Lactobacillus* são descritos como um gênero de bactérias Gram-positivas, em forma de bastonete ou cocos, heterofermentativo e anaeróbico ou aerotolerante, catalase negativa, que produzem ácido lático como principal produto final da fermentação de carboidratos, e, portanto, pertencem ao grupo das bactérias lácticas (BAL) (BINTSIS, 2018).

Entre as BAL, o gênero *Lactobacillus* é o maior e o mais utilizado em humanos por incluir um grande número de espécies reconhecidas como GRAS (geralmente reconhecidas como seguras), devido ao seu longo histórico de uso seguro como culturas iniciadoras em produtos lácteos fermentados e sua presença em diferentes localizações do corpo humano, incluindo TGI, sistema urinário e genital (SALVETTI *et al.*, 2018).

O gênero *Lactobacillus* é classificado no filo *Firmicutes*, classe *Bacilli*, ordem *Lactobacillales*, família *Lactobacillaceae*, e atualmente, compreende 261 espécies (até março de 2020), e foi reclassificado em 25 gêneros diferentes (ZHENG *et al.*, 2020). Assim, a partir da sequência de DNA de cada uma das espécies de *Lactobacillus*, na nova reclassificação taxonômica avaliada, *Lactobacillus fermentum* foi renomeado como *Limosilactobacillus fermentum*, sendo amplamente encontrado em cereais fermentados e outros materiais vegetais fermentados, laticínios, esterco, esgoto, fezes e vagina de humanos (ZHENG *et al.*, 2020).

Entre as espécies de *Lactobacillus*, tem sido reportado que cepas de *L. fermentum* exercem propriedades probióticas, devido a algumas funções demonstradas em estudos pré-clínicos (*in vitro* e *in vivo*) e clínicos (NAGHMOUCHI *et al.*, 2020), como por exemplo, habilidade de reduzir o colesterol sanguíneo (YADAV *et al.*, 2019), capacidade de modular o sistema imunológico intestinal, devido a propriedade de interagir com células epiteliais intestinais e macrófagos, estimulando a liberação de imunoglobulina A (IgA) (GARCIA-CASTILLO *et al.*, 2019), redução da inflamação intestinal (JANG *et al.*, 2019), além de propriedades antioxidantes demonstradas pela atividade aumentada de enzimas antioxidantes como SOD, catalase e GSH peroxidase em nível tecidual e sanguíneo (DE LUNA FREIRE *et al.*, 2021; WU *et al.*, 2021).

Um dos mecanismos pelos quais os probióticos promovem benefícios para a saúde humana é inibindo o crescimento de bactérias patogênicas, devido a propriedades específicas como: capacidade de aderir às células do epitélio intestinal e colonizá-lo, exercendo o mecanismo de exclusão competitiva com patógenos pela adesão ao epitélio e por nutrientes, além de inibir a produção de patógenos entéricos através da produção de substâncias antimicrobianas como ácido lático, peróxido de hidrogênio, bacteriocinas, ácidos graxos de cadeia curta (acetato, propionato e butirato), e produção de mucinas (AZAD *et al.*, 2018; PLAZA-DIAZ *et al.*, 2019).

Os efeitos imunomoduladores dos probióticos são atribuídos ao mecanismo de ativação das células T reguladoras (macrófagos e células dendríticas), estimulando a síntese e liberação de citocinas anti-inflamatórias, especialmente interleucina 10 (IL-10), e reduzindo a atividade inflamatória através da inibição da sinalização da via do fator de transcrição nuclear kappa B (NF-kB) nos enterócitos, além de estimular a secreção de IgA (MALDONADO GALDEANO *et al.*, 2019; WANG; ZHANG; ZHANG, 2021). Outros mecanismos de ação dos probióticos à nível intestinal incluem melhora da integridade da barreira intestinal, estimulando a proliferação das células epiteliais intestinais, inibindo sua apoptose, por meio dos receptores *Toll-like* (TLR), secreção de muco pelas células epiteliais e aumento da estabilidade das *tight junctions* (WAN *et al.*, 2016; LEMME-DUMIT *et al.*, 2018).

Em relação às propriedades hipocolesterolêmicas dos probióticos, alguns mecanismos envolvidos na redução dos níveis séricos de colesterol foram propostos, incluindo a capacidade de assimilação do colesterol, adesão epitelial do cólon e inibição da captação de colesterol pelas células epiteliais do cólon (Caco-2), reduzindo assim, a disponibilidade de colesterol no lúmen intestinal, bem como por meio da atividade da enzima hidrolase de sais biliares (BSH) (PALANIYANDI *et al.*, 2020). A desconjugação dos sais biliares é catalisada pela enzima BSH, e os sais biliares desconjugados apresentam baixa solubilidade e são absorvidos em menor eficiência no lúmen intestinal, resultando no aumento da excreção de ácidos biliares nas fezes e diminuição dos níveis de colesterol sérico (KHARE; GAUR, 2020).

A funcionalidade dos probióticos é determinada pelas características específicas de cada cepa, não devendo ser extrapolada, inclusive para outras cepas de uma mesma espécie ou gênero (BUBNOV *et al.*, 2018). A dose ideal do probiótico também é um ponto importante a ser considerado, pois o consumo de uma dose elevada não confere necessariamente um benefício maior para a saúde do que uma dose mais baixa. Além disso, outros fatores devem ser considerados na determinação da dose diária como, frequência diária, duração da

intervenção, forma de administração (alimento fermentado, bebida, cápsula ou sachê) e a viabilidade da cepa probiótica (FORSSTEN *et al.*, 2020).

No geral, os produtos contendo micro-organismos probióticos devem conter uma concentração mínima de células viáveis, com eficácia comprovada com base em ensaios clínicos humanos, estimadas entre 10^6 e 10^8 UFC/g do produto final ou entre 10^8 e 10^{10} UFC/dia (considerando 100g ou 100mL do produto final). A legislação brasileira recomenda uma concentração mínima viável de probióticos entre 10^8 e 10^9 UFC/dia no final da vida de prateleira do produto. Canadá e Itália exigem uma concentração mínima de bactérias probióticas viáveis de 10^9 UFC/dia para conferir um efeito terapêutico à microbiota intestinal (MARTINEZ; BEDANI; SAAD, 2015). No entanto, a maioria dos estudos clínicos com probióticos utiliza doses entre 10^8 e 10^{11} UFC, sendo estabelecida como uma faixa de dosagem eficaz, segura e econômica (OUWEHAND, 2017).

Outro ponto relevante é que a eficácia das formulações probióticas pode ser influenciada por vários mecanismos de ação desempenhados por cepas isoladas ou múltiplas cepas combinadas (McFARLAND, 2021). Evidências têm demonstrado que a utilização de formulações probióticas com múltiplas cepas pode ser mais efetiva do que probióticos de única cepa, devido ao efeito sinérgico das cepas constituintes, resultando em alta adesão às mucosas e aumento da inibição de patógenos no TGI, sendo mais eficaz no tratamento da disbiose. Por outro lado, formulações com múltiplas cepas também podem ter a eficácia reduzida devido à inibição mútua por diferentes cepas probióticas, possivelmente pela produção de agentes antagonistas, pela competição por nutrientes ou por sítios de ligação no TGI (KWOJI *et al.*, 2021).

Embora o uso de probióticos tenha sido inicialmente associado à melhora da saúde gastrointestinal, evidências de estudos experimentais e clínicos têm demonstrado uma relação bem estabelecida entre os efeitos dos probióticos na pressão arterial através da modulação positiva da microbiota intestinal, revelando que a suplementação em uma dosagem ideal e a escolha de uma cepa apropriada pode desempenhar um papel importante na redução da pressão arterial na condição de hipertensão, no entanto, os mecanismos que relacionam a ingestão de probióticos e a diminuição dos níveis de pressão arterial ainda não foram totalmente elucidados (KHALESİ *et al.*, 2014; ROBLES-VERA *et al.*, 2017).

Mecanicamente, estudos em modelos animais têm demonstrado a capacidade dos probióticos de reduzir a PA. Alguns desses mecanismos incluem a liberação de peptídeos bioativos por bactérias lácticas durante os processos de fermentação do leite, tais como os peptídeos inibidores da enzima conversora da angiotensina (ECA), ocasionando a diminuição

na síntese da angiotensina II e atenuação da vasoconstrição, consequentemente reduzindo os níveis pressóricos (SIPOLA *et al.*, 2002). Adicionalmente, os probióticos podem reduzir a endotoxemia metabólica, através da restauração da microbiota intestinal e diminuição da permeabilidade intestinal, reduzindo os níveis plasmáticos de lipopolissacarídeos (LPS) e estresse oxidativo vascular, contribuindo para a melhora da disfunção endotelial (TORAL *et al.*, 2014).

Outras hipóteses incluem diminuição dos níveis de endotelina e angiotensina II e aumento dos níveis de óxido nítrico, reduzindo a PAS (YANG *et al.*, 2015), além de favorecer o aumento da produção de ácidos graxos de cadeia curta (AGCC), diminuição da atividade de NADPH oxidase, prevenindo a disbiose intestinal e o desenvolvimento de disfunção endotelial e hipertensão, que demonstraram estar relacionados à capacidade dos probióticos de reduzir a endotoxemia e ao acúmulo de células Treg na vasculatura (ROBLES-VERA *et al.*, 2020).

2.5.1 Efeitos da intervenção com probióticos na hipertensão arterial: achados experimentais e clínicos

Em ratos espontaneamente hipertensos (SHR), a administração do probiótico *L. fermentum* CECT5716 (LC40), a uma dosagem de 3.3×10^{10} UFC/dia durante 5 semanas, demonstrou uma redução na PAS e melhora na disfunção endotelial, inflamação vascular, estresse oxidativo vascular, hipertrofia cardíaca e renal. Além disso, esses efeitos protetores foram acompanhados por alterações na microbiota cecal, com contagens elevadas de *Lactobacillus spp. cluster* em detrimento de *Bacteriodes spp.* e *Clostridium spp.*, restaurando a disbiose intestinal (GOMEZ-GUSMAN *et al.*, 2015).

A suplementação com *L. fermentum* CECT5716 (LC40) a uma dosagem de 5×10^8 UFC/dia durante 7 dias consecutivos em camundongos preveniu o desenvolvimento da hipertensão e disfunção endotelial induzida por tacrolimo, favorecendo o aumento da diversidade microbiana, redução da razão *Firmicutes/ Bacteroidetes* e aumento de bactérias produtoras de butirato. Esses efeitos benéficos foram associados a uma melhora no acoplamento eNOS e diminuição na produção de NOX2, além de restaurar o equilíbrio entre as células Th17 e as células T reguladoras, prevenindo a inflamação (TORAL *et al.*, 2018).

Em adição, a intervenção com a cepa probiótica *L. fermentum* MTCC: 5898 em leite fermentado durante 90 dias em ratos com hipercolesterolemia induzida por dieta rica em colesterol, apresentou uma redução dos níveis séricos de CT, LDL, VLDL, TG, atenuando o

estresse oxidativo pelo aumento da atividade das enzimas antioxidantes SOD, CAT e GPx e inibindo a peroxidação lipídica, além de diminuir as citocinas inflamatórias IL-6 e TNF- α (YADAV *et al.*, 2019).

Em um ensaio clínico, randomizado, placebo-controlado, duplo-cego com 40 pacientes com DMT2, a suplementação diária com 200mL de leite de soja probiótico contendo *L. planetarium* A7 (2×10^7 UFC) durante 8 semanas, reduziu significativamente os níveis de PAS e PAD, no entanto, não apresentou alteração nos parâmetros de IMC e relação cintura-quadril (RCQ) (HARIRI *et al.*, 2015). Por outro lado, em outro ensaio clínico, randomizado, placebo-controlado, duplo-cego realizado com 156 homens e mulheres adultos com sobrepeso, a suplementação com *L. acidophilus* La5 e *B. animalis subsp lactis* Bb12 a uma dosagem de 3×10^9 UFC/dia durante 6 semanas, não demonstrou efeitos significativos na PA, FC, CT, LDL, HDL e TG (IVEY *et al.*, 2015).

Em adição, em um ensaio clínico randomizado, triplo-cego, controlado por placebo realizado com 40 mulheres adultas diagnosticadas com HA, foi demonstrado que a suplementação diária com um sachê contendo *L. paracasei* LPC-37, *L. rhamnosus* HN001, *L. acidophilus* NCFM e *B. lactis* HN019 (10^9 UFC de cada cepa) durante 8 semanas reduziu significativamente os níveis de glicose em jejum e os níveis de CT, aumentou os níveis de HDL, além de reduzir LF e a razão LF/HF, sugerindo uma melhora na modulação autonômica (ROMÃO DA SILVA *et al.*, 2020). No entanto, uma meta-análise realizada com 9 ensaios clínicos randomizados controlados, demonstrou que a suplementação com múltiplas cepas probióticas durante um período de intervenção ≥ 8 semanas e dosagens diárias superiores a 10^{11} UFC pode ser efetiva na redução da PA (KHALESI *et al.*, 2014).

Nessa perspectiva, cepas de *Lactobacillus fermentum* foram isoladas e caracterizadas para testar o potencial probiótico *in vitro*. As cepas de *L. fermentum* 139, *L. fermentum* 263 e *L. fermentum* 296 foram recuperadas de subprodutos do processamento de frutas brasileiras, respectivamente, da manga (*Mangifera indica*), do abacaxi (*Ananas comosus*) e do morango (*Fragaria vesca*) (GARCIA *et al.*, 2016). Todas essas três cepas apresentaram bom desempenho para funcionalidades fisiológicas de adesão, agregação, coagregação, antagonismo e sobrevivência à exposição a condições gastrointestinais simuladas, além de não apresentar atividades hemolíticas e mucinolíticas e resistência a antibióticos (DE ALBUQUERQUE *et al.*, 2018).

Investigações iniciais do nosso laboratório demonstraram que a administração isolada de *L. fermentum* 296 (1×10^9 UFC/mL/dia) durante 4 semanas atenuou os níveis séricos de CT e TG e reduziu a hiperatividade simpática e PAS em ratos machos alimentados com uma

HFD, mas não aumentou os níveis de HDL e não melhorou a tolerância à glicose e resistência à insulina (CAVALCANTE *et al.*, 2019). Nessa perspectiva, a avaliação da relevância cardiometabólica de uma formulação probiótica na função autonômica, pressão arterial e parâmetros glicolipídicos na prole de ratos expostos à dislipidemia materna pode representar uma estratégia promissora e inovadora para proteger os filhos contra hipertensão programada e distúrbio metabólico.

A nossa hipótese é que a intervenção diária com uma formulação probiótica de *L. fermentum* (139, 263 e 296) do pós-desmame à vida adulta, diminui a produção de espécies reativas de oxigênio, através da interrupção da sinalização de citocinas pró-inflamatórias, atenuando a peroxidação lipídica e reduzindo os níveis pressóricos, promovendo a melhora dos parâmetros cardiovasculares na prole de ratos expostos à dislipidemia materna.

3 ABORDAGEM METODOLÓGICA

3.1 CARACTERIZAÇÃO DO ESTUDO

Trata-se de um estudo experimental, realizado na prole de ratos *Wistar* machos e fêmeas. O mesmo foi realizado no Laboratório de Controle Neural da Circulação e Hipertensão Arterial (LACONCHA) da Universidade Federal da Paraíba (UFPB).

3.2 ANIMAIS E ASPECTOS ÉTICOS

Foram utilizados ratos *Wistar* (*Rattus norvegicus*) provenientes do biotério do Instituto de Pesquisas em Fármacos e Medicamentos (IPeFarM) da UFPB. 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.

Todos os procedimentos experimentais foram submetidos e aprovados pela Comissão de Ética no Uso de Animais da Universidade Federal da Paraíba (CEUA/UFPB) sob o protocolo nº 9492260418 e seguiram as orientações do Conselho Nacional para o Controle de Experimentação Animal (CONCEA) e os Princípios Internacionais para Pesquisas Biomédicas Envolvendo Animais (Anexo A).

3.3 CEPAS PROBIÓTICAS

As cepas de *L. fermentum* 139, *L. fermentum* 263 e *L. fermentum* 296 foram gentilmente cedidas pelo Laboratório de Microbiologia de Alimentos do Departamento de Nutrição da Universidade Federal da Paraíba (João Pessoa, Brasil).

Essas cepas foram previamente identificadas usando a análise de sequenciamento de genes 16S rRNA (GARCIA *et al.*, 2016). Os estoques foram armazenados a -20°C em caldo Mann, Rogosa e Sharpe (MRS) (HiMedia, Mumbai, Índia) contendo glicerol (Sigma-Aldrich, St Louis, EUA; 20 mL por 100 mL).

3.4 PREPARAÇÃO DA SUSPENSÃO DE CÉLULAS PROBIÓTICAS

Cada inóculo foi obtido preparando suspensões em solução tampão fosfato-salino (PBS) estéril de culturas cultivadas em caldo MRS (HiMedia, Mumbai, Índia) e incubadas anaerobicamente (Anaerobic System Anaerogen, Oxoid Ltd, Wade Road, Reino Unido) a 37°C. As células foram colhidas por centrifugação (8.000 g, 10 min, 4°C), lavadas duas vezes em solução PBS estéril, ressuspensas em solução PBS e homogeneizadas usando um vórtex (30 segundos) para obter suspensões celulares padrão com densidade óptica (OD) leitura a 660 nm (OD₆₆₀) de 1.0 de absorbância, o que forneceu contagens viáveis de aproximadamente 9 log UFC / mL. O inóculo misto foi obtido misturando a suspensão preparada de cada cepa probiótica na proporção de 1: 1: 1.

3.5 GRUPOS EXPERIMENTAIS E INTERVENÇÃO COM FORMULAÇÃO DE *L. fermentum*

Ratas *Wistar* primíparas pesando 250±10g (n=14) foram acasaladas com machos na proporção de 1:1. A presença de espermatozóides no esfregaço vaginal foi utilizada como marcador do 1º dia de gestação. Posteriormente, ratas prenhes foram transferidas para gaiolas individuais e submetidas a manipulações dietéticas durante os períodos de gestação e lactação: grupo controle (CTL, n=7) e grupo dieta dislipidêmica (DLP, n=7) e água *ad libitum*.

A dieta controle (CTL) foi elaborada no Laboratório de Nutrição Experimental (LANEX), Departamento de Nutrição da Universidade Federal da Paraíba de acordo com o Instituto Americano de Nutrição (AIN-93G) (REEVES; NIELSEN; FAHEY, 1993). A dieta dislipidêmica (DLP) foi adquirida pela empresa Rhostrer® (Araçoiaba da Serra, São Paulo, Brasil). As dietas foram oferecidas durante os períodos de gestação e lactação. O óleo de soja foi a fonte de gordura da dieta de controle (AIN-93G; 15,92% de gordura), enquanto a dieta dislipidêmica com altas concentrações de lipídios e colesterol continha 31,11% de gordura distribuída em óleo de soja, colesterol, banha e gordura vegetal hidrogenada como fonte de lipídios (Tabela 1).

Tabela 1. Composição nutricional dietética (g/100g).

Nutrientes	Dieta Controle	Dieta Dislipidêmica
Caseína (85%)*	20	19,86
Amido dextrinizado	13	15,5
Celulose	5	5
Sacarose	10	6
Amido de milho	39,7	33,6
Óleo de soja	7	3
Banha de porco	0	6
Gordura vegetal hidrogenada	0	5
Ácido cólico (Sigma)	0	0,5
Colesterol (Sigma)	0	0,5
Colina	0,25	0,25
Metionina	0,3	0,3
Formulação de vitaminas	1	1
Formulação de minerais	3,5	3,5
T-BHQ	0,014	0,014

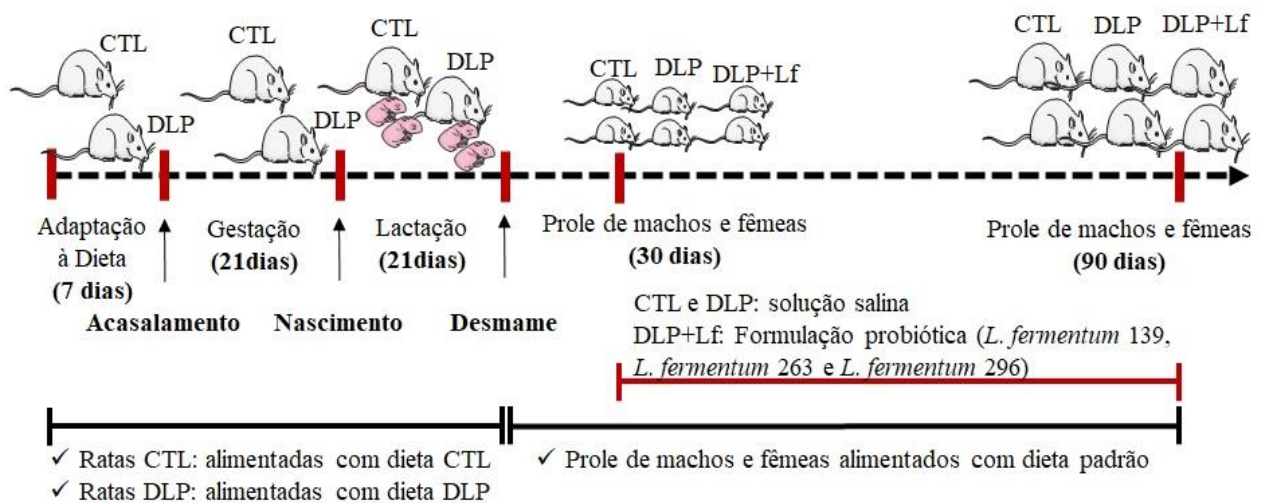
*A caseína possui 85% de pureza (sendo 85 g de caseína realmente, em cada 100 g).

Às 24h após o nascimento, a prole de ratos foi ajustada para 8 filhotes (4 machos e 4 fêmeas) por ninhada para garantir a nutrição padronizada até o desmame. Após o período de lactação (21º dia pós-natal), as proles de fêmeas (CTL, n=7 e DLP, n=14) e machos (CTL, n=7 CTL e DLP, n=14) foram alocadas separadamente (4 por gaiola) e receberam dieta comercial (Presence Purina®, Paulínea, Brasil) e água *ad libitum* até os 90 dias de vida, enquanto as mães foram sacrificadas e amostras de soro foram coletadas para quantificação de colesterol total (CT), LDL-colesterol, HDL-colesterol e triglicerídeos (TG) utilizando kits comerciais (Bioclin, Belo Horizonte, Minas Gerais, Brasil).

Os grupos experimentais CTL e DLP foram distribuídos aleatoriamente em três grupos: grupo controle (CTL, n=14; 7 machos e 7 fêmeas), grupo dislipidêmico (DLP, n=14; 7 machos e 7 fêmeas) e grupo dislipidêmico + formulação probiótica de *L. fermentum* (DLP + Lf, n=14; 7 machos e 7 fêmeas). Nos grupos CTL e DLP, solução salina foi administrada como placebo durante 8 semanas. No grupo DLP + Lf, a formulação probiótica contendo *L. fermentum* 139, *L. fermentum* 263 e *L. fermentum* 296 em uma solução de aproximadamente 1×10^9 UFC/ mL foi administrada diariamente por 8 semanas. A administração do placebo ou formulação probiótica contendo *L. fermentum* foi realizada por gavagem na dosagem de 1mL para cada rato após o desmame (30 dias de idade).

Após 8 semanas de tratamento com solução salina ou formulação probiótica contendo *L. fermentum*, foram realizadas as medições bioquímicas, testes de tolerância à glicose e insulina, contagens microbianas nas fezes, registro da pressão arterial (PA) e frequência cardíaca (FC) basal, análise espectral da pressão arterial sistólica e intervalo cardíaco e avaliação do balanço simpático-vagal e tônus vascular simpático em cada grupo aos 90 dias de idade (Figura 1).

Figura 1. Protocolo experimental do estudo.



3.6 AVALIAÇÃO DO PESO CORPORAL E DO CONSUMO ALIMENTAR

O peso corporal e o consumo alimentar dos ratos machos e fêmeas foram mensurados semanalmente até o final dos experimentos. O peso corporal e o consumo alimentar foram acompanhados utilizando uma balança eletrônica (modelo AS-1000; Marte, Santa Rita MG, Brasil).

3.7 TESTE ORAL DE TOLERÂNCIA À GLICOSE E TESTE DE TOLERÂNCIA À INSULINA

Os testes de tolerância oral à glicose (TOTG) e de tolerância à insulina (TTI) foram realizados em ratos em jejum noturno de 12 horas. No TOTG, uma carga oral de glicose (2g/kg) foi administrada via gavagem. Amostras de sangue foram retiradas da veia da cauda antes da administração de glicose e, posteriormente, aos 15, 30, 60, 90 e 120 minutos. Após 24 horas do TOTG, o TTI foi realizado após uma injeção intraperitoneal de insulina (0,75 UI

por kg de peso corporal) e as concentrações de glicose sanguínea foram medidas antes (0 minuto) e após (30, 60, 90 e 120 minutos) da injeção de insulina peritoneal (GUIMARÃES *et al.*, 2017). Todas as medições da concentração de glicose capilar foram realizadas com um glicosímetro Accu-Check (Bayer®, São Paulo, Brasil).

3.8 ANÁLISES BIOQUÍMICAS

As medidas séricas foram realizadas 24 horas após os experimentos de TOTG e TTI. Os ratos machos e fêmeas passaram por um jejum de 12 horas durante o período noturno e amostras de sangue (aproximadamente 2 mL) foram coletadas por rompimento do plexo venoso orbital (GUIMARÃES *et al.*, 2017). As amostras foram centrifugadas a 300 g, 25°C durante 15 minutos e medidas séricas para concentrações de colesterol total (TC), colesterol lipoproteína de alta densidade (HDL-c), colesterol lipoproteína de muito baixa densidade (VLDL-c) e triglicerídeos (TG) foram realizadas utilizando kits colorimétricos enzimáticos específicos, conforme instruções do fabricante (Bioclin, Belo Horizonte, Minas Gerais, Brasil).

A concentração de malondialdeído (MDA), um produto final da peroxidação lipídica, foi medida como um indicador de estresse oxidativo. Neste ensaio, o MDA reage com o ácido tiobarbitúrico para produzir um complexo de cor vermelha. Resumidamente, 400 µL de ácido perclórico (7%) foram adicionados a 250 µL de soro plasmático, misturados e centrifugados a 600 g, 4°C, durante 20 minutos. O sobrenadante foi coletado, adicionado a 400 µL de ácido tiobarbitúrico (0,6%), aquecido a 100°C durante 1 hora e lido a 532 nm (GUIMARÃES *et al.*, 2017).

3.9 PROCEDIMENTO CIRÚRGICO PARA REGISTRO DA PRESSÃO ARTERIAL

Aos 90 dias de vida, os ratos machos e fêmeas (n=7 por grupo) foram anestesiados com 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), para registro da pressão arterial e infusão de drogas, respectivamente. Os cateteres foram tunelizados e exteriorizados pela região dorsal e uma dose de anti-inflamatório (cetoprofeno, 5 mg/kg) foi injetada por via subcutânea (GUIMARÃES *et al.*, 2017). Após 24 horas de recuperação cirúrgica, os ratos foram colocados em uma caixa pletismográfica, onde a pressão arterial (PA) e a frequência cardíaca (FC) foram registradas nos animais conscientes conectando a cânula arterial a um transdutor

de pressão (ML866/P, ADInstruments, Power Lab, Bella Vista, NSW, Austrália), conforme descrito anteriormente (DE BRITO ALVES *et al.*, 2014).

A pressão arterial pulsátil (PAP) e a FC foram registradas por 50–60 minutos em condições basais, e os valores de pressão arterial sistólica (PAS), pressão arterial diastólica (PAD), pressão arterial média (PAM) e FC foram calculados por uma seleção de intervalos de 10 minutos para cada animal (LabChart™ Pro, ADInstruments, Bella Vista, NSW, Austrália).

3.10 ANÁLISE ESPECTRAL

Utilizando o mesmo segmento de 10 minutos dos registros basais de PA e FC, a análise espectral no domínio de frequência de PAS e do intervalo de pulso (IP) foi realizada através de um programa de software apropriado (CardioSeries-v.2.4; www.danielpenteado.com).

Os espectros de PAS foram integrados nas bandas de baixa frequência (LF) (0,2–0,75 Hz) e de alta frequência (HF) (0,75–3 Hz). Para avaliar o índice simpato-vagal, foi calculada a relação LF / HF do intervalo cardíaco (IC). Além disso, a sensibilidade barorreflexa espontânea (SBRs) foi calculada através de um método sequencial por meio do método de sequência (GUIMARÃES *et al.*, 2017).

3.11 ATIVAÇÃO DOS QUIMIORRECEPTORES PERIFÉRICOS

Após 60 minutos do registro cardiovascular, o quimiorreflexo periférico foi ativado através da injeção intravenosa de cianeto de potássio (KCN; 40 µg para 0,1 mL por rato; Merck) conforme descrito previamente (DE BRITO ALVES *et al.*, 2015). A diferença entre a PAM e a FC basais e o pico de resposta após o estímulo (Δ PAM; Δ FC) foi avaliada para os componentes simpáticos e parassimpáticos, respectivamente.

3.12 AVALIAÇÃO DO CONTROLE BARORREFLEXO

Após 10 minutos da ativação dos quimiorreceptores periféricos, as respostas cardiovasculares da PA e da FC foram obtidas pela injeção sequencial das drogas vasoativas fenilefrina (8 µg/kg, i.v.) e nitroprussiato de sódio (25 µg/kg, i.v.) para avaliar a sensibilidade do barorreceptor, conforme descrito anteriormente (CARVALHO-GALVÃO *et al.*, 2018).

3.13 AVALIAÇÃO DO TÔNUS SIMPÁTICO SOBRE O SISTEMA CARDIOVASCULAR

Por fim, após 10 minutos da administração de nitroprussiato de sódio, a contribuição do tônus vascular simpático no sistema cardiovascular foi avaliada por meio da administração intravenosa de um bloqueador neuronal pré-ganglionar (hexametônio; 30 mg/kg, Sigma-Aldrich®, St Louis, MO, EUA). O tônus simpático foi calculado pelas alterações na PAM (Δ PAM) após a administração do bloqueador e a PAM basal, conforme descrito anteriormente (GUIMARÃES *et al.*, 2019). Ao final dos experimentos, os animais foram eutanasiados com overdose de 0,5 mL de cetamina (i.v.).

3.14 CONTAGEM DE MICRO-ORGANISMOS VIÁVEIS NAS FEZES

Amostras de fezes da prole de machos e fêmeas foram coletadas aos 90 dias de vida para contagem do número de micro-organismos viáveis. As amostras fecais foram homogeneizadas em água peptonada (1mg peptona por mL) e em seguidas foram diluídas em série no mesmo diluente (seis diluições). Alíquotas de 20 μ L das respectivas diluições foram inoculadas usando a técnica de microgotas (MILES; MISRA; IRWIN, 1938) em placas Petri estéreis contendo Man, Rogosa e Sharpe (MRS) ágar (HiMedia, Índia) para contagem de *Lactobacillus* spp; MRS + cisteína (0,05g por 100mL, Sigmam-Aldrich, Milão, Itália) (cMRS) para contagem de *Bifidobacterium* spp; MacConkey ágar (HiMedia, Índia) para contagem de *Enterobacteriaceae* e Bacteroides Bile Esculina (BBE) ágar (Acumedia, EUA) para contagem de *Bacteroides* spp. A incubação foi realizada em condições anaeróbicas (Anaerobic System Anaerogen, Okoid Ltd, Basingstoke, Hampshire, Reino Unido) para contagem de *Bifidobacterium* spp., *Lactobacillus* spp. e *Bacteroides* spp. e em condições aeróbicas para a contagem de *Enterobacteriaceae*. Após um período de incubação de 24-48 horas, o número de colônias visíveis em cada meio seletivo foi contado e os resultados foram expressos como log UFC/g. O limite para detecção do teste foi de 2 log UFC/ g (DA SILVA *et al.*, 2013).

3.15 ANÁLISES ESTATÍSTICAS

Os resultados foram expressos como média $\pm$ erro padrão. O teste de Kolmogorov-Smirnov foi usado para avaliar a normalidade dos dados. O teste paramétrico ANOVA *one way* e pós-teste de Tukey foram usados para as análises dos parâmetros bioquímicos, microbiológicos e cardiovasculares. ANOVA *two way* e o teste *post hoc* de Bonferroni foram usados para a análise das curvas do TOTG e TTI e medidas de peso corporal. A análise estatística foi realizada usando o software computacional Prism 5 (GraphPad Software, San Diego, CA). A diferença foi considerada significativa quando $p < 0,05$.

4 RESULTADOS

De acordo com o Artigo nº 55 da Resolução nº 06/2019 do Conselho Superior de Ensino, Pesquisa e Extensão (CONSEPE) da Universidade Federal da Paraíba, é preconizado que, para a defesa do trabalho final, deve ser gerado, no mínimo, dois artigos oriundos da tese de doutorado, comprovadamente submetidos para publicação em periódico inserido nos quatro estratos superiores do sistema *Qualis* na área de avaliação do programa junto a CAPES.

Os resultados desta tese serão apresentados na forma de dois artigos publicados, apresentados nos apêndices A e B, além da publicação de um capítulo de livro, apresentado no apêndice C.

No artigo I (Apêndice A), “*Gut microbiota and probiotic intervention as a promising therapeutic for pregnant women with cardiometabolic disorders: Present and future directions*”, objetivamos destacar as novas percepções da microbiota intestinal na fisiopatologia dos distúrbios cardiometabólicos maternos e o papel dos probióticos como uma estratégia terapêutica potencial para desordens cardiometabólicas durante a gestação. Inicialmente, esta revisão apresenta como os distúrbios metabólicos maternos podem impactar com um risco aumentado para o desenvolvimento de doenças cardiometabólicas na vida adulta da prole. Em seguida, são discutidas as principais alterações da microbiota intestinal na diabetes mellitus gestacional (DMG), pré-eclâmpsia, obesidade materna e dislipidemia materna. Por fim, apresentamos os possíveis efeitos benéficos de intervenções com probióticos nos distúrbios metabólicos maternos, bem como os benefícios potenciais da intervenção probiótica materna na saúde da prole.

O artigo II, apresentado no Apêndice B, “*Oral administration of *Lactobacillus fermentum* post-weaning improves the lipid profile and autonomic dysfunction in rat offspring exposed to maternal dyslipidemia*”, teve por objetivo avaliar os efeitos da administração pós-desmame de uma formulação probiótica de *Lactobacillus fermentum* sobre a pressão arterial, tônus simpático, barorreflexo, quimiorreflexo periférico, contagem de micro-organismos viáveis e parâmetros de glicolipídicos na prole de ratos machos e fêmeas de mães expostas à dislipidemia materna durante os períodos de gestação e lactação. Nossos resultados demonstraram pela primeira vez que a intervenção com uma formulação probiótica contendo *L. fermentum* 139, *L. fermentum* 263 e *L. fermentum* 296 (10^9 UFC/mL) durante 8 semanas apresentou efeitos hipocolesterolêmicos, induzindo melhorias no perfil lipídico, com redução dos níveis séricos de CT e aumento dos níveis séricos de HDL-c em ambos os sexos,

enquanto o efeito hipotrigliceridêmico foi observado apenas na prole de machos. Adicionalmente, a administração pós-desmame com *L. fermentum* atenuou os níveis de MDA dos grupos dislipidêmicos de ambos os sexos, sugerindo uma melhoria no equilíbrio oxidativo, no entanto, não foi eficaz na melhoria da tolerância à glicose e na redução da resistência à insulina na prole dislipidêmica de ambos os sexos. Outro achado deste estudo é que a formulação probiótica testada reduziu significativamente as oscilações LF da PAS, reduziu o tônus simpático e atenuou a via simpática do quimiorreflexo periférico na prole dislipidêmica de ambos os sexos, mas sem efeitos na via do barorreflexo. Além disso, a terapia com formulação de *L. fermentum* foi capaz de restaurar a população fecal de *Lactobacillus* spp. na prole dislipidêmica masculina e reduziu o crescimento de *Enterobacteriaceae* em filhotes machos e fêmeas de mães alimentadas com uma dieta rica em lipídios e colesterol durante a gestação e lactação, sugerindo uma melhora da inflamação intestinal.

No capítulo de livro (Apêndice C), “*Probiotics for humans: Current status and future prospects*”, apresentamos o uso terapêutico dos probióticos como uma estratégia inovadora para o fornecimento de bactérias intestinais favoráveis com potenciais efeitos benéficos para a saúde materna, fetal, infantil e adulta. Neste sentido, discutimos os principais efeitos da administração de probióticos para a saúde humana, abordando diversas condições clínicas específicas durante a gravidez, infância e idade adulta, considerando evidências de ensaios clínicos, bem como a influência de diferentes cepas, dose utilizada e tempo de intervenção dos resultados clínicos.

5 CONCLUSÃO

Foi demonstrado que o consumo de uma dieta rica em gordura e colesterol durante os períodos de gestação e lactação é capaz de alterar a composição das bactérias intestinais, ocasionando o desenvolvimento de RI, disfunção autonômica e HA na prole de machos e fêmeas aos 90 dias de vida expostos à dislipidemia materna.

Por outro lado, a intervenção com uma formulação probiótica contendo as cepas *L. fermentum* 139, *L. fermentum* 263 e *L. fermentum* 296 na prole masculina e feminina durante 8 semanas produziu efeitos hipocolesterolêmicos, induzindo melhorias no perfil lipídico e nos níveis séricos de MDA, mas não atenuou a RI induzida pela dislipidemia materna. Além disso, a formulação probiótica proposta demonstrou melhora nas contagens fecais de *Lactobacillus* spp. e *Enterobacteriaceae* spp., PA e tônus simpático, sem afetar a modulação barorreflexa.

Concluiu-se, portanto, que a administração de uma formulação probiótica de *L. fermentum* melhorou o perfil lipídico e a disfunção autonômica na prole masculina e feminina de mães expostas à dislipidemia materna durante a gestação e lactação, indicando que a formulação probiótica de *L. fermentum* pode efetivamente atenuar a resposta simpato-excitatória induzida pelo quimiorreflexo periférico, bem como reduzir o tônus simpático, sendo uma estratégia terapêutica coadjuvante para prevenir o desenvolvimento de HA e distúrbios metabólicos na vida adulta da prole.

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APÊNDICE A – ARTIGO I

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Review

Gut microbiota and probiotic intervention as a promising therapeutic for pregnant women with cardiometabolic disorders: Present and future directions



José Luiz de Brito Alves^{a,*}, Yohanna de Oliveira^{a,1}, Nara Nóbrega Crispim Carvalho^a, Raíssa Georgianna Silva Cavalcante^a, Marília Marques Pereira Lira^a, Luciana Caroline Paulino do Nascimento^a, Marciane Magnani^b, Hubert Vidal^c, Valdir de Andrade Braga^d, Evandro Leite de Souza^a

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

^b Department of Food Engineering, Technology Center, Federal University of Paraíba, João Pessoa, Brazil

^c Univ-Lyon, CarMeN(Cardio, Metabolism, Diabetes and Nutrition) Laboratory, INSERM U1060, INRA U1397, Université Claude Bernard Lyon 1, INSA Lyon, Oullins, France

^d Department of Biotechnology, Biotechnology Center, Federal University of Paraíba, João Pessoa, PB, Brazil

A B S T R A C T

Maternal cardiometabolic disorders, such as gestational diabetes mellitus, pre-eclampsia, obesity, and dyslipidemia, are the most common conditions that predispose offspring to risk for future cardiometabolic diseases, needing appropriate therapeutic approach. The implications of microbiota in the pathophysiology of maternal cardiometabolic disorders are progressively emerging and probiotics may be a simple and safe therapeutic strategy for maternal cardiometabolic management. In this review, we argue the importance of cardiometabolic dysfunction during pregnancy and/or lactation on the offspring risk for cardiometabolic disease in later life. In addition, we comprehensively discuss the microbial diversity observed in maternal cardiometabolic disorders and we present the main findings on probiotic intervention as a potential strategy for management of maternal cardiometabolic disorders. Current data reveal that gut microbiota may be transmitted from mother to offspring. Whether targeting microbiota with probiotic intervention during the periconceptional period prevents or delays the onset of cardiometabolic disorders in adult offspring should be tested in future clinical trials.

1. Introduction

Pregnancy is a biological process that involves several physiological and hormonal alterations necessary to ensure appropriate weight gain and fetal growth [1]. During this period adequate nutritional supply

exerts a key role for maintenance of maternal-fetal health. Nonetheless, cardiometabolic disorders also associated with poor diet, such as obesity, gestational diabetes mellitus (GDM), pre-eclampsia or dyslipidemia, experienced *in utero* and/or during lactation could be involved with adverse dual-burden outcomes to mother and offspring [2–4].

Abbreviations: ARH, Arcuate nucleus of the mediobasal hypothalamus; BBB, blood–brain barrier; BMI, body mass index; *Buk*, butyrate kinase; CFU, colony forming unit; CRP, C-reactive protein; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GPCRs, G protein-coupled receptors; GDM, gestational diabetes mellitus; GWG, gestational weight gain; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; GIP, glucose-dependent insulinotropic polypeptide; GSH, glutathione; GPx, glutathione peroxidase; HbA1c, glycosylated hemoglobin; HDL-c, high-density lipoprotein cholesterol; HDP, hypertensive disorders of pregnancy; HOMA-IR, homeostatic model assessment of insulin resistance; IGF-2, insulin-like growth factor 2; IL-1, interleukin 1; IL-6, interleukin 6; IL-10, interleukin 10; LPS, lipopolysaccharide; LDL-c, low-density lipoprotein cholesterol; NHANES, National Health and Nutrition Examination Survey; NPY, neuropeptide Y; NO, nitric oxide; PPAR-γ, peroxisome proliferator-activated receptor gamma; PAI-1, plasminogen activator inhibitor-1; PIH, pregnancy-induced hypertension; PTB, preterm birth; POMC, proopiomelanocortin; PUFAs, polyunsaturated fatty acids; RF, respiratory frequency; SFAs, saturated fatty acids; SCFAs, short chain fatty acids; SOD, superoxide dismutase; SBP, systolic blood pressure; *TIMP3*, TIMP metalloproteinase inhibitor 3; TC, total cholesterol; TG, triglycerides; TNF-α, tumor necrosis factor-α; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus

* Corresponding author at: Federal University of Paraíba, Department of Nutrition, Campus I – Jd. Cidade Universitária, 58051-900, João Pessoa, PB, Brazil.

E-mail addresses: jose_luiz_61@hotmail.com, joseluiz@cbiotec.ufpb.br (J.L. de Brito Alves).

¹ The authors contributed equally.

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Alterations in the microbiota composition during pregnancy may occur naturally and healthy in a variety of body sites, including gut, vagina, placenta, oral cavity or even in the breast milk [5,6]. However, the relationship between maternal microbiota and gestational physiometabolic conditions are still complex and has not been fully elucidated. Some research has begun to link microbial dysbiosis and specific microbial populations to unhealthy complications in pregnancy.

Maternal gut microbiota alters progressively over the course of each trimester of the gestational period [7]. In the first trimester of pregnancy, the abundance of gram-positive Firmicutes and the gram-negative Bacteroidetes phyla are almost unaltered. However, the composition of the gut microbiota of healthy pregnant women is substantially remodeled during the third trimester of pregnancy, exhibiting a low bacterial diversity with decreased abundance of *Faecalibacterium*, which is a butyrate-producer with anti-inflammatory effects, and increased richness of Actinobacteria and Proteobacteria [8], which are associated with dysbiosis and inflammatory condition [9].

Placental microbiota is composed of nonpathogenic bacteria belonging to the phylum Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes and Fusobacteria [10]. Factors, such as maternal obesity, vaginal infection, GDM, and antibiotic therapy alter the placental microbiota composition [11]. For example, excess gestational weight gain (GWG) has been associated with dysbiosis of placental microbiota and a changeable risk factor for preterm birth [12]. In the postnatal environment, an additional complex connection has been seen between the breast milk microbiome and infant gut microbiota [13]. A prospective study demonstrated that bacterial species found in maternal breast milk areolar skin are the most abundant in infant's gut microbiota during the first month of life [13], suggesting that gut microbiota in early life could be acquired through maternal breastfeeding.

Moreover, maternal diet poor quality has been related as an important risk factor for gut microbiota derangements in mothers and their offspring, [14–16], and augmented risk for the development to late cardiometabolic disorders in offspring [17,18]. These findings show that the microbiota during pregnancy and lactation may be altered by the various external factors, impacting on the establishment of a lifelong host-microbe symbiosis and human health.

It is reasonable to infer that strategies capable of modulating the host-microbial interactions in early life could represent an innovative therapeutic for the prevention and/or control of maternal metabolic dysfunction and their consequences on the healthy offspring. In this way, maternal microbiota modulation with probiotic intervention has emerged as a safe strategy capable of recovering the community of commensal microbiota and provide beneficial effects for maternal, fetal and infant health [19,20]. The concept set by Food and Agriculture Organization of the United Nations/ World Health Organization (FAO / WHO) and recognized worldwide, defines probiotics as “live microorganisms, that when consumed in adequate amounts, confer a health benefit on the host” [21]. Currently, the most studied probiotic species belong to *Bifidobacterium* (e.g., *B. bifidum*, *B. breve*, *B. infantis*, *B. lactis*, *B. longum* and *B. thermophilum*) and *Lactobacillus* genera (e.g., *L. acidophilus*, *L. casei*, *L. paracasei*, *L. fermentum*, *L. reuteri*, *L. plantarum*, *L. rhamnosus* and *L. salivarius*).

In order to highlight these new insights, this review will firstly present how maternal metabolic disorders can impact on the risk of cardiometabolic disease in later life offspring. Secondly, the main gut microbiota alterations in GDM, pre-eclampsia, maternal obesity and dyslipidemic pregnant will be discussed. Lastly, the possible benefits of probiotic interventions on maternal metabolic disorders, as well as the potential benefits of maternal probiotic intervention on offspring health will be presented.

2. Gestational diabetes, microbiota, and probiotic intervention

GDM is one of the most common pregnancy complications, is defined as any degree of glucose intolerance with onset or first diagnostic

during gestational period and does not meet the diagnostic criteria for pre-existing diabetes mellitus [22]. The prevalence of GDM has increased by more than 30% in the last two decades, becoming an emerging epidemic worldwide [23]. Excess of fat and sugar consumption, pre-gestational obesity [24], excessive GWG [25], advanced maternal age and occurrence of previous pregnancy with GDM are considered the main risk factors for the increasing prevalence of GDM [26].

GDM increases maternal and offspring risk for cardiometabolic diseases. In short-term, GDM has been associated with increased risk of preeclampsia and probability of newborn with macrosomia and excess adiposity [27,28]. Additionally, women with GDM present seven-fold increase in the risk of developing type 2 diabetes mellitus (T2DM) after the gestational period [29].

Experimental and clinical findings have demonstrated that GDM is a risk factor for the development of obesity, reproductive function impairment, hypertension, renal dysfunction, cardiovascular diseases, and T2DM in the later life of offspring [30–34]. A preclinical study demonstrated that offspring from diabetic mothers displayed glucose intolerance and high arterial blood pressure with no major effects on lipid metabolism at 12 months of age [35]. Another study showed that adult male but not female rat offspring from dams exposed to maternal diabetes during the last third of gestation developed hypertension not associated with a reduction in cardiovascular baroreflex control [36], but with relevant impairment in renal function [31,37]. Additionally, the exposure to GDM has been shown to induce neuroinflammation, derangement of hippocampal neurons and cognitive changes in rat offspring [38].

Clinical trials have also investigated the effects of GDM on cardiometabolic health of offspring. A recent clinical study demonstrated that adult man (n = 27) and woman (n = 31) exposed to maternal type 1 diabetes mellitus (T1DM) displayed pancreatic β -cell dysfunction in adult life, which was more pronounced in female than male offspring [39].

A randomized study performed with children at 7 (n = 109) and 9 years-old (n = 99) from pregnant women with GDM revealed that total and abdominal body fat and metabolic parameters [e.g., fasting plasma glucose (FPG), triglycerides (TG), insulin, insulin resistance, glycosylated hemoglobin (HbA1c), cholesterol, liver transaminases, leptin, and adiponectin)] were similar in children from women with GDM treated with metformin or insulin [40]. However, metformin-exposed children were larger at 9 years. Although the FDA approves metformin for the treatment of GDM, there are doubts about the impact of this medication on offspring. The follow-up of this cohort should be helpful to elucidate if the development of metabolic disorders occurs at later ages or if pharmacological hypoglycemic treatment is effective to protect the offspring against the development of cardiometabolic dysfunction.

The available literature strongly indicates that GDM exerts harmful dual-burden for mothers and their offspring [41–43]. Periconceptional therapies with the potential to counteract adverse GDM outcomes for mothers and their offspring are of great importance. Nowadays, dietary and lifestyle modifications comprise the primary therapeutic approach for women with GDM [44,45]. If diet and lifestyle management are insufficient to maternal glycemic control, insulin therapy or oral anti-diabetic pharmacological therapies are prescribed to GDM conditions [46].

Growing evidence indicates that disruption of intestinal permeability may be involved in the development of T2DM [47–50], inflammation, high-fat mass, hyperglycemia and insulin resistance in pregnant women [51]. In most cases, the impaired gut barrier function is associated with inadequate gut microbiota composition [47,52,53].

A study demonstrated that the profile of the placental microbiota from women with GDM is significantly distinct of non-diabetic pregnant women [54]. Relative abundance of bacteria belonging to the Pseudomonadales order and Acinetobacter genus were lower in women with GDM compared to healthy pregnant women. Moreover, in women with GDM, a lower relative abundance of Acinetobacter was associated with

Table 1
Summary of clinical trials investigating the relationship between probiotic supplementation in pregnant women and clinical responses.

Studies (Number of participants)	Probiotics	Dosage	Time of Intervention	Primary Endpoints	Other parameters
[60] n = 256	<i>L. rhamnosus</i> GG and <i>B. lactis</i> Bb12	10 ¹⁰ CFU/day each	From the first trimester of pregnancy to the end of exclusive breastfeeding 6 weeks	Reduced the frequency of GDM	Provided a normal growth rates in children receiving probiotics
[63] n = 64	<i>L. acidophilus</i> LA-5, <i>B. BB-12</i> , <i>Streptococcus thermophilus</i> STY-31 and <i>L. delbrueckii bulgaricus</i> LBY-27	4 × 10 ⁹ CFU/g each	6 weeks	Decreased FPG and HOMA-IR	Decreased significantly the gestational weight gain
[69] n = 149	<i>L. salivarius</i> UCC118	10 ⁹ CFU/g	6 weeks	Not detected any beneficial glycemic effect	Reduced TC and LDL-c levels
[62] n = 60	<i>L. acidophilus</i> , <i>L. casei</i> , and <i>B. bifidum</i>	2 × 10 ⁹ CFU/g each	6 weeks	Decreased FPG, HOMA-IR, HOMA-B and increased QUICKI	Decreased TG and VLDL levels
[68] n = 82	Probiotic VSL #3 (<i>Streptococcus thermophilus</i> , <i>B. breve</i> , <i>B. longum</i> , <i>B. infantis</i> , <i>L. acidophilus</i> , <i>L. plantarum</i> , <i>L. paracasei</i> , and <i>L. delbrueckii subsp. Bulgaricus</i>)	112.5 × 10 ⁹ CFU/capsule	8 weeks	FPG, HbA1c, HOMA-IR, and insulin levels remained unchanged	Decreased in levels of IL-6, TNF-α and hs-CRP
[61] n = 60	<i>L. acidophilus</i> , <i>L. casei</i> , <i>B. bifidum</i>	2 × 10 ⁹ CFU/g each	12 weeks	Decreased serum insulin levels, HOMA-IR, HOMA-B and increased QUICKI	Decreased TG, hs-CRP and increased NO, TAC and GSH levels
[64] n = 423	<i>L. rhamnosus</i> HN001	6 × 10 ⁹ CFU/day	From 14 to 16 weeks' gestation	Reduced a 3-fold in the prevalence of GDM in the older group	No associated with maternal anthropometric measures or infant birth weight
[65] n = 64	<i>L. acidophilus</i> LA-5, <i>Bifidobacterium</i> BB-12.S, <i>thermophilus</i> STY-31 and <i>L. delbrueckii bulgaricus</i> LBY-27	> 4 × 10 ⁹ CFU/capsule/ daily	8 weeks	Prevented an increase in SBP as the pregnancy progressed and significantly decreased DBP after 2 weeks of consumption	SBP was influenced by weight changes, yet these did not affect longitudinal changes in DBP
[66] n = 60	<i>L. acidophilus</i> , <i>L. casei</i> and <i>B. bifidum</i>	2 × 10 ⁹ CFU/g each/day	6 weeks	Decreased in FPG, CRP, MDA, MDA/TAC ratio, and increased in TAC levels	Did not affect plasma NO, GSH levels and pregnancy outcomes
[67] n = 57	Inflozan® (<i>L. acidophilus</i> and <i>B. bifidum</i>)	1000 million CFU/each/day	4 weeks	Decreased in FPG and HOMA-IR levels	Did not affect gestational weight gain

CFU: colony forming unit; GDM: gestational diabetes mellitus; FPG: fasting plasma glucose; HOMA-IR: homeostatic model assessment of insulin resistance; TC: total cholesterol; LDL-c: low density lipoprotein cholesterol; HOMA-b: QUICKI; TG: triglycerides; VLDL: very low density lipoprotein; HbA1c: glycosylated hemoglobin; IL-6: Interleukin 6; TNF-α: hs-CRP; NO: nitric oxide TAC: total antioxidant capacity; GSH: glutathione; SBP: systolic blood pressure DBP: diastolic blood pressure; MDA: malondialdehyde.

lower blood eosinophil count and lower placental expression of several anti-inflammatory cytokines, including interleukin 10 (*IL10*) and metalloproteinase inhibitor 3 (*TIMP3*) [54]. Gut microbiota composition of women with GDM diagnosed in the third trimester of pregnancy was previously characterized by an aberrant microbiota composition typical of dysbiosis, which persists even after about 8 months postpartum [55]. These findings indicate that gut microbiota could play a role in the development of GDM, and, therefore, could constitute a new candidate target for GDM therapy.

2.1. Probiotic intervention: a new therapeutic approach for GDM women?

Strategies capable of recovering the community of commensal microbiota has been highlighted [56,57]. Probiotic, prebiotic or symbiotic interventions have been used in the improvement of gut microbiota dysfunction and management of GDM [58,59]. Clinical outcomes of the use of probiotics through randomized clinical trials are presented in Table 1.

A double-blind, placebo-controlled study performed with 256 pregnant women demonstrated that probiotic intervention (*L. rhamnosus* GG and *B. lactis* Bb12, 10^{10} CFU/day each), from the first trimester of pregnancy until the end of exclusive breastfeeding had no adverse effects on the duration of pregnancy and reduced the frequency of GDM when compared to pregnant that received placebo intervention ($p = 0.003$) [60]. Similarly, a different study with 60 women aged 18–37 years in the first half of pregnancy and receiving an intervention with *L. acidophilus*, *L. casei* and *B. bifidum* (2×10^9 CFU/g each, for 12 weeks) reduced serum insulin concentrations and increased insulin sensitivity and total antioxidant capacity in comparison to women pregnant that received placebo intervention [61].

A study involving 60 pregnant women diagnosed with GDM at 24–28 weeks of gestation, including primigravida aged 18–40 years, showed that a daily intervention with *L. acidophilus*, *L. casei* and *B. bifidum* (2×10^9 CFU/g each, for 6 weeks) decreased plasma levels of FPG, TG, very low-density lipoprotein and cholesterol, as well as, reduced maternal insulin resistance when compared to the placebo group [62].

Another randomized clinical trial with 64 pregnant women presenting GDM between 24 and 28 weeks, showed that a daily intervention with probiotic capsules containing *L. acidophilus* LA-5, *B. BB-12*, *S. thermophilus* STY-31 and *L. delbrueckii bulgaricus* LBY-27 (4×10^9 CFU/g each, for 2 weeks) reduced approximately 15% in FPG and 6.74% in homeostatic model assessment for insulin resistance (HOMA-IR) when compared with the placebo group [63].

Data from a double-blind, placebo-controlled and randomized study demonstrated that probiotic *L. rhamnosus* HN001 (6×10^9 CFU/day) initiated at 14th to 16th week of gestation until 6 months post birth was not associated with improvement in maternal anthropometric measures or infant's birth weight, but reduced approximately 3-fold the prevalence of GDM in comparison to women that received placebo [64].

An 8-weeks randomized, double-blind and placebo-controlled trial carried out with 64 pregnant women with GDM diagnosed at 24–28 weeks demonstrated that a daily intervention with capsules containing 180 mg of a standard powder, including freezer dried cultures of *L. acidophilus* LA-5, *Bifidobacterium* BB-12, *S. thermophilus* STY-31 and *L. delbrueckii bulgaricus* LBY-27 (4×10^9 CFU), reduced systolic blood pressure (SBP) and diastolic blood pressure (DBP) in GDM [65].

In addition, a 6-weeks randomized, double-blind and placebo-controlled study performed with 60 pregnant women diagnosed with GDM and aged 18–40 years observed that a daily intervention with probiotic capsules (containing *L. acidophilus*, *L. casei* and *B. bifidum*, at dose of 2×10^9 CFU/g each) reduced FPG, c-reactive protein (CRP) and malondialdehyde serum levels when compared with pregnant patients that received placebo intervention [66].

Another randomized, double-blind and placebo-controlled trial involving 57 women with GDM at 24–28 weeks of gestation demonstrated

that a daily administration of the commercial probiotic Inflan® (containing 1000 million CFU of *L. acidophilus* and 1000 million CFU of *B. bifidum*, for 4 weeks) lowered FPG and increased insulin sensitivity in comparison to the placebo group [67]. The changes in metabolic parameters after randomization showed a significant improvement in glucose metabolism in the probiotic group in comparison to placebo group, including FPG ($p = 0.03$), fasting plasma insulin ($p = 0.001$) and HOMA-IR ($p = 0.001$).

A double-blind, placebo-controlled and randomized study carried out with 82 pregnant women with GDM demonstrated that an intervention twice a day with probiotic formulation VSL#3 containing 112.5×10^9 CFU of *Streptococcus thermophilus*, *B. breve*, *B. longum*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. paracasei* and *L. delbrueckii* subsp. *bulgaricus* for 8 weeks reduced interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) and CRP serum levels and improved insulin resistance through HOMA-IR test compared to placebo group [68].

Despite the positive findings demonstrating that probiotic intervention could improve glycemic control in GDM, a double-blind, placebo-controlled and randomized trial performed with 149 pregnant women diagnosed with GDM at 24th to 28th week of gestation showed that probiotic daily intervention for 6 weeks (capsule containing 100 mg *L. salivarius* UCC118 at a dose of 10^9 CFU) neither reduce maternal FPG nor improve lipid profile when compared to placebo group [69].

The available literature demonstrates that probiotic therapy may be a relevant supporting approach to improve glycemic control in pregnant women diagnosed with GDM. However, additional experimental and clinical studies are needed to elucidate the underlying mechanisms by which probiotic therapy may improve the glycemic control in GDM, as well as determine the type, dose, and duration of probiotic that is safe for administration during pregnancy. In addition, is necessary to know whether probiotic intervention during the gestational period could have a positive impact on prevention or reduction of cardiometabolic disorders in later life of offspring from diabetic mothers.

3. Pre-eclampsia, microbiota and probiotic intervention

Pregnancy-associated hypertensive disorders include pre-pregnancy hypertension that persists throughout gestation (chronic/preexisting hypertension) and *de novo* hypertension that is diagnosed after 20 weeks of gestation and disappear after birth (gestational hypertension). Additionally, they include *de novo* hypertension that is diagnosed after 20 weeks of gestation with or without proteinuria and end-organ damage (pre-eclampsia and eclampsia) and chronic hypertension with superimposed pre-eclampsia during gestation [70]. Pre-eclampsia is a syndrome affecting 3–5% of pregnancies worldwide and considered one of the main causes of maternal, fetal and neonatal mortality. Pre-eclampsia is clinically diagnosed by the combined occurrence of high arterial blood pressure and proteinuria [71].

Diastolic blood pressure > 80 mmHg, family history of pre-eclampsia, increased maternal age (mainly > 35 years), multi-fetal gestation, nulliparous women, obesity (mainly BMI > 35 kg/m²), previous pregnancy with pre-eclampsia and proteinuria on at least one occasion or ≥ 0.3 g/24 h are well-known risk factors for the development of pre-eclampsia [72].

Altered immune response, with consequent increase of inflammatory cytokines; anti-angiogenic responses; abnormal trophoblast invasion of placenta; as well as oxidative stress are mechanisms in which they occur in pre-eclampsia and are widely considered to contribute to its severity [72]. Evidence indicates that placenta may play a critical role in the pathogenesis of pre-eclampsia and deleterious effects on the offspring [73,74]. In pre-eclampsia condition, the utero-placental blood flow is compromised and the risk of preterm delivery and low birth weight is increased [75].

In recent years, has been demonstrated that hypertensive disorders in pregnancy lead to increased risk of complications not only during

pregnancy, but also for mother and fetal health in short and long-term [76]. Observational studies have shown that children from mothers who had pre-eclampsia are subject to long-term dysfunctions related to cardiovascular risk factors, such as increased SBP (approximately 2.39 mmHg) and DBP (about 1.35 mmHg) [77], use of anti-hypertensives between 34–44 years of age [78], increased BMI, 0.62 kg/m² [79,80], psychic complications [81], reduced cognitive function [82] and negative association with bone mass in the hip at 17 years of age [83].

The Helsinki Birth Cohort Study performed with the database from 6410 births between 1934–1944 verified that 244 mothers had pre-eclampsia and 1592 had gestational hypertension. The results of the study showed an increase in the occurrence of stroke in adults from mothers who had pre-eclampsia and gestational hypertension, but no increase in coronary artery disease occurrence was observed [84].

The evaluation of the placental microbiome in pregnant women with pre-eclampsia had shown that several bacterial genera associated with gastrointestinal infection (e.g. *Bacillus*, *Listeria*, *Salmonella* and *Escherichia*), respiratory tract infections (e.g. *Klebsiella* and *Anoxybacillus*) or with periodontitis (*Variovorax*, *Prevotella shahii*, *Porphyromonas* and *Dialiste*) are present in placental tissues [85]. These findings suggest a possible role of placental microbiome dysbiosis in pre-eclampsia development.

Gut microbiota dysbiosis in hypertension is characterized by a reduced bacterial diversity, increased Firmicutes/Bacteroidetes ratio, decreased population of acetate- and butyrate-producing bacteria and increased population of lactate-producing bacteria as well [86,87].

During pregnancy, Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria are the phyla relatively stable in gut microbiota in healthy pregnant women, being Bacteroidetes the most predominant phylum, while Firmicutes population are relatively dropped. Verrucomicrobia population typically decreases in the third trimester, while Tenericutes population increases in late pregnancy. In contrast, pregnant women with pre-eclampsia exhibit an increased population of pathogenic bacteria, such as *Clostridium perfringens* and *Bulleidia moorei*, and a reduction of in population of the bacteria *Coprococcus catus*. These gut microbiota modifications have been associated with the progress of pre-eclampsia during pregnancy, suggesting the intestinal dysbiosis as a new potential biological marker for monitoring pregnant women, as well as a target for intervention [88].

A number of experimental studies and clinical trials have demonstrated a beneficial relationship between probiotics intervention and reduced arterial blood pressure levels [87,89,90]. Actually, there is no evaluation of the use of probiotic intervention for the treatment of pre-eclampsia. A prospective cohort study, the Norwegian Mother and Child Cohort, showed that high intake of probiotic dairy products containing *L. acidophilus* LA-5, *B. lactis* Bb12 and *L. rhamnosus* GG (> 200 mL/day) were associated with a lower risk of pre-eclampsia (20%) and severe pre-eclampsia in primiparous women [91]. However, this study did not assess the effects of the consumption of probiotic dairy products on gut microbiota of pregnant women with pre-eclampsia.

Further studies envisaging the modulation of gut microbiota by different probiotic strains in pregnant women will help to clarify whether the probiotic intervention could be an effective strategy to maintain satisfactory blood pressure levels and prevent or reduce the harmful effects on the offspring.

4. Obesity during pregnancy, microbiota and probiotic intervention

Prevalence of obesity in women in reproductive age has been increasing worldwide. The database from the US *National Health and Nutrition Examination Survey* (NHANES) in 2011–2012 showed an estimated obesity prevalence of 31.8% in the USA for women aged 20–39 years with BMI > 30 Kg/m². Current estimates suggest that more than

21% of women in the world could be obese by 2025 [92].

Maternal obesity is associated with increased risk for GDM, cesarean delivery, pre-eclampsia, delivery of large for gestational age neonates, postpartum hemorrhage and endometritis [93]. Maternal obesity is also associated with increased risk for adverse maternal and neonatal outcomes, such as fetal growth abnormalities, intrauterine growth restriction and macrosomia, prematurity, and higher frequency of maternal placental vascular lesions [94,95].

Genetic predisposition and postnatal environment are key factors for the development of chronic disease, but the gestational period may also influence the long-term risk unaccounted by genetic tendency or postnatal environment alone [96]. Although evidence from clinical and experimental studies has associated maternal obesogenic environment with increased risk for development of obesity and related cardiometabolic disorders in offspring in adulthood [97,98], the possible mechanisms underlying these effects are still unclear [6,97].

The physiological insulin resistance observed in late normal pregnancy is ordinarily increased in pregnant women with obesity and diabetes. The exposure of the fetus to high levels of metabolic substrates, such as glucose and lipids, might affect the placenta and influence the development and functionality of organs and fetal systems, leading to increased fetal size, fat storage and increased risk for post-natal diseases [99–101].

Programming of hypothalamic appetite-regulating centers by maternal obesity is described as a potential mechanism by which maternal obesity contributes to adverse metabolic effects in offspring [99]. Hormonal factors, including insulin, leptin and ghrelin, have been already identified as potential mediators of early developmental misprogramming, as a consequence of maternal overnutrition. For example, it was demonstrated that the blood-brain barrier in offspring from obese dams exhibit increased permeability to hormonal factors, which are directed to the arcuate nucleus of the mediobasal hypothalamus, augmenting the risk of obesity and metabolic disorders [102].

Maternal obesity has been also associated with hyperlipidemia in offspring, which occurs in parallel with increased expression of hypothalamic proopiomelanocortin (POMC) mRNA, reduced expression of neuropeptide Y (NPY) and energy sensors (including mTOR and pAMPK). These alterations may be expanded in the offspring when exposed to a postnatal obesogenic environment [97]. In addition, maternal diet-induced obesity has been considered an important risk factor for hypertension development, left ventricular pathological remodeling and ventricular dysfunction in adult offspring [103].

The occurrence of congenital malformation in offspring has been related to maternal obesity. A meta-analysis study showed that this disorder is an important risk factor for neural tube defects in offspring [104]. A retrospective case-control study involving 37,168 live births identified maternal obesity as a strong predictor for increased nervous system anomalies, facial defects, genitourinary anomalies and cardiac malformation in offspring [105]. A population-based case-control study observed an association between congenital abnormalities in the kidney and urinary tract and pre-existing maternal obesity [106].

Considering the kidney injury, maternal obesity can lead to detrimental effects in the offspring, including increased inflammation, oxidative stress [96,107] and fibrotic changes [108]. Oxidative stress is an important factor for the occurrence of renal fibrosis, being reported to act synergistically with pro-inflammatory cytokines to cause renal damage [96,107]. Furthermore, maternal BMI status should be associated with increased inflammation and IgM levels [109], as well as with increased risk of wheeze, cough and lower respiratory tract infection in the infancy of offspring [110].

A recent study examined the gut, oral and placental microbiome from 37 overweight or obese pregnant women at 38th to 40th week of gestation by using 16S rRNA sequencing. Interestingly, the genera *Veillonella*, *Streptococcus* and *Prevotella* were commonly present in either placental, oral or gut microbiome. Therefore, it is contacted that both the oral and the intestinal microbiome can interfere in the microbiome

of the placenta, being this colonization coming from several regions [111]. A relationship between gut microbiome composition and circulating metabolic hormones was demonstrated in overweight and obese pregnant women at 16 weeks gestation [112]. The authors demonstrated that insulin, HOMA-IR, leptin and c-peptide were positively associated with growing BMI, while that the glucagon-like peptide-1 (GLP-1) and resistin levels were negatively linked with the BMI in overweight and obese pregnant women. Interestingly, the authors found that HOMA-IR was positively associated with relative fecal abundance of the phyla *Actinobacteria* and negatively linked with the phyla *Tenericutes*. Maternal hormones involved with energy metabolism also were associated with changes in relative abundance of bacterial taxa. For example, leptin levels were positively associated with *Firmicutes* family members, *Lachnospiraceae* and *Ruminococcaceae*, while resistin levels were negatively associated with *Firmicutes* abundance [112].

Another study showed an association between pre-pregnancy overweight/obese or excessive GWG with reduced bacterial diversity in maternal gut microbiota and reduced the abundance of the genera *Lachnospira*, *Parabacteroides* and *Bifidobacterium* and increased abundance of the genera *Blautia* [113]. However, these maternal characteristics did not show association with overall differences in the infant gut microbiota composition over the first 2 years of life.

In a study with 205 overweight and obese pregnant women at 16 weeks gestation, the abundance of the butyrate-producers *Odoribacter* and *Clostridiaceae* in the gut microbiota and expression of butyrate kinase (*Buk*), a bacterial butyrate-producing gene, were inversely correlated with SBP. Additionally, the increased *Odoribacter* abundance and *Buk* expression were associated with lower serum levels of the inflammatory marker plasminogen activator inhibitor-1 (PAI-1), which is typically increased in severe pre-eclampsia [114].

In addition to changes in the composition of the microbiota, initial evidence indicates that intestinal permeability and LPS activity may be increased in obese pregnancy woman [115–117]. LPS can lead to metabolic endotoxemia, a potential mediator of low-grade inflammation associated with metabolic disorders [118]. It is known that multiple events responsible for low-grade inflammation and metabolic disorders may be modulated by probiotics, especially by the increase of the intestinal barrier integrity and modulation in gut microbiota composition [119].

Based on current literature, it seems reasonable that the modulation of the gut microbiome by specific probiotic strains could exert positive effects for obese pregnant.

4.1. Probiotic intervention: a new therapeutic approach for obese pregnant?

In a placebo-controlled, double-blind, randomized trial, the probiotic intervention with capsules containing 100 mg *L. salivarius* UCC118 (10^9 CFU/day, for 4 weeks) in 138 obese pregnant women had no effect on maternal-neonatal anthropometric measures, markers of glucose or lipid metabolism, GWG and delivery outcomes [120]. Another randomized clinical trial carried out with 200 overweight pregnant women demonstrated that intestinal permeability and LPS serum levels were correlated with higher maternal weight gain. In this study, the daily intervention with probiotic (*B. animalis* ssp. *lactis* 420 and *L. rhamnosus* HN001, containing a 2×10^{10} CFU) from 14th to 35th week of gestation was not associated with improvement in gut permeability or LPS serum levels [121]. We highlight that the potential limitation of the studies was the lack of fecal samples analysis. A recent double-blind, randomized, controlled trial carried in 411 overweight and obese pregnant women demonstrated that probiotic intervention with capsules containing a mixture of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* subspecies *lactis* at a daily dose of 1×10^9 CFU from the second trimester of gestation until birth did not prevent GDM in overweight and obese pregnant women [122].

Conversely, a placebo-controlled, double-blind, randomized trial

found that dietary counseling and probiotic intervention with capsules containing *L. rhamnosus* GG (ATCC 53,103) and *B. lactis* Bb12 at a dose of 10^{10} CFU/day from the first trimester of pregnancy until 6 months of postpartum reduced central adiposity in women, being indicated as a supporting therapy for the prevention and management of obesity in pregnant women [123].

Recently, a prospective study conducted with 965 women pregnant suggested that consumption of more than 5 cups of yogurt per week may be associated with reduced preterm delivery among lean women, but this effect was not observed in maternal overweight status [124]. We highlight that yogurt has many other nutrients that could be beneficial for pregnant women.

Due to the lack of consensus on the beneficial effects of probiotic intervention for overweight or obese pregnant, experimental and larger well-designed clinical trials are required to prove or reject a causal link between probiotic intervention and body composition and metabolic parameters for overweight or obese pregnant.

5. Dyslipidemia during pregnancy, microbiota and probiotic intervention

During the gestational period, changes in lipid and lipoprotein metabolism have been implicated in the development of physiological hyperlipidemia. This clinical condition has been characterized by increase of approximately 42% in low-density lipoprotein cholesterol (LDL-c) serum levels at 36 weeks of gestation and a three-fold increase in TG serum level from 14th to 36th week of gestation [125]. These changes are considered beneficial and necessary for satisfactory fetal growth and fetal nervous system development. Although pregnancy has been recognized as a period of life favorable to increased serum lipid levels, the diagnostic of maternal dyslipidaemia is complex and their effects on the offspring are poorly understood.

A prospective longitudinal cohort study carried out with 1310 pregnant women demonstrated that both hypertriglyceridemia and hypercholesterolemia during gestation constitute important risk factors for GDM and hypertensive disorders [126]. Additionally, triglyceride serum levels > 300 mg/dL in the first trimester of pregnancy are strongly associated with gestational hypertension, pre-eclampsia, pre-term birth, and fetuses considered large for gestational age [127,128].

Maternal cholesterol has been shown to cross the placenta and enter the fetal circulation, contributing to the fetal cholesterol pool in offspring. A recent study showed that maternal supraphysiological hypercholesterolemia (established by total cholesterol above 280 mg/dL and LDL-c exceeding 190 mg/dL) at delivery impaired placental microvascular endothelial function [129]. Fetal exposure to high cholesterol serum levels has been shown to increase the predisposition for the development of atherosclerosis in later life of offspring [130]. Recently, our research group demonstrated that rats exposed to maternal dyslipidemia during pregnancy and lactation developed severe arterial hypertension and metabolic dysfunction in adult life [14,17,131].

Understanding the effects of maternal dyslipidemia on offspring is timely based on the gaps in clinical practice. For example, the lipid profile is not included in obstetric routine exams, and there is no established consensus regarding reference lipid values per trimester of pregnancy. Additionally, there is no standardization for diagnostic criteria and determination of risk groups. The impact of maternal dyslipidemia on offspring risk for future cardiometabolic diseases also remains unclear.

Maternal dyslipidemia should be treated with diet and exercise intervention, as well as with glycemic control when appropriate. Bile acid sequestrates, such as colestevam or mipomersen, may be used for the treatment of familial hypercholesterolemia during pregnancy and severe hypertriglyceridemia should be treated with omega-3 fatty acids, parenteral nutrition, plasmapheresis and other lipid-lowering agents in the last trimester of pregnancy [127].

It has been demonstrated that pregnant mice fed with high-fat diet

during periconceptional period exhibit distinctive shifts in the pregnant gut microbiota characterized by increased microbial abundance of *Allobaculum*, *Sarcina*, *Biophila*, *Coprobaillus*, *Staphylococcus*, *Akkermansia* and *Bifidobacterium* when compared to health mice pregnant [51]. Although the study has not demonstrated the exact role of these microbial alterations on maternal metabolism, numerous metabolic signaling pathways, such as fatty acid, ketone, cysteine, methionine, and vitamin metabolism were significantly augmented in pregnant mice fed maternal high fat before and during pregnancy [51].

It is suggested that the intestinal microbiota is a major mediator in the development of dyslipidemia, therefore the use of probiotics is noted as an important resource for the prevention or treatment of this condition [132].

5.1. Probiotic intervention: a new therapeutic approach for the dyslipidemic pregnant women?

In a randomized, single-blinded, controlled clinical trial, the supplementation with 200 g/day of a probiotic yogurt containing *Streptococcus thermophilus*, *L. bulgaricus*, *L. acidophilus* LA-5 and *B. animalis* BB12 (1×10^7 CFU, for 9 weeks) among 70 pregnant women in the third trimester of gestation, resulted in a significant reduction in total cholesterol (TC), LDL-c, and HDL-c levels as well as serum TG concentrations [133].

In another study, 256 pregnant women allocated into three groups, including dietary counseling with probiotics (capsule containing *L. rhamnosus* GG and *B. lactis*), placebo dietary counseling and without counseling (control group) from the first trimester of pregnancy to 12 months postpartum, exhibited similar lipid serum levels during pregnancy. However, maternal dietary counseling group demonstrated to be more efficient in reducing postpartum TC and LDL-c serum levels in comparison to pregnant control [134].

Small-scale, double-blind and placebo-controlled studies have observed beneficial effects of probiotic supplementation in dyslipidemia [132]. Therefore, it is important to be exploited in the near future, the probiotic intervention as a coadjuvant therapeutic for maternal dyslipidemias.

6. Disturbances in gut microbiota in early life could be considered a risk factor for the onset of cardiometabolic disorders in the later life of offspring?

This is an intriguing question and one that certainly deserves a lot of attention for future research. The gut microbiota is a dynamic metabolic organ associated with host health and diseases phenotypes. Pregnancy and lactation represent a time period where the microbiota composition may be modified in mother and newborn [135,136]. The initial establishment of the microbiome in infants may be structured by a combination of host genetics and environmental influences, such as mode of birth, colonization of placenta, type of infant feeding and maternal diet. There are still few studies examining the impact of cardiometabolic disorders experienced *in utero* or during lactation on the microbiota, immune function and cardiometabolic parameters in offspring.

Using an animal model, was demonstrated that substantial disturbances in gut microbiota in early life by antibiotic therapy has been associated with increased adiposity, glucose-dependent insulinotropic polypeptide (GIP) serum levels and upregulation in pathways linked to lipogenesis and triglyceride synthesis [137].

A study showed that offspring from obese dams display impaired gut microbiota diversity in early stages of life and persistent gut microbiota dysbiosis and metabolic disorder into young adulthood [138]. Recently, our research group demonstrated that high fat and cholesterol consumption during pregnancy and lactation markedly caused a decline in fecal counts of *Lactobacillus* spp. and *Bifidobacterium* spp. and increase in *Bacteroides* spp. counts in dams. Interestingly, this phenotypic trait of

maternal fecal microbiota also was observed in the neonate, which progressively displayed malfunctions in lipid metabolism and cardiovascular control in the later life of offspring [14,17,131]. These pre-clinical discoveries suggest that the development of cardiometabolic disorders in the later life of offspring exposed to nutritional insults may be, at least in part, affected by the colonization of offspring gut microbiota in early life. In this way, strategies that alleviate the adverse changes provoked by maternal cardiometabolic disorders on the gut microbiota of offspring should be recommended at the beginning of life as potential therapeutic capable of mitigates the pathological disturbances during pregnancy and later life of offspring. Two recent studies demonstrated that maternal administration of probiotic or prebiotic in dams fed on high fat or high fructose consumption prevented adult rat offspring against the development of gut microbiota dysbiosis, metabolic disorder and arterial hypertension [138,139].

7. Conclusion and perspectives

We pointed out that the first choice for pregnant women with cardiometabolic disorders should be performed through lifestyle modifications, including regular physical activity, maintenance of normal pre-gestational bodyweight, appropriate weight gain during pregnancy and adoption of a diet rich in fruit and vegetables and low in saturated, trans and total fat associated with pharmacological intervention, when appropriate. Accumulating evidence from experimental and clinical studies has demonstrated that altered composition of the microbiota is observed in pregnant women with cardiometabolic disorders. Probiotics have received increased attention in the last years as a promise simple and safe intervention to reduce the prevalence of cardiometabolic disorders during pregnancy (Fig. 1).

Despite the relevant findings presented in this new field of research, we understand that some critical limitations, such as i) the absence of data on the microbiota composition of pregnant women at baseline and after probiotic intervention to evaluate the impact on intestinal microbial communities; ii) the lack of consensus about the appropriate doses of probiotics to be administered safely; and iii) discrepancies on intervention time (what would be the better intervention time? Pre-pregnancy? Only pregnancy? Pregnancy and lactation?), should be the focus of further studies.

Among several functions, commensal intestinal bacteria play an essential role in the development of intestinal functions and structures, especially the gut-associated immune system. Experimental findings revealed that gut microbiota may be transmitted from mother to offspring for four generations [140]. Based on this observation, we postulated that predicting the gut microbiota composition of offspring exposed to cardiometabolic disorders during pregnancy and/or lactation could be used to target intervention strategies more effectively for the prevention of undesirable outcomes in later life. In this perspective, we have proposed that a focused intergenerational microbiome investigation (Fig. 2) conducted in rodent species could be useful to understand how cardiometabolic disorders experienced *in utero* impact gut microbiota composition and cardiometabolic parameters in offspring over several generations, and also to demonstrate whether targeting microbiota with probiotic intervention during the periconceptional period could be a safe therapeutic strategy to promote beneficial effects to mother and their offspring at long-term. A recent study has shown that genes related to weight gain or obesity may present changes in their methylation status after probiotic supplementation during pregnancy [141]. Thus, it could be suggested that maternal probiotic intervention may be, in the near future, a coadjuvant innovative strategy for management of cardiometabolic disorders in the pregnant woman.

Conflict of interest

The study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of

Microbiota dysfunction in maternal cardiometabolic disorders: an opening for probiotic therapy

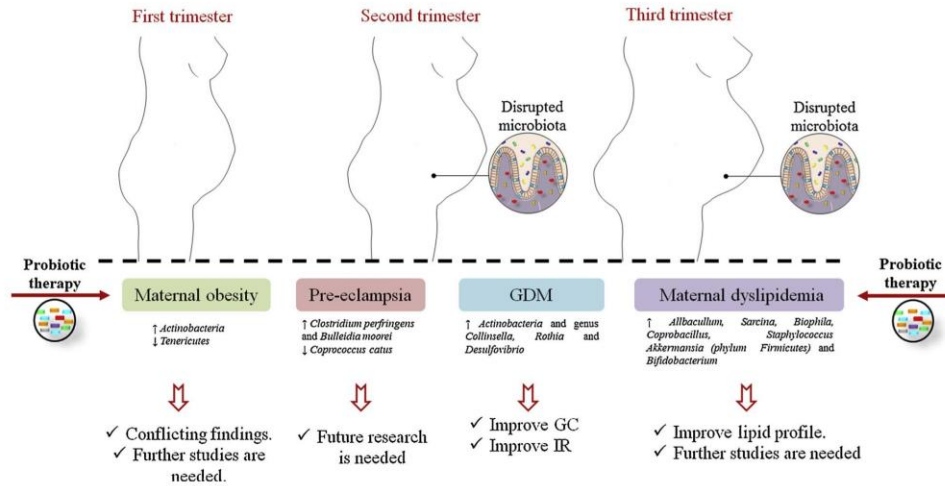


Fig. 1. Schematic drawing showing that maternal cardiometabolic disorders, such as gestational diabetes mellitus (GDM), pre-eclampsia, obesity and dyslipidemias, are associated with disrupted microbiota and that probiotic intervention could improve glycemic control (GC), insulin resistance (IR) and lipid profiles in pregnant women.

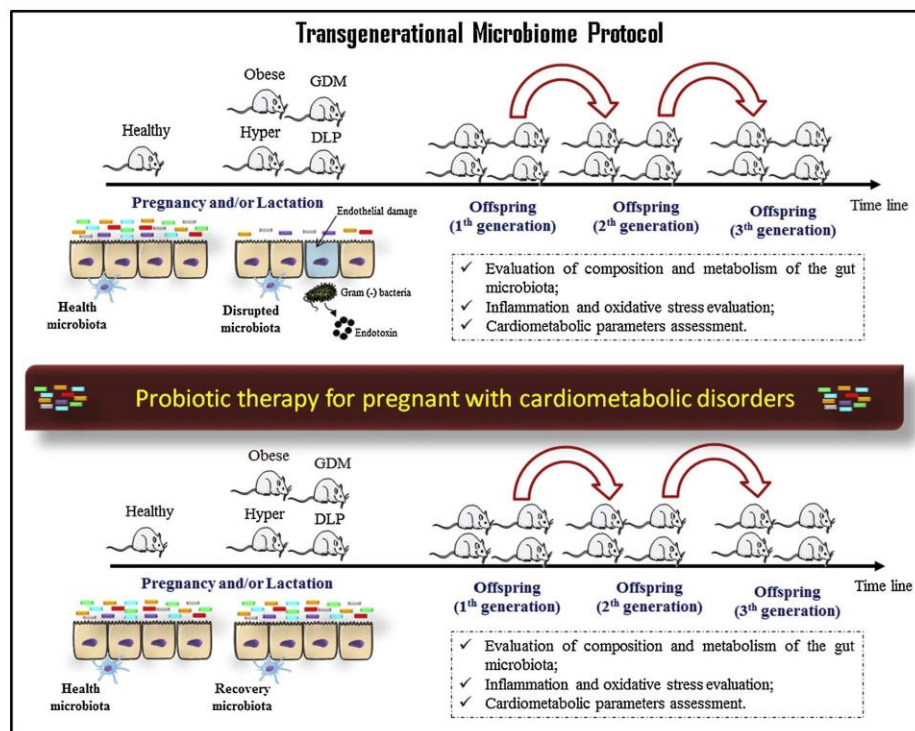


Fig. 2. Schematic drawing proposing "The Transgenerational Microbiome Project" as a future direction for investigations: i) the transgenerational impact of maternal cardiometabolic disorders on the microbiota, immune function and cardiometabolic parameters in offspring; and ii) evaluate if probiotic therapy in the course of cardiometabolic disorders in pregnancy could exert beneficial effects on microbiota, immune function and cardiometabolic parameters in offspring. Hypertension (Hyper); Gestational diabetes mellitus (GDM); Dyslipidemia (DLP).

interest.

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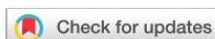
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APÊNDICE B – ARTIGO II

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Oral administration of *Lactobacillus fermentum* post-weaning improves the lipid profile and autonomic dysfunction in rat offspring exposed to maternal dyslipidemia

Yohanna de Oliveira,^a Raissa Georgianna Silva Cavalcante,^a Marinaldo Pacifico Cavalcanti Neto,^b Marciane Magnani,^c Valdir de Andrade Braga,^{d,e} Evandro Leite de Souza^a and José Luiz de Brito Alves^{d,*a}

Background: Maternal dyslipidemia alters the gut microbiota composition and contributes to the development of arterial hypertension (AH) in offspring. Here, we evaluated the effects of a new *Lactobacillus fermentum* probiotic formulation given post-weaning on cardiometabolic parameters and gut microbiota in male and female rat offspring from dams exposed to maternal dyslipidemia during pregnancy and lactation. **Methods:** Wistar rats ($n = 14$) were fed with a control diet (CTL = 7) or a dyslipidemic diet (DLP = 7) during pregnancy and lactation. After weaning, male and female offspring received a standard diet up to 90 days of life. Rats were allocated into three groups: CTL group + saline solution ($n = 14$); DLP group + saline solution ($n = 14$) and DLP group receiving a probiotic cocktail ($n = 14$). A vehicle or probiotic formulation containing *L. fermentum* 139, *L. fermentum* 263 and *L. fermentum* 296 (ratio 1:1:1, 1×10^9 CFU mL⁻¹) was administered daily by oral gavage for 8 weeks. **Results:** The intervention with the probiotic formulation of *L. fermentum* in male and female offspring reduced total cholesterol (TC) and increased HDL-c, but did not affect the insulin resistance induced by maternal dyslipidemia. Additionally, the male and female rats that received the probiotic formulation of *L. fermentum* demonstrated improvement in fecal *Lactobacillus* sp. counts, blood pressure and sympathetic tone, without affecting baroreflex modulation. **Conclusion:** The probiotic formulation containing *L. fermentum* improved the lipid profile and autonomic dysfunction in male and female offspring exposed to maternal dyslipidemia.

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1. Introduction

Maternal high fat diet consumption during the critical stages of development, such as pregnancy, lactation and first infancy, is a risk factor for the development of arterial hypertension and metabolic disorders in adult offspring.^{1,2} The programmed hypertension observed in adult offspring fed on a high fat diet during pregnancy and/or lactation has been

linked with sympathetic hyperactivity, impaired baroreflex and augmented peripheral chemoreceptor sensitivity.^{3–5}

More recently, it was demonstrated that high-fat diet intake during pregnancy and/or lactation shifts the composition of maternal–fetal gut microbiota, impairs the integrity of the gut barrier and leads to developmental programming of hypertension.^{6–8} In part, gut dysbiosis may increase sympathetic activity⁹ and plays a key role in the development of arterial hypertension,^{10,11} suggesting that gut microbiota-targeted interventions could be a reprogramming strategy to protect against programmed hypertension and metabolic disorders.

The therapeutic modulation of gut microbiota through the administration of probiotics would be a safe strategy to prevent the development of arterial hypertension and metabolic disorders in offspring from dams fed on a high fat diet. Probiotics have been defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”.¹² The most commonly used probiotics belong to the genera *Lactobacillus*, with various strains demon-

^aDepartment of Nutrition, Health Sciences Center, Federal University of Paraíba, João Pessoa, Brazil. E-mail: jose_luiz_61@hotmail.com,

jose.luiz@academico.ufpb.br; Fax: +55 81 9 98455485; Tel: +55 81 9 98455485

^bLaboratory of Cell Signaling and Metabolic Modulation, Institute of Health Biotechnology, Federal University of Amazonas, Coari, Brazil

^cDepartment of Food Engineering, Technology Center, Federal University of Paraíba, João Pessoa, Brazil

^dDepartment of Biotechnology, Biotechnology Center, Federal University of Paraíba, João Pessoa, Brazil

^eDepartment of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden

strating properties associated with beneficial effects on host health *in vitro* and *in vivo*.¹³

In this way, *Lactobacillus* strains isolated from fruit processing by-products were identified using 16S rRNA gene sequence analysis¹⁴ and characterized for a set of probiotic *in vitro* properties.¹⁵ Among the strains previously isolated, *L. fermentum* 139, *L. fermentum* 263, and *L. fermentum* 296 displayed optimal performance regarding adhesion, aggregation, co-aggregation, antagonism, survival upon exposure to simulated gastrointestinal conditions and absence of hemolytic and mucinolytic activities.¹⁵ However, the cardiometabolic relevance of these strains remains to be elucidated.

There have been, to date, few studies examining the effects of probiotics on cardiac dysautonomia, but early discoveries have found that probiotic therapy may attenuate sympathetic hyperactivity and reduce blood pressure in hypertensive rats.^{16,17} In this perspective, the assessment of the cardiometabolic relevance of a new probiotic cocktail on autonomic function, blood pressure and glucolipid variables in offspring exposed to maternal dyslipidemia may represent a promising and innovative strategy to protect offspring against programmed hypertension and metabolic disorders.

Here we evaluated the effects of post-weaning administration of a new probiotic formulation containing *L. fermentum* 139, *L. fermentum* 263 and *L. fermentum* 296 on blood pressure, sympathetic tone, baroreflex, peripheral chemoreflex and glucolipid parameters in male and female rat offspring exposed to maternal dyslipidemia during pregnancy and lactation.

2. Methods

2.1. Probiotic strains

The *L. fermentum* 139, *L. fermentum* 263 and *L. fermentum* 296 strains were kindly provided by the Laboratory of Food Microbiology, Department of Nutrition, Federal University of Paraíba (João Pessoa, Brazil). These strains were previously identified using 16S rRNA analysis.¹⁴ Stocks were stored at -20°C in Mann, Rogosa and Sharpe (MRS) broth (HiMedia, Mumbai, India) containing glycerol (Sigma-Aldrich, St Louis, USA; 20 mL per 100 mL).

2.2. Preparation of the probiotic cell suspension

The probiotic cell suspension was obtained from overnight cultures grown on MRS broth (HiMedia, Mumbai, India) and anaerobically incubated (Anaerobic System Anaerogen, Oxoid Ltd, Wade Road, UK) at 37°C . Cells were harvested by centrifugation (8000g, 10 min, 4°C), washed twice with sterile saline solution and re-suspended and homogenized using a vortexer (30 s) in sterile saline solution to obtain standard cell suspensions with an optical density (OD) reading at 660 nm (OD_{660}) of 1.0, which provided viable counts of approximately $9 \log \text{CFU mL}^{-1}$. The mixed cell suspension was obtained by mixing the prepared suspension of each probiotic strain in a ratio of 1:1:1.

2.3. Ethical aspects and animals

All rats used in the experiment received water and diet *ad libitum* and were maintained in polypropylene cages under controlled temperature ($21 \pm 1^{\circ}\text{C}$), with humidity between 50–55% and a 12 h light–dark cycle. 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 # 9492260418) and followed the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) and the International Principles for Biomedical Research Involving Animals.

2.4. Experimental design

Primiparous Wistar rats (*Rattus norvegicus*) weighing $250 \pm 10 \text{ g}$ ($n = 14$) were mated with 1:1 fertile male rats. The presence of sperm in the vaginal smear was used to define the first day of pregnancy. Subsequently, the pregnant dams were placed in single cages and subjected to dietary manipulations during the pregnancy and lactation periods: control diet (CTL group, $n = 7$) and dyslipidemic diet (DLP group, $n = 7$).

The control diet was prepared according to the American Institute of Nutrition – AIN-93G¹⁸ and the dyslipidemic diet was purchased from Rhoster® Company (Araçoiaba da Serra, São Paulo, Brazil). The diets were offered during the pregnancy and lactation periods. Soybean oil was the fat source of the control diet (AIN-93G; 15.92% fat), while the dyslipidemic diet having high-fat and high-cholesterol concentrations contained 31.11% fat distributed in soybean oil, cholesterol, lard and non-hydrolysed vegetable fat as lipid sources (Table 1).

Table 1 Composition of the control and dyslipidemic diets offered to dams during pregnancy and lactation

Ingredients (g per 100 g)	Diets	
	Control (AIN-93G) ^a	Dyslipidemic ^b
Corn starch	39.75	33.09
Dextrinized corn starch	13.20	15.50
Casein ^d	20.00	19.86
Sucrose	10.00	6.00
Soybean oil	7.00	3.00
Animal fat (lard)	—	6.00
Non-hydrolyzed vegetable fat	—	5.00
Sigma cholesterol	—	1.00
Sigma cholic acid	—	0.50
Cellulose	5.00	5.00
Mineral mix 93G	3.50	3.50
Vitamin mix	1.00	1.00
L-Cystine	0.30	0.30
Choline bitartrate	0.25	0.25
<i>t</i> -BHQ ^c	0.014	0.014
Calories (kcal g ⁻¹)	3.96	4.34
Carbohydrates (kcal%)	63.62	50.32
Proteins (kcal%)	20.47	18.58
Lipids (kcal%)	15.92	31.11

^a Adapted from Reeves, Nielsen and Fahey (1993).¹⁸ ^b Rhoster – Industry and Trade Ltd. ^c *t*-BHQ: *tert*-butylhydroquinone. ^d Casein showed 85% purity (85 g protein for each 100 g casein).

At 24 h after birth, rat offspring were adjusted to eight pups (4 males and 4 females) per litter to ensure standardized nutrition up to weaning. At weaning (postnatal day 21), the female (CTL, $n = 7$ and DLP, $n = 14$) and male (CTL, $n = 7$ and DLP, $n = 14$) rats were housed separately (4 per cage) and had free access to a commercial diet (Presence Purina®, Paulínea, Brazil) and water *ad libitum* up to 90 days of age, while the dams were euthanized and serum samples were collected for quantification of total cholesterol (TC), LDL-cholesterol, HDL-cholesterol and triglycerides with commercial kits (Bioclin, Belo Horizonte, Minas Gerais, Brazil).

The CTL and DLP experimental groups were randomly assigned into three groups: (i) control group (CTL, $n = 14$; 7 females and 7 males); (ii) dyslipidemic group (DLP, $n = 14$; 7 females and 7 males) and (iii) dyslipidemic group that received the probiotic formulation of *L. fermentum* (DLP + Lf, $n = 14$; 7 females and 7 males). In the CTL and DLP groups, saline solution was administered as a placebo for 8 weeks. In the DLP + Lf group, the probiotic suspension containing *L. fermentum* 139, *L. fermentum* 263 and *L. fermentum* 296 in a solution of approximately 1×10^9 CFU mL⁻¹ was administered daily for 8 weeks. The administration of the vehicle or probiotic suspension containing *L. fermentum* was performed by oral gavage after weaning (30 days of age). The body weight was measured weekly during all experiments using an appropriate scale (model AS-1000; Marte, Santa Rita, MG, Brazil). After 8 weeks of treatment with saline solution or probiotic suspension containing *L. fermentum*, biochemical measurements, glucose and insulin tolerance tests, microbial counts in feces, baseline blood pressure (BP) and heart rate (HR) recording, spectral analysis of systolic arterial pressure and cardiac interval, and evaluation of sympathetic-vagal balance and sympathetic vascular tone were performed in each group at 90 days of age.

2.5. Glucose and insulin tolerance test

The oral glucose tolerance (OGTT) and insulin tolerance (ITT) tests were performed in rats fasted overnight. In the OGTT, an oral load was administered *via* gavage of glucose (2 g kg⁻¹). Blood samples were taken from the tail vein before glucose administration and, subsequently, at 15, 30, 60, 90 and 120 min. OGTT and ITT were performed after 24 h, following an intraperitoneal insulin injection (0.75 UI per kg body weight); blood glucose concentrations were measured before (0 min) and after (30, 60, 90 and 120 min) the peritoneal insulin injection. Measurements of blood glucose concentration were performed with an Accu-Check glucometer (Bayer®).

2.6. Serum measurement assays

Serum measurements were performed 24 h after the glucose homeostasis experiments. Both female and male rats were fasted overnight and blood samples were collected by orbital venous plexus disruption.³ The samples were centrifuged at 300g, 25 °C, for 15 min and serum measurements for TC, HDL-c, VLDL-c and TG concentrations were performed using appropriate enzymatic colorimetric kits according to the man-

ufacturer's instructions (Bioclin, Belo Horizonte, Minas Gerais, Brazil).

Malondialdehyde (MDA) concentration was measured as an indicator of oxidative stress. Briefly, 400 µL of perchloric acid (7%) was added to 250 µL of serum, and mixed and centrifuged (600g, 4 °C, 20 min). The supernatant was collected, added to 400 µL of thiobarbituric acid (0.6%), heated at 100 °C for 1 h and read at 532 nm. In this assay, MDA reacts with thiobarbituric acid to produce a red-colored complex.³

2.7. Surgical procedure and blood pressure recording

At 90 days of age, both female and male rats ($n = 7$ per group) were anesthetized with ketamine (80 mg kg⁻¹, i.p.) and xylazine (10 mg kg⁻¹, i.p.) for insertion of polyethylene catheters into the femoral artery and vein. The catheters were tunneled through the back of the neck and ketoprofen (5 mg kg⁻¹) was injected subcutaneously. The rats underwent a period of surgical recovery for 24 h. The arterial pressure (AP) and HR were recorded in conscious animals connecting the arterial catheter to a pressure transducer (LabChart™ Pro, ADInstruments, Bella Vista, NSW, Australia).

2.8. Measurements and analysis of arterial blood pressure

The pulsatile arterial pressure (PAP) and HR were recorded for 50–60 min under baseline conditions. The values for systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP) and HR were calculated offline by selection of 10 min intervals for each animal (LabChart™ Pro, ADInstruments, Bella Vista, NSW, Australia).

2.9. Spectral analysis

Using the same 10 min-segment of baseline AP and HR recordings, the spectral analysis in the frequency domain of SAP and pulse interval (PI) was performed using appropriate software (CardioSeries-v.2.4; <http://www.danielpenteado.com>). The spectra were integrated into the low frequency (LF) (0.2–0.75 Hz) and high frequency (HF) bands (0.75–3 Hz). To assess the sympathovagal index, the LF/HF ratio of the cardiac interval was calculated. Additionally, the spontaneous baroreflex sensitivity (SBRS) was calculated through the sequence method.³

2.10. Evaluation of the peripheral chemoreflex

The peripheral chemoreflex was activated through the intravenous injection of potassium cyanide (KCN; 40 µg per 0.1 mL per rat; Merck) in accordance with previous reports.¹⁹ The difference between the baseline MAP and HR and the peak response after the stimulus (Δ MAP; Δ HR) was assessed for sympathetic and parasympathetic components, respectively.

2.11. Evaluation of the sympathetic tone on the vascular system

The contribution of the sympathetic vascular tone on the cardiovascular system was evaluated by an intravenous injection of a ganglionic blocker (hexamethonium; 30 mg kg⁻¹, Sigma-Aldrich®, St Louis, MO, USA). The sympathetic tone was calculated by the changes in the MAP (Δ MAP) following

administration, as previously described.²⁰ At the end of the experiments, the animals were euthanized with 0.5 mL overdose of ketamine (i.v.).

2.12. Microbial counts in feces

Fecal samples were homogenized in peptone water (1 mg peptone per mL) and serially diluted in the same diluent. Twenty μL aliquots of the respective dilutions were inoculated using the microdrop technique²¹ on de Man, Rogosa and Sharpe (MRS) agar (HiMedia, India) to count *Lactobacillus* spp.; MRS + cysteine (0.05 g per 100 mL, Sigma-Aldrich, Milan, Italy) (cMRS) to count *Bifidobacterium* spp.; MacConkey agar (HiMedia, India) to count Enterobacteriaceae and Bacteroides Bile Esculina (BBE) agar (Acumedia, USA) to count *Bacteroides* spp. Incubation was performed under anaerobic conditions (Anaerobic System Anaerogen, Oxoid Ltd, Basingstoke, Hampshire, UK) for counting *Bifidobacterium* spp., *Lactobacillus* spp. and *Bacteroides* spp. and under aerobic conditions for counting Enterobacteriaceae. After an incubation period of 24–48 h, the number of visible colonies on each selective medium was counted and the results were expressed as log CFU g^{-1} . The detection limit of the test was 2 log CFU g^{-1} .

2.13. Statistical analysis

The results were expressed as mean $\pm$ standard errors. The Kolmogorov–Smirnov test was used to assess the normality of data in cardiovascular and biochemical parameters. Most of the variables required the one-way ANOVA parametric test and Tukey's post-test. Two-way ANOVA and Bonferroni's *post hoc* tests were used for the analysis of curves of the glucose and insulin tolerance test and body weight measurements during pregnancy and lactation. Statistical analysis was performed using the computational software Prism 5 (GraphPad Software, San Diego, CA). The difference was considered significant when $p < 0.05$.

3. Results

Body weight was measured weekly during pregnancy and lactation. The body weights were similar between the CTL and DLP dams, except in week 1 postpartum, where the DLP dams displayed reduced body weight compared to the CTL dams (Table 2). The dyslipidemic diet consumption during pregnancy and lactation was effective for the development of maternal dyslipidemia (Table 2).

3.1 Effects of *L. fermentum* supplementation on body weight and biochemical measurements

The DLP male offspring presented increased body weight and triglyceride levels when compared to the CTL group ($p < 0.05$, Table 3). Interestingly, these differences were not observed in the DLP female offspring. The DLP male offspring that received the probiotic formulation of *L. fermentum* exhibited reduced body weight when compared with the DLP group ($p <$

Table 2 Body weight and biochemical variables in dams that received a control (CTL) or dyslipidemic (DLP) diet during pregnancy and lactation

Variables	CTL	DLP
Body weight (g)		
Pre-gestation	206 $\pm$ 1.8	206 $\pm$ 5.3
Week 1 of gestation	236 $\pm$ 3.7	224 $\pm$ 7.2
Week 2 of gestation	258 $\pm$ 6.2	249 $\pm$ 5.9
Week 3 of gestation	300 $\pm$ 7.2	285 $\pm$ 8.0*
Week 1 postpartum	262 $\pm$ 6.0	234 $\pm$ 7.7
Week 2 postpartum	271 $\pm$ 8.3	249 $\pm$ 5.7
Week 3 postpartum	270 $\pm$ 7.1	255 $\pm$ 4.5
Biochemical variables		
Triglycerides (nmol L^{-1})	1.3 $\pm$ 0.2	2.3 $\pm$ 0.6
Total cholesterol (nmol L^{-1})	2.2 $\pm$ 0.1	7.5 $\pm$ 1.2*
LDL-cholesterol (nmol L^{-1})	0.6 $\pm$ 0.1	6.3 $\pm$ 1.7*
HDL-cholesterol (nmol L^{-1})	1.7 $\pm$ 0.2	1.1 $\pm$ 0.1*

0.05, Table 3). On the other hand, the probiotic intervention did not cause significant changes in the body weight in the DLP female offspring (Table 3).

Administration of the probiotic formulation of *L. fermentum* to the DLP male and female offspring for 8 weeks significantly reduced the serum levels of TC ($p < 0.05$, Table 3); however, only the DLP male offspring presented reduced triglyceride levels when compared to the DLP group ($p < 0.05$, Table 3). Additionally, male and female offspring that received the probiotic formulation of *L. fermentum* displayed increased levels of serum HDL ($p < 0.05$, Table 3) when compared to the CTL and DLP groups.

Serum MDA levels were increased in the DLP male and female offspring when compared to the CTL groups ($p < 0.05$, Table 3). The probiotic intervention in the DLP groups caused a reduction in serum levels of MDA in male and female offspring ($p < 0.05$, Table 3).

3.2 Effects of *L. fermentum* supplementation on glucose and insulin tolerance tests

In male offspring, the areas under the curve (AUC) during OGTT and ITT was similar among groups (Table 3). In female offspring, the AUC after OGTT and ITT was increased in the DLP group compared to the CTL group (Table 3). Administration of *L. fermentum* for 8 weeks did not improve glucose tolerance and insulin resistance in the DLP offspring (Table 3).

3.3 Effects of *L. fermentum* supplementation on cardiovascular parameters

The SAP, DAP and MAP were increased in the DLP male ($p < 0.05$, Fig. 1A, B and C) and female ($p < 0.05$, Fig. 1E, F and G) offspring when compared to the respective CTL groups. Administration of the probiotic formulation of *L. fermentum* for 8 weeks reduced the SAP and MAP in male offspring when compared to the DLP group ($p < 0.05$, Fig. 1A and C), although this did not reduce these parameters in female offspring when compared to the DLP group (Fig. 1E and G). The HR were similar among the three groups of both sexes (Fig. 1D and H).

Table 3 Body weight, biochemical parameters and microbial counts in feces

Parameters	Males			Females		
	CTL	DLP	DLP + Lf	CTL	DLP	DLP + Lf
Body weight (g)	336 ± 4.9	360 ± 4.7*	320 ± 5.2 [#]	218 ± 4.8	220 ± 7.7	210 ± 3.2
Tryglycerides (mg dL ⁻¹)	92 ± 11.9	174 ± 35.2*	85 ± 7.9 [#]	53 ± 14.8	78 ± 8.2	55 ± 5.2
Cholesterol (mg dL ⁻¹)	56 ± 3.7	62 ± 3.4	43 ± 2.9 [#]	70 ± 3.2	79 ± 7.1	57 ± 5.3 [#]
VLDL (mg dL ⁻¹)	18.4 ± 2.3	31.7 ± 6.7	18.8 ± 1.1	14 ± 4.6	16 ± 1.6	12 ± 1.1
HDL (mg dL ⁻¹)	23 ± 1.9	24 ± 0.7	33 ± 2.3 [#]	28 ± 1.3	30.1 ± 2.4	48 ± 3.4 [#]
TBARS (nmol mL ⁻¹)	6.7 ± 0.5	11.1 ± 1.5*	5.1 ± 1.0 [#]	6.0 ± 0.3	10.8 ± 1.3*	4.0 ± 1.3 [#]
OGTT (AUC)	10 553 ± 444.8	10 044 ± 352.3	9473 ± 244.1	9888 ± 335.4	11 198 ± 408.9*	10 093 ± 132.1
ITT (AUC)	2650 ± 262.2	2740 ± 275.6	3206 ± 51.13	2202 ± 169.3	2794 ± 164.6*	3097 ± 140.6 [#]
Microbiological analyses: viable count (log cfu g⁻¹ fecal content)						
<i>Lactobacillus</i> spp.	7.76 ± 0.13	6.86 ± 0.12*	7.76 ± 0.01 [#]	7.08 ± 0.35	6.80 ± 0.28	7.48 ± 0.13
<i>Bifidobacterium</i> spp.	7.35 ± 0.19	6.78 ± 0.36	7.66 ± 0.08	6.37 ± 0.27	6.32 ± 0.37	7.38 ± 0.02
<i>Bacterioides</i> spp.	7.14 ± 0.15	7.17 ± 0.24	7.09 ± 0.14	6.51 ± 0.10	6.50 ± 0.12	6.45 ± 0.43
Enterobacteriaceae spp.	4.95 ± 0.19	5.77 ± 0.39	>2 [#]	5.31 ± 0.18	5.8 ± 0.30	>2 [#]

Body weight and biochemical parameters in male and female rat offspring at 90 days of age from dams exposed to maternal dyslipidemia during pregnancy and lactation. Groups: control group (CTL, $n = 14$; 7 females and 7 males), dyslipidemic group (DLP, $n = 14$; 7 females and 7 males) and the DLP group that received the probiotic formulation of *L. fermentum* (DLP + Lf, $n = 14$; 7 females and 7 males). Data are shown as mean ± SEM, analyzed by one-way ANOVA, and followed by Tukey's post-test. * $p < 0.05$ indicates significant difference in mean values of the variable between the DLP and CTL groups. [#] $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and DLP groups. ^ψ $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and CTL groups.

The LF oscillations of the SAP were increased in the DLP male and female offspring when compared to the CTL groups ($p < 0.05$, Fig. 2A and E); however, the LF/HF ratio of the cardiac interval was increased only in the DLP female offspring when compared to the CTL group ($p < 0.05$, Fig. 2G). HF oscillations of the SAP were similar among the three groups of both sexes (Fig. 2B and F). The intervention with the probiotic formulation of *L. fermentum* significantly reduced the LF oscillations in the DLP groups ($p < 0.05$, Fig. 2A and E).

Additionally, the SBRS was lower in the DLP male and female offspring when compared to the respective CTL groups ($p < 0.05$, Fig. 2D and H). Administration of the probiotic formulation of *L. fermentum* did not improve the SBRS in the DLP group ($p < 0.05$, Fig. 2D and H).

3.4 Effects of *L. fermentum* supplementation on the peripheral chemoreflex

Peripheral chemoreflex activation produced pressor and bradycardic response in male and female offspring (Fig. 3). The pressor response was significantly greater in the DLP male and female offspring than in the CTL group ($p < 0.05$, Fig. 3A and C). Administration of the probiotic formulation of *L. fermentum* for 8 weeks reduced the pressor response in male and female offspring when compared to the DLP group ($p < 0.05$, Fig. 3A and C). The magnitudes of the decrease in the HR were similar among the groups of both sexes (Fig. 3B and D).

3.5 Effects of *L. fermentum* supplementation on the sympathetic tone

To test whether the sympathetic tone may be modulated by the probiotic formulation of *L. fermentum*, we evaluated the

changes in the MAP (Δ MAP) after the administration of a pharmacological ganglionic blocker in all groups. After ganglionic blockade, the non-treated DLP male group presented a larger fall in the MAP compared to the CTL group ($p < 0.05$, Fig. 4A). This result suggests that the DLP male offspring from dams exposed to maternal dyslipidemia displayed higher sympathetic tone. Administration of the probiotic formulation of *L. fermentum* reduced significantly the pressor response in the DLP male offspring ($p < 0.05$, Fig. 4A). Similar results were observed for the DLP female offspring that received the probiotic intervention in relation to the non-treated DLP group ($p < 0.05$, Fig. 4B). Taken together, these results suggest that probiotic therapy with *L. fermentum* may be effective in reducing the sympathetic tone in male and female offspring exposed to maternal dyslipidemia.

3.6 Effects of *L. fermentum* supplementation on microbial counts in feces

The DLP male offspring had lower counts of *Lactobacillus* spp. in feces when compared to the CTL group ($p < 0.05$, Table 3). The DLP male offspring that received the probiotic formulation of *L. fermentum* for 8 weeks exhibited a significant increase in fecal counts of *Lactobacillus* spp. ($p < 0.05$, Table 3); however, no differences were observed for these counts among the three female experimental groups after the intervention. Additionally, the intervention with the probiotic formulation of *L. fermentum* in the DLP male and female offspring decreased the fecal counts of Enterobacteriaceae ($p < 0.05$, Table 3) when compared to the CTL and DLP groups. No differences were observed in the fecal counts of *Bifidobacterium* spp. and *Bacterioides* spp. among male and female offspring groups after the probiotic intervention ($p > 0.05$, Table 3).

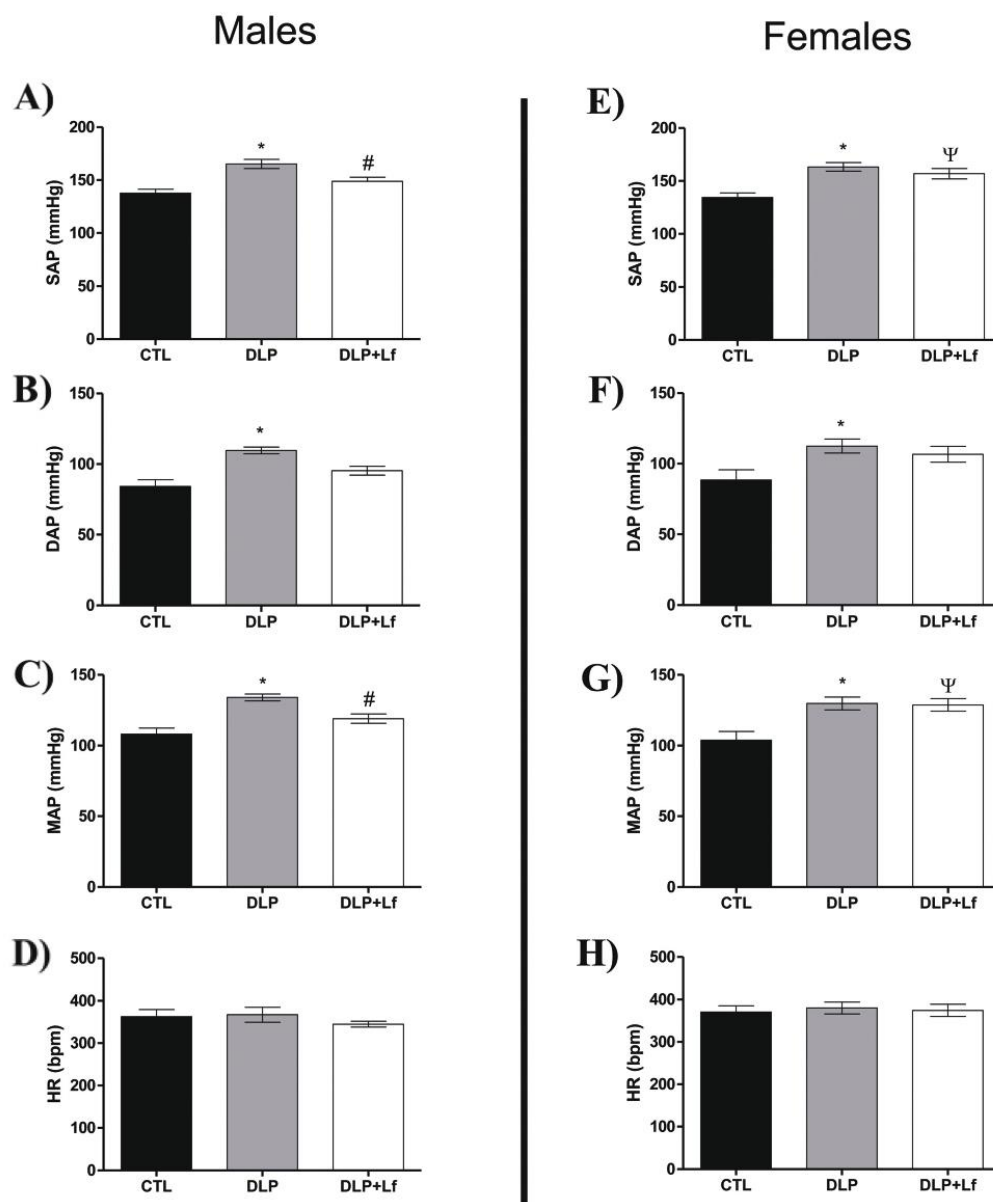


Fig. 1 Effects of the probiotic formulation of *L. fermentum* on resting blood pressure and heart rate in male and female offspring from dams exposed to maternal dyslipidemia. Evaluation of resting systolic blood pressure (SAP), diastolic blood pressure (DAP), mean arterial pressure (MAP), and heart rate (HR) in male (A, B, C, D) and female (E, F, G, H) offspring at 90 days of age that received or did not receive intervention with *L. fermentum* after both were exposed to maternal dyslipidemia during pregnancy and lactation. Groups: control group (CTL, $n = 14$; 7 females and 7 males), dyslipidemic group (DLP, $n = 14$; 7 females and 7 males) and the DLP group that received the probiotic formulation of *L. fermentum* (DLP + Lf, $n = 14$; 7 females and 7 males). Data are shown as mean $\pm$ SEM, analyzed by one-way ANOVA, and followed by Tukey's post-test. * $p < 0.05$ indicates significant difference in mean values of the variable between the DLP and CTL groups. # $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and DLP groups. Ψ $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and CTL groups.

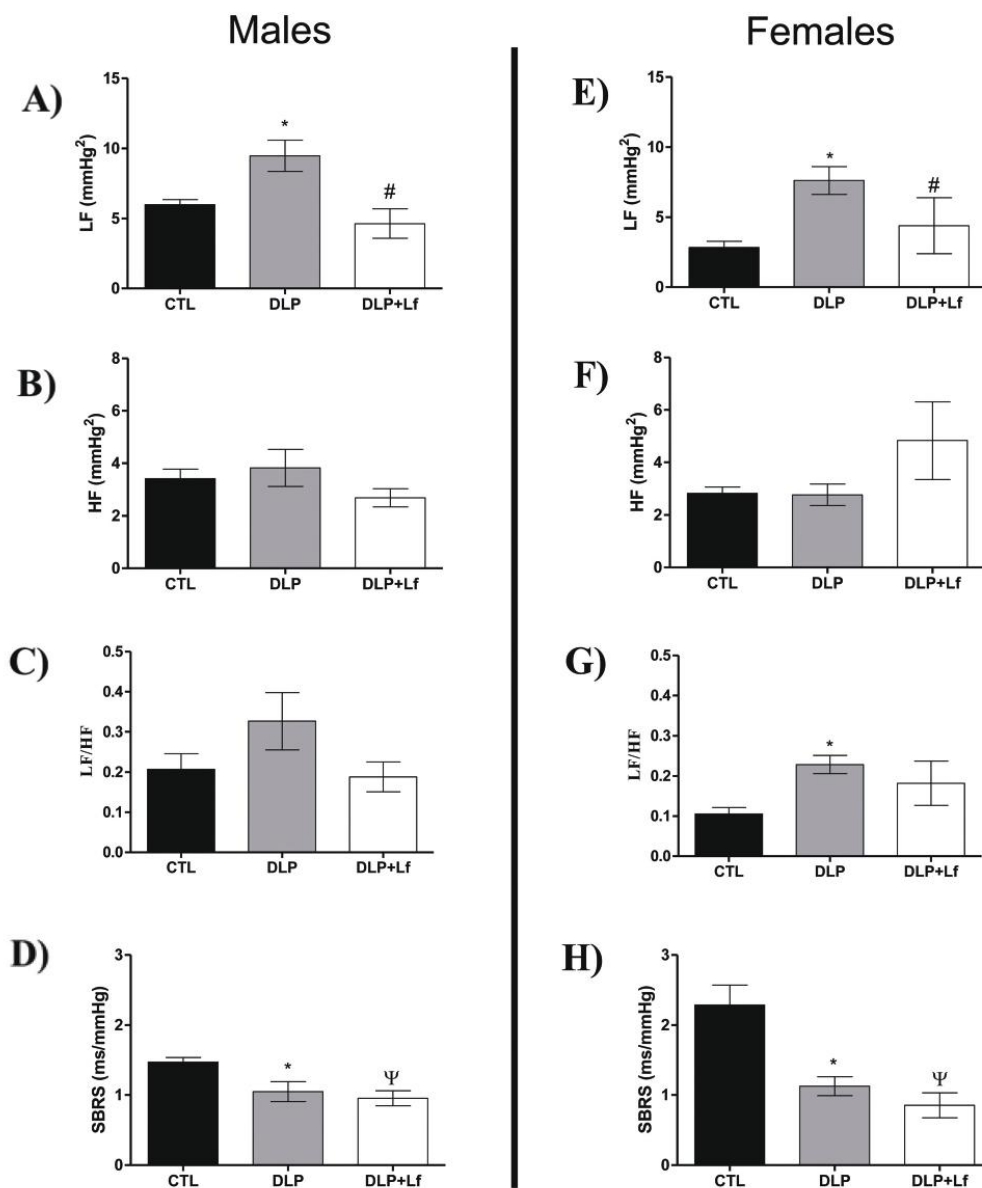


Fig. 2 Effects of the probiotic formulation of *L. fermentum* on the spectral analysis of systolic arterial pressure (SAP), cardiac interval and spontaneous baroreflex sensitivity (SBRS) in male and female offspring from dams exposed to maternal dyslipidemia. Evaluation of low frequency (LF) power, high frequency (HF) power, LF/HF ratio and spontaneous baroreflex sensitivity (SBRS) in male (A, B, C, D) and female (E, F, G, H) offspring at 90 days of age that received or did not receive intervention with *L. fermentum* after both were exposed to maternal dyslipidemia during pregnancy and lactation. Groups: control group (CTL, $n = 14$; 7 females and 7 males), dyslipidemic group (DLP, $n = 14$; 7 females and 7 males) and the DLP group that received the probiotic formulation of *L. fermentum* (DLP + Lf, $n = 14$; 7 females and 7 males). Data are shown as mean $\pm$ SEM, analyzed by one-way ANOVA, and followed by Tukey's post-test. * $p < 0.05$ indicates significant difference in mean values of the variable between the DLP and CTL groups. # $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and DLP groups. Ψ $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and CTL groups.

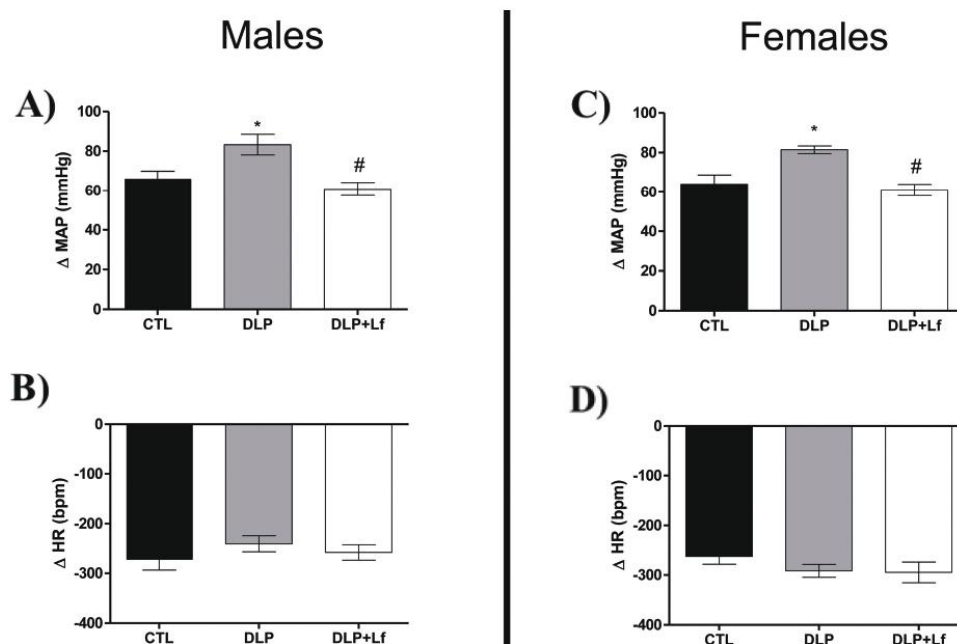


Fig. 3 Effects of the probiotic formulation of *L. fermentum* on peripheral chemoreflex evaluation in male and female offspring from dams exposed to maternal dyslipidemia. Evaluation of the variation in mean arterial pressure (MAP) and heart rate (HR) during peripheral chemoreflex activation (KCN, 0.04%) in male (A, B) and female (C, D) offspring at 90 days of age that received or did not receive intervention with *L. fermentum* after both were exposed to maternal dyslipidemia during pregnancy and lactation. Groups: control group (CTL, $n = 14$; 7 females and 7 males), dyslipidemic group (DLP, $n = 14$; 7 females and 7 males) and the DLP group that received the probiotic formulation of *L. fermentum* (DLP + Lf, $n = 14$; 7 females and 7 males). Data are shown as mean $\pm$ SEM, analyzed by one-way ANOVA, and followed by Tukey's post-test. * $p < 0.05$ indicates significant difference in mean values of the variable between the DLP and CTL groups. # $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and DLP groups. $\Psi p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and CTL groups.

4. Discussion

Similar to previous studies, the high-fat and high cholesterol diet consumption during pregnancy and lactation was effective for the development of maternal dyslipidemia.^{3,22} Our findings provide evidence for the first time that probiotic therapy with *L. fermentum* 139, *L. fermentum* 263 and *L. fermentum* 296, for 8 weeks, reduced the serum levels of TC and MDA, increased the serum levels of HDL-c, reduced LF oscillations of the SAP, attenuated the sympathetic pathway of the peripheral chemoreflex and reduced the sympathetic tone in male and female rat offspring of dams fed on a high fat-high cholesterol diet during pregnancy and lactation. Additionally, we found that probiotic therapy restored the fecal population of *Lactobacillus* spp. in the DLP male offspring and reduced the growth of Enterobacteriaceae in the DLP offspring of both sexes.

The abundance of Enterobacteriaceae has been associated with inflammation, such as colitis in mice²³ and in patients with diverticular disease.²⁴ A recent *in vivo* study showed that co-administration of *L. fermentum* GOS57 and *L. plantarum*

GOS42 at a dose of 5×10^7 to 1×10^8 CFU per day for 7 weeks significantly decreased the fecal counts of Enterobacteriaceae and improved the abundance of *Lactobacillus*.²⁵

In adult life, the DLP male group displayed higher body weight compared to the CTL offspring, while the body weights in female offspring were similar. The effects of maternal high fat diet consumption during pregnancy and/or lactation on adult body weight are conflicting. For example, there are studies finding increased body weight in both male and female offspring, increased body weight only in male offspring and similar body weights in both sexes.^{3,22,26,27} In our understanding, these findings demonstrate that maternal high fat diet may modulate a of anthropometric parameters in a sex-dependent manner. In this way, a recent study demonstrated low-grade and high-grade pathogenic obesity in females and males after maternal high fat diet consumption, respectively.²⁷ In the present study, the intervention with the probiotic formulation of *L. fermentum* was able to reduce the body weight in the DLP male offspring, but failed to reduce the body weight in female offspring. In contrast to our findings, an

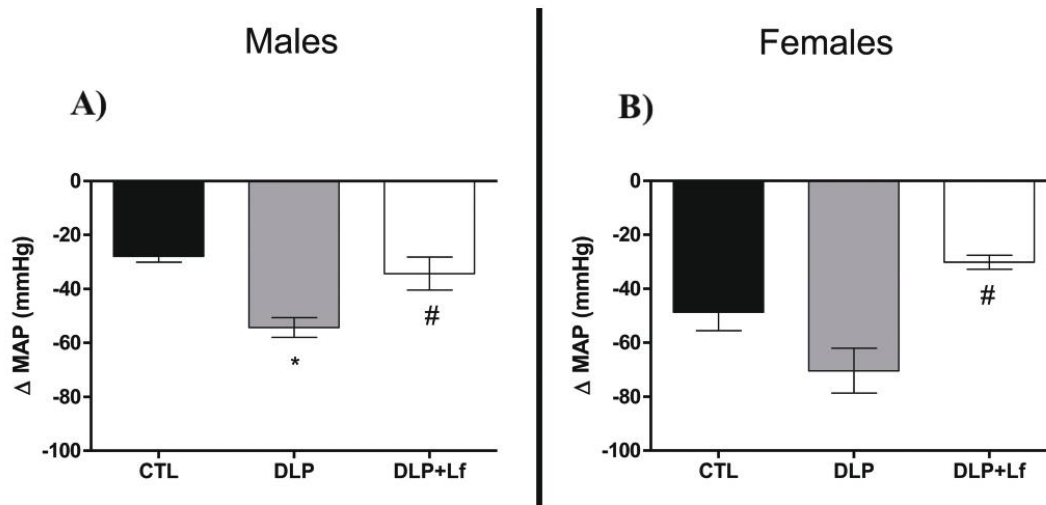


Fig. 4 Effects of the probiotic formulation of *L. fermentum* on the sympathetic tone in male and female offspring from dams exposed to maternal dyslipidemia. Assessment of delta change of the mean arterial pressure (MAP) after blockade with hexamethonium ($30 \mu\text{g kg}^{-1}$, i.v.) in male (A) and female (B) offspring at 90 days of age that received or did not receive intervention with *L. fermentum* after both were exposed to maternal dyslipidemia during pregnancy and lactation. Groups: control group (CTL, $n = 14$; 7 females and 7 males), dyslipidemic group (DLP, $n = 14$; 7 females and 7 males) and the DLP group that received the probiotic formulation of *L. fermentum* (DLP + Lf, $n = 14$; 7 females and 7 males). Data are shown as mean $\pm$ SEM, analyzed by one-way ANOVA, and followed by Tukey's post-test. * $p < 0.05$ indicates significant difference in mean values of the variable between the DLP and CTL groups. # $p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and DLP groups. $\Psi p < 0.05$ indicates significant difference in mean values of the variable between the DLP + Lf and CTL groups.

early double-blind, placebo-controlled, randomized trial revealed that supplementation with two capsules per day containing *Lactobacillus rhamnosus* CGMCC1.3724 (1.62×10^8 CFU per capsule) was more effective in reducing the body weight loss in women than in men.²⁸

The intervention with the probiotic formulation of *L. fermentum* in the DLP male and female offspring was able to decrease the serum levels of TC and increase HDL-c in both sexes, while the hypotriglyceridemic effect was observed only in the DLP male offspring. Probiotic strains of *L. fermentum* have been shown to exert hypocholesterolemic effects in animal models (e.g., *L. fermentum* FTDC 8312, *L. fermentum* MJM60397 and *L. fermentum* MTCC:5898)^{29,30} and humans (e.g., *L. fermentum* ME-3).³¹ However, it is noteworthy that the probiotic properties are strain specific and cannot be extrapolated to another strain even belonging to the same species.³²

It has been documented that the administration of specific probiotic *L. fermentum* strains, such as *L. fermentum* NCIMB 5221, *L. fermentum* MTCC5689 and *L. fermentum* MTCC:5898, should be beneficial for improving glucose metabolism and insulin resistance.^{33,34} Thus, OGTT and ITT were performed to assess whether the probiotic formulation of *L. fermentum* could exert effects on the regulation of glucose homeostasis in male and female offspring from dams exposed to maternal dyslipidemia. The intervention with the probiotic formulation of *L. fermentum* for 8 weeks was not effective in improving

glucose tolerance and in reducing insulin resistance in the DLP offspring of both sexes. In our understanding, these findings are important because not all probiotic strains have the same efficacy to improve biochemical variables and, perhaps, an international bank of well-cataloged probiotic strains with well-defined experimental and clinical studies and information about the qualification of the deposited strains to a specific use could assist the development of more effective products and successful applications for human health promotion.

In agreement with other studies that used treatment with different *L. fermentum* strains for reducing the SAP in hypertensive rats,^{17,35,36} we observed, particularly in male offspring, that the intervention with the probiotic formulation of *L. fermentum* caused a reduction in the SAP and MAP. On the other hand, the tested probiotic formulation did not reduce the SAP and MAP in female offspring, which could be probably associated with hormonal variations and unbalanced gut microbiota. While the DLP male offspring that received the probiotic intervention of *L. fermentum* showed a reduction of 7.6 mmHg in the MAP, the DLP female offspring that received the same probiotic intervention showed a reduction of 3.3 mmHg in the MAP when compared to the respective DLP groups. Taken together, our results suggest that male offspring are more responsive to the intervention with the probiotic formulation of *L. fermentum* than female offspring.

Recent evidence has proposed an association between gut microbiota and gender differences in cardiometabolic dysfunction,³⁷ suggesting that gut microbiota could be modulated by estrogen and testosterone levels. For example, the BP is typically higher in women after menopause than in men, probably due to sex hormone levels (e.g., decline in estrogen), which play a central role in the marked rise in arterial stiffness after menopause. Furthermore, inflammation has also been reported to contribute to the development of hypertension after menopause, which could be an additional factor to the potential sex-differential BP effects imposed by the gut microbiota composition.^{38,39} These findings suggest that gut microbiota-targeted interventions by prebiotic, probiotic or symbiotic therapy may display different results in accordance with the sex of the recipient, but few studies have taken recipient sex into account and the results are inconsistent.⁴⁰

The intervention with the probiotic formulation of *L. fermentum* reduced the LF component of the SAP in offspring of both sexes, although it has not been demonstrated to reduce the LF:HF ratio. The LF component has been considered as an index of sympathetic modulation,⁴¹ indicating that oral treatment with the probiotic formulation of *L. fermentum* could reduce sympathetic hyperactivity in the DLP groups.

In addition, the administration of the probiotic formulation of *L. fermentum* reduced the sympathetic tone in the DLP male and female offspring after hexamethonium infusion, suggesting that the tested probiotic intervention was capable of attenuating the sympathetic hyperactivity in the DLP groups. The results of ganglionic blockade and spectral analysis together indicate that the probiotic formulation of *L. fermentum* effectively reduces sympathetic hyperactivity in male and female rat offspring from dams exposed to maternal dyslipidemia.

Oxidative stress increases chemoreflex sensitivity, impairs baroreflex sensitivity and contributes to sympathetic hyperactivity, playing a role in the development of AH in adult offspring.⁴² Furthermore, gut dysbiosis has been associated with excessive production of free radicals, causing damage in key organs for blood pressure control and favoring the development of AH.^{43,44} The intervention with the probiotic formulation of *L. fermentum* in the DLP male and female offspring reduced the serum levels of MDA, suggesting improvements in pro-oxidant species. This indicates that exposure of the gut microbiota to microorganisms such as *L. fermentum* strains may contribute to minimize reactive oxygen species (ROS) production via stopping pro-inflammatory cytokine signaling and

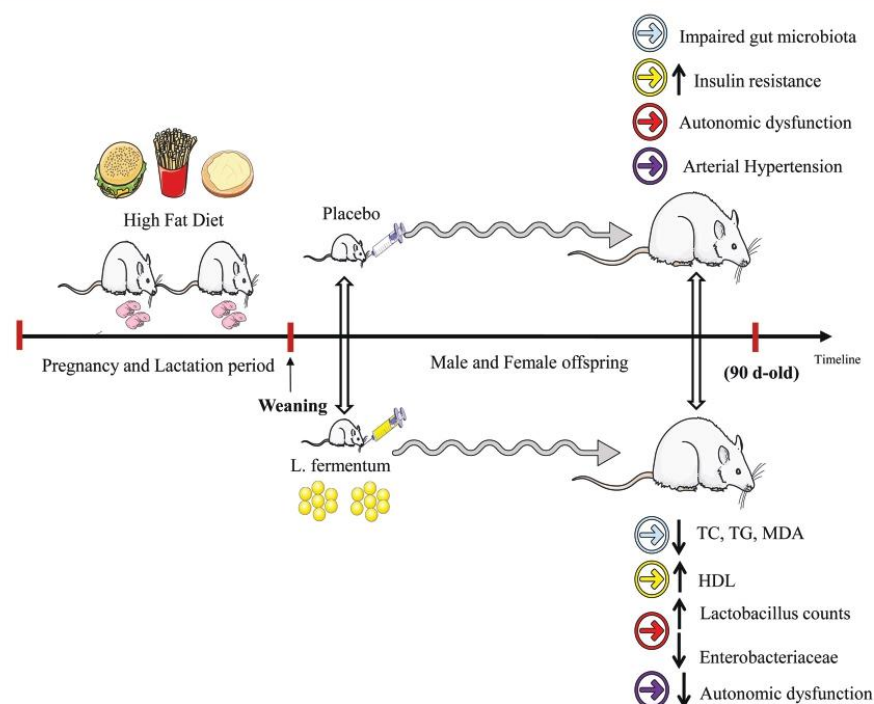


Fig. 5 Schematic drawing showing the impact of the probiotic formulation with *L. fermentum* on the fecal microbiota, biochemical variables, blood pressure and autonomic function in male and female rats exposed to maternal dyslipidemia. HDL (high density lipoprotein); L (*Lactobacillus*); MDA (malondialdehyde); TC (total cholesterol); TG (triglycerides).

reducing lipid peroxidation,^{45–47} being associated with an improvement of cardiovascular parameters.

Consistent with the literature,^{3,4} cardiovascular responses to hypoxia were increased in the DLP male and female rat offspring exposed to maternal dyslipidemia during pregnancy and lactation. Therefore, it could be suggested that the increased chemoreceptor sensitivity observed in the DLP groups was mainly associated with nutritional insults experienced during pregnancy and lactation. For the first time, the present study revealed that the administration of the probiotic formulation of *L. fermentum* effectively reduced the pressor response in offspring of both genders, demonstrating a blunted sympathetic response to the peripheral chemoreflex.

Although the administration of the probiotic formulation of *L. fermentum* has shown to attenuate the increase in the sympathetic component of the peripheral chemoreflex, no effects were observed on baroreflex sensitivity. Otherwise, a recent study demonstrated that oral administration of a newly synthesized carboxymethyl-glucan (a water-soluble derivative of *Saccharomyces* spp. cell wall $\beta(1-3)(1-6)$ glucan, with well-known antioxidant properties) at a dose of 40 mg kg⁻¹ daily for 2 weeks improved the baroreflex sensitivity in rats with renovascular hypertension,⁴⁸ suggesting that the identification of antioxidant compounds with baroreceptor modulation properties could be a potential therapy for AH.

An early investigation reported that *L. fermentum* 263 and *L. fermentum* 296 presented outstanding response to the presence of quercetin and resveratrol, with improvements in their *in vitro* probiotic aptitudes. These findings were considered to indicate that quercetin and resveratrol could boost some properties associated with the functionalities of selected probiotic *L. fermentum* strains.⁴⁹ Considering the antioxidant capacity of these polyphenols, our next step will be to investigate if the combined administration of quercetin or resveratrol with this probiotic formulation of *L. fermentum* could improve the baroreflex functionality in male and female offspring from dams exposed to maternal dyslipidemia.

5. Conclusion

The intervention with a new probiotic formulation containing *L. fermentum* 139, *L. fermentum* 263 and *L. fermentum* 296 strains for 8 weeks produced hypocholesterolemic effects, inducing improvements in the lipid profile and serum MDA levels in male and female rat offspring from dams exposed to maternal dyslipidemia during pregnancy and lactation. Moreover, the proposed probiotic formulation reduced the SAP and MAP only in male offspring (Fig. 5).

This study was the first to show that the administration of a probiotic formulation of *L. fermentum* may effectively attenuate the sympathoexcitatory response induced by the peripheral chemoreflex, as well as reduce the baseline sympathetic tone and oxidative stress, being a promising strategy to prevent hypercholesterolemia and, consequently, the development of arterial hypertension and oxidative stress in adult offspring.

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Author contributions

Y. de Oliveira and J.L. de Brito Alves designed the experiments. Y. de Oliveira and R.G.S. Cavalcante performed the experiments. Y. de Oliveira and J.L. de Brito Alves analyzed the data and wrote the manuscript. Y. de Oliveira, R.G.S. Cavalcante, J.L. de Brito Alves, V.A. Braga, M.P. Cavalcanti Neto and E.L. de Souza reviewed the manuscript.

Abbreviations

AH	Arterial hypertension
AP	Arterial pressure
BP	Blood pressure
CVD	Cardiovascular diseases
CFU	Colony forming unit
CTL	Control
DAP	Diastolic arterial pressure
DBP	Diastolic blood pressure
DLP	Dyslipidemia
HR	Heart rate
HDL-c	High-density lipoprotein cholesterol
HF	High frequency
IR	Insulin resistance
ITT	Insulin tolerance test
LDL-c	Low-density lipoprotein cholesterol
LF	Low frequency
MDA	Malondialdehyde
MAP	Mean arterial pressure
OGTT	Oral glucose tolerance test
PAP	Pulsatile arterial pressure
PI	Pulse interval
SBRS	Spontaneous baroreflex sensitivity
SAP	Systolic arterial pressure
SBP	Systolic blood pressure
TC	Total cholesterol
TG	Triglycerides
VLDL-c	Very low density lipoprotein cholesterol

Conflicts of interest

The authors declare no conflicts of interest.

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APÊNDICE C – CAPÍTULO DE LIVRO



Chapter 16

Probiotics for humans: Current status and future prospects

José Luiz de Brito Alves, Yohanna de Oliveira, Vanessa Polyana de Sousa, Evandro Leite de Souza

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

16.1 Introduction

The term probiotic was first used to describe compounds produced by the protozoa class with ability to stimulate the growth of other organisms (Lilly and Stillwell, 1965). In 2001, scientists and experts of Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) conceptualized probiotic as “live nonpathogenic microorganisms, which when administered in adequate amounts confer a health benefit to the host” (Hill et al., 2014). This description incorporates a broad range of viable microbes that confer benefit to human health and excludes commensal microorganisms that have not been isolated and characterized as probiotics, live starter cultures present in fermented food, fecal microbiota transplantation, and products as mattresses, shampoos, and aftershave, which sometimes have been inappropriately given the term probiotic.

For the last decades, the effects of probiotics on human health have attracted increasing interest of researchers and industry worldwide. This interest in understanding the effects of probiotics has been primarily associated with the increasing available information about the important role of the gut microbiota on human health. Two phenomena have been perceived in the last decades: (i) a large number of basic, experimental and clinical studies evaluating the effects of probiotic administration on different clinical conditions; and (ii) a huge commerce involving the probiotic market, which was estimated to mean up to \$15 billion annually, with an estimated annual growth of 5%–30% depending on the country and product type (Caselli et al., 2013).

The scientific and commercial burst unfortunately raised some concerns, such as the misuse of the term probiotic, safety of probiotic products for human consumption, and role of regulatory agencies in protecting consumers. In 2013, the International Scientific Association for Probiotic and Prebiotic (ISAPP) reexamined the concept of probiotic microbes and achieved a consensus document to help the maintenance of advances in probiotic research and ensure safety and health benefit of probiotics to consumers (Hill et al., 2014). In this chapter, we will address only the main findings related to the effects of the probiotics administration on human, which properly meet the updated ISAPP recommendation.

A strain to be considered as probiotic needs to meet a number of relevant aspects related to functionality and safety, such as survivability to simulated acid gastric and intestinal conditions, adhesion to intestinal epithelial cells, coaggregation and cell surface hydrophobicity properties, antagonism against pathogens, susceptibility to common antibiotics, and absence of potential virulence genes and hemolytic and mucin degradation activity (Saarela, Mogensén et al., 2000; de Albuquerque et al., 2018).

Most of the lactic acid bacteria strains are generally recognized as safe (GRAS) or presumably qualified as safe (QPS), naturally found in the intestine, and typically recognized as potential probiotics due to their ability to promote host health benefits (Wojtyniak and Szajewska, 2017). Lactic acid bacteria constitute a group of 13 genera, including, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Lactoshaepa*, *Leuconostoc*, *Oenococcus*, *Paralactobacillus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*, and *Lactobacillus* and *Bifidobacterium* genera include the probiotics most commonly studied and available on market (Champagne et al., 2011), although other bacteria, such as *Bacillus*, *Escherichia coli*, and some yeasts (e.g., *Saccharomyces*), have been also exploited as probiotics.

(Vijaya Kumar et al., 2015). In particular, *L. acidophilus*, *L. casei*, *L. paracasei*, *L. gasseri*, *L. johnsonii*, *L. fermentum*, *L. reuteri*, *L. plantarum*, *L. rhamnosus* and *L. salivarius*, *B. bifidum*, *B. breve*, *B. lactis*, *B. longum*, *B. thermophilum*, *B. adolescentis*, and *B. animalis* are cited among the main species of probiotics worldwide (Vijaya Kumar et al., 2015).

Probiotic strains are available in the market comprising food formulations (e.g., dairy products) and pharmaceutical products in the forms of tablets, capsules, or sachets. Although there have been differences concerning the optimum amount of probiotics need for providing health benefits on host, regulatory approaches have demonstrated that daily consumption of a dose of 1×10^8 – 1×10^{10} colony forming units per serving may be effective to support a healthy gut microbiota and health benefits (Backhed et al., 2012; Hill et al., 2014; Smug et al., 2014).

Biological properties of probiotics have been strain-specific and the success or failure of one strain in modulating gut microbiota or improving specific physio-pathological conditions should not be extrapolated to another strain. In fact, some strains may be detrimental when given to certain patients, since they may aggravate the health problems of these individuals. The beneficial effects of probiotics on human health seem to occur through different mechanisms, including induction of immunomodulation, protection against physiological stress, suppression of pathogens, gut microbiome modulation, and improvement of the barrier function of gut epithelium (Suez et al., 2019).

16.2 Probiotic therapy for humans

Probiotics have emerged as an innovative strategy to provide improved gut bacterial communities with potential beneficial effects for maternal, fetal, infant, and adult health. The main effects of probiotic administration on specific conditions during pregnancy, childhood, and adulthood will be discussed in the following sections.

16.2.1 Clinical effects of probiotic therapy on pregnancy

The available literature has demonstrated that probiotics administration during pregnancy neither increases nor decreases the risk of preterm birth or other infant and maternal adverse pregnancy outcomes (Jarde et al., 2018), indicating that a safe prescription of probiotic may effectively be proposed for pregnant women.

The probiotic administration to women during pregnancy and lactation has been shown capable of changing the composition of breast milk, and consequently, to promote benefits to the child, such as the reduction of the occurrence of gastrointestinal disorders (Baldassarre et al., 2018b). An investigation examining the effects of probiotics on maternal and infant health demonstrated that administration of supplements without probiotics or enriched with *L. rhamnosus* CGMCC 1.3724 and *B. lactis* CNCC I-3446 from the third trimester of pregnancy for up to at least 2 months postdelivery in healthy pregnant women at 24–28 weeks gestation was well-tolerated and safe. However, the administration had no significant effects on the incidence of infant diarrhea and pregnancy-related or fetal adverse outcomes (Mantaring et al., 2018).

Considering the high prevalence of constipation during pregnancy, a randomized, controlled clinical trial conducted on 60 constipated pregnant women found that the consumption of 300 g/day of probiotic yogurt enriched with *L. acidophilus* La-5 and *B. lactis* Bb-12, at a dose of 4.8×10^{10} CFU for 4 weeks, showed a significant increase in the frequency of defecation and amount of stool. In addition, the consumption of probiotics decreased the straining to defecate, sensation of anorectal obstruction, and need for manipulation to facilitate defecation, suggesting an alleviation of the constipation symptoms during pregnancy (Mirghafourvand et al., 2016).

The use of antibiotics or caesarean delivery has been considered as an important risk factor for development of metabolic, inflammatory, and immunological diseases due to disruption of health gut microbiota during a critical developmental time window (Mueller et al., 2015; Korpela et al., 2016). A recent randomized double-blind, placebo-controlled clinical trial demonstrated that administration of two daily capsules with multiprobiotic strains, containing *B. breve* Bb99 (2×10^8 CFU), *Propionibacterium freundenreichii* subsp. *shermanii* JS (2×10^9 CFU), *L. rhamnosus* Lc705 (5×10^9 CFU), and *L. rhamnosus* GG (5×10^9 CFU) during pregnancy, as well as for the first 6 months following birth, restored the gut microbiota composition and function in antibiotic-treated and caesarean-born infants (Korpela et al., 2018).

Colonization with *Streptococcus agalactiae* has been described as one of the most important causes of severe neonatal early-onset sepsis (Martin et al., 2019). A recent study demonstrated that daily administration of *L. salivarius* CECT 9145, at a dose of 1×10^9 CFU, from the 26th to 38th week of gestation reduced the number of rectal and vaginal *S. agalactiae* population in healthy pregnant women. These data suggested that administration of *L. salivarius* CECT 9145 for pregnant women is a safe and successful strategy to decrease the *S. agalactiae* colonization (Martin et al., 2019).

Probiotics have also been prescribed for maternal cardiometabolic disorders. The main cardiometabolic disorders experienced during pregnancy include obesity, gestational diabetes mellitus (GDM), preeclampsia, or dyslipidemia. These cardiometabolic disorders experienced in utero may negatively impact the mother and children health (de Brito Alves et al., 2019).

Recent studies have commonly reported that maternal cardiometabolic disorders may be linked with relevant alteration in gut microbiota composition and probiotic therapy could be a relevant supporting approach to improve maternal gut microbiota and dysmetabolic conditions (de Brito Alves et al., 2019).

16.2.1.1 Effects of probiotics on gestational diabetes mellitus

In gestational diabetes mellitus (GDM), probiotic therapy for 4–8 weeks has been proposed as a promising strategy to support glycemic control and metabolic management (Taylor et al., 2017; de Brito Alves et al., 2019). A recent meta-analysis of 11 randomized clinical trials involving 719 participants showed that probiotic supplementation in women with GDM improved four biomarkers for glycemic control (fasting blood glucose, fasting serum insulin, homeostasis model assessment insulin resistance, and quantitative insulin sensitivity check index), two biomarkers for lipid profile (triglycerides and HDL-cholesterol), four biomarkers related to inflammation and oxidative stress (total glutathione, malondialdehyde, nitric oxide, and total antioxidant capacity), and reduced the incidence of hyperbilirubinemia in newborns by 74% (Zhang et al., 2019). However, the probiotic supplementation was cited to not affect total cholesterol and LDL-cholesterol at maternal levels, preterm delivery, macrosomia, and newborns' hypoglycemia.

A randomized, double-blind, and placebo-controlled clinical trial performed with 48 women with GDM demonstrated that the intervention with probiotic capsule containing *L. acidophilus*, *L. casei*, *B. bifidum*, and *L. fermentum* (2×10^9 CFU/g each) for 6 weeks had beneficial effects on the expression of genes related to insulin and inflammation and significantly improved insulin sensitivity, fasting plasma glucose (FPG), triglycerides, and HDL-cholesterol levels (Babadi et al., 2018).

Despite the results of available studies, investigation considering duration to determine the ideal strain or strains' composition, safety use, optimal dose, and duration of probiotic administration are still needed to support their routine use and effective recommendation for GDM.

16.2.1.2 Effects of probiotics on obese pregnant women

The use of probiotic to control or alleviate the complications of gestational obesity has not been a consensus yet. The results of an available study have demonstrated that probiotic intervention in obese pregnant women had no effects on maternal neonatal anthropometric measures, markers of glucose, lipid metabolism, improvement in gut permeability or LPS serum levels and did not prevent GDM in overweight and obese pregnant women (de Brito Alves et al., 2019). Results of a placebo-controlled, double-blind, randomized trial with 138 obese pregnant women supplemented with a single probiotic strain of *L. salivarius* UCC118 (10^9 CFU per capsule; one capsule per day) for 4 weeks from the 24th week of gestation onward showed that the probiotic supplementation reduced the body mass index (BMI) of obese pregnant women, but did not affect blood glucose levels and metabolic profile (Lindsay et al., 2014).

On the other hand, a placebo-controlled, double-blind, and randomized trial found that dietary counseling and probiotic intervention with capsules containing *L. rhamnosus* GG (ATCC 53103) and *B. lactis* Bb12, at a dose of 10^{10} CFU/day, from the first trimester of pregnancy up to 6 months of postpartum reduced central adiposity in women, being indicated as a supporting therapy for the prevention and management of obesity in pregnant women (Ilmonen et al., 2011).

16.2.1.3 Effects of probiotics on maternal dyslipidemia

Probiotic therapy could also be a promising strategy to treat maternal dyslipidemia. The consumption of probiotic yogurt containing *L. acidophilus* LA-5 and *B. animalis* BB12 (1×10^7 CFU, for 9 weeks) resulted in reduction of total cholesterol (TC), LDL-c and triglycerides levels in pregnant women in the third trimester of gestation (Asemi et al., 2012). On the other hand, a randomized study with 256 pregnant women reported that the dietary counseling and supplementation with probiotic capsule containing *L. rhamnosus* GG and *B. lactis* in the first trimester of pregnancy caused no effect on serum lipid levels during the third trimester (Hopppu et al., 2014). Additionally, the results from a meta-analysis of six randomized clinical trials involving 579 pregnant women demonstrated that probiotic capsule intake could decrease total cholesterol and triglycerides levels in pregnant women (Han et al., 2019). Additional double-blind and placebo-controlled trials should be needed to clarify the outcomes of probiotic therapy on maternal dyslipidemias.

16.2.1.4 Effects of probiotics on preeclampsia

There has been no available study on the use of probiotic intervention for preeclampsia treatment (de Brito Alves et al., 2019). The unique available prospective cohort study has been the Norwegian Mother and Child Cohort, which demonstrated that high intake of probiotic dairy products containing *L. acidophilus* LA-5, *B. lactis* Bb12, and *L. rhamnosus* GG (> 200 mL/day) was associated with a reduction by 20% of the risk of preeclampsia and severe preeclampsia in primiparous women. The no evaluation of the effects of the consumption of probiotic dairy products on gut microbiota of pregnant women with preeclampsia was a considerable limitation for this study (Brantsaeter et al., 2011).

Further investigation using specific probiotic strains with the use of randomized controlled trials should be necessary to clarify the effects of probiotics on preeclampsia.

16.2.2 Clinical effects of probiotic therapy in infant and children

16.2.2.1 Effects of probiotics on necrotizing enterocolitis in infant

The early supplementation of probiotics in the perinatal and postnatal periods seems to have overall a positive impact on future health of infants (Baldassarre et al., 2018b). Neonatal late-onset sepsis and/or necrotizing enterocolitis (NEC) are gastrointestinal diseases that mainly affect premature newborns. Considering the potential benefits of probiotic supplementation for preterm infants, a large-scale randomly controlled trial carried on 4556 Indian breast-fed infants demonstrated that consumption of an oral preparation containing *L. plantarum* PP 11-217 and prebiotic fructooligosaccharides protected infants against neonatal sepsis and death (Panigrahi et al., 2017). However, another study examining the effects of an enteral formula containing *B. breve* BBG-001 in a trial with 1310 very preterm English infants did not find significant effects on prevention of NEC or sepsis (Costeloe et al., 2016). Although there have been divergence in the conclusions of systematic reviews and meta-analysis studies on the efficacy of probiotics for prevention of late-onset sepsis in preterm infants (Zhang et al., 2016a; Aceti et al., 2017), a recent study examining 25 systematic review and meta-analyses studies of randomized controlled trials suggested that probiotics should help to reduce the risk of all-cause mortality, NEC $\geq$ Stage II, late-onset sepsis, and feeding intolerance in preterm infants, in addition to indicate probiotics as a standard prophylaxis for this group (Athalye-Jape and Patole, 2019). In addition, a systematic review of 24 randomized controlled trials indicated that supplementation with *Lactobacillus* or with the combination of *Lactobacillus* and *Bifidobacterium* probiotic strains should reduce the incidence of NEC in premature infants (AlFaleh and Anabrees, 2014).

In a systematic review investigating the effects of *B. breve* M-16V in preterm neonates, it was reported that findings of nonrandomized controlled trials indicate benefits on late-onset sepsis, mortality, and postnatal age at full feeds; however, the current evidence is still limited regarding the role of *B. breve* in this group (Athalye-Jape et al., 2018). Further investigations are still needed to provide strong evidence on the effects of probiotics in preterm infants. These studies could design experiments to give an understanding of the impact of feeding (formula, breastfeeding, and donors milk) on the relationship between probiotic supplementation and clinical outcomes, efficacy of multistrain probiotics or single-strain probiotic, safety issues, and long-term effects for such vulnerable population (Aceti et al., 2018).

A randomized, double-blind, placebo-controlled clinical trial demonstrated that daily supplementation of mothers and their infants for 3 months with a multispecies probiotic formulation containing *B. breve* Bb99 (2×10^8 CFU), *P. freudenreichii* subsp. *shermanii* JS (2×10^9 CFU), *L. rhamnosus* Lc705 (5×10^9 CFU), and *L. rhamnosus* GG (5×10^9 CFU) caused increases in fecal bifidobacteria population and decreases in *Proteobacteria* and *Clostridia* population in breast-fed caesarean-born infants. These findings indicate that breastfeeding together with the probiotic supplementation could impact positively on the gut microbiota of infants delivered by caesarean section, as well as that these commercially available strains could be adopted into clinical practice (Korpela et al., 2018).

16.2.2.2 Effects of probiotics on colic infants

In a recent randomized, double-blind, clinical trial conducted in 53 exclusively breast-fed colic infants showed that the group that received the intervention with a mixture of eight different probiotic strains (*L. paracasei* DSM 24733, *L. plantarum* DSM 24730, *L. acidophilus* DSM 24735, *L. delbrueckii* subsp. *bulgaricus* DSM 24734, *B. longum* DSM 24736, *B. breve* DSM 24732, *B. infantis* DSM 24737, and *Streptococcus thermophilus* DSM 24731) decreased the crying episodes per day at the end of the 3-week treatment. However, this probiotic intervention did not modify anthropometric data, bowel movements, stool consistency, and microbiota composition of infants (Baldassarre et al., 2018a). In addition, two meta-analyses concluded that *L. reuteri* DSM17938, at daily dose of 10^8 CFU, should be considered for the management of breast-fed colic infants, but the efficacy of this probiotic in formula-fed infants is still limited (Dryl and Szajewska, 2018; Sung et al., 2018).

An observational study demonstrated that the consumption of a probiotic formula rich in *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* probiotic strains during early life was associated with high abundance of *Bifidobacterium* population in infants at 1 week of age, but without changes in breast milk immune markers and overall health outcomes. On the other hand, the results of this study showed that probiotics consumption was associated with increased mucosal infections at the first 2 years of life when compared with infants who did not consume probiotics. Although this study had been performed with variation of brands and doses of probiotics and with a reduced sample size, the findings suggested caution in probiotic administration during infancy and demonstrate that more rigorous controlled follow-up studies are needed for elucidating the reported outcomes (Quin et al., 2018).

16.2.2.3 Effects of probiotics on allergy and food hypersensitivity in children

Three different meta-analyses have evaluated the effects of the intervention with probiotics on the prevention of atopic syndrome, allergic disease, and hypersensitivity related to food in children (Osborn and Sinn, 2007; Zuccotti et al., 2015; Zhang et al., 2016b). Two of these meta-analyses suggested that use of probiotics, especially when administered prenatally to pregnant mother and postnatally to child, could be a feasible way to prevent atopy and food hypersensitivity in young children (Zuccotti et al., 2015; Zhang et al., 2016b). However, although the findings of one of the available meta-analyses had indicated a reduction in clinical eczema in infants and all of them had indicated benefits from the use of supplements containing *L. rhamnosus*, the authors reported that these effects were not consistent enough to recommend the incorporation of probiotics to infant feed in order to prevent allergic disease or food hypersensitivity (Osborn and Sinn, 2007).

16.2.2.4 Effects of probiotics on gastroenteritis and diarrhea in children

Acute gastroenteritis is a current problem in pediatric population. Meta-analyses and systematic reviews have demonstrated that probiotic therapy, especially with formulations containing *S. boulardii* and *L. rhamnosus*, may be effective for prevention or treatment of acute diarrhea, hospitalization, nosocomial diarrhea, and respiratory tract infection in children (Van Niel et al., 2002; Feizizadeh et al., 2014; Hojsak, 2017; Szajewska et al., 2019). A meta-analysis carried out with 60 randomized clinical trials in children (0–18 years) receiving antibiotics demonstrated that administration of probiotic formulations containing *Bacillus* spp., *Bifidobacterium* spp., *Lactobacillus* spp., *Lactococcus* spp., *Leuconostoc cremoris*, *Saccharomyces* spp., and *Streptococcus* spp., in a single or mixed-strain composition and dose ≥ 5 billion CFU/day, should prevent the occurrence of antibiotic-associated diarrhea (Johnston et al., 2011). However, there has been no consensus about the probiotics for this end, since some studies have not found beneficial effects of probiotic use for diarrhea prevention in children (Olek et al., 2017; Freedman et al., 2018; Schnadower et al., 2018).

16.2.2.5 Effects of probiotics on functional constipation in children

A recent systematic review with six randomized clinical trials reported that no evidence exists to support the use of *L. casei rhamnosus* LCR35, *B. lactis* DN173 010, *B. longum*, *L. reuteri* DSM 17938, as well as of a mix of *B. infantis*, *B. breve*, and *B. longum* for children with functional constipation. It was stated that the detection of no benefit from probiotic use could be associated with the experimental design of the studies, since the interventions and follow-up were characterized as short-term and the number of individuals were small and heterogeneous (Wegh et al., 2018). Another systematic review including seven randomized clinical trials with five different probiotic strains and one mixture of seven probiotic strains demonstrated that probiotics are ineffective for the management of functional constipation in children when considered the defecation, fecal incontinence, and abdominal pain frequency, which could make a recommendation for clinical use not justified (Wojtyniak and Szajewska, 2017).

16.2.3 Clinical effects of probiotic therapy in adult

A growing body of studies has demonstrated that administration of probiotics should be beneficial in a variety of pathophysiological conditions. In this section, the effects of probiotics administration on management of the most common diseases in adults are summarized considering evidence from clinical trials and discussed concerning the influence of different probiotics, administered dose, and intervention time on measured clinical outcomes.

16.2.3.1 Effects of probiotics on constipation

A meta-analysis with 14 randomized clinical trials demonstrated that administration of approximately 10^8 – 10^{10} CFU/day of specific probiotic strains decreased the intestinal transit time by 12 h, increased stool frequency by 1.5 stools/week, and improved some constipation-related symptoms (e.g., bloating, incomplete evacuation sensation, occurrence of hard stools, and ease of stool expulsion), indicating beneficial effects, particularly of *B. lactis* strains (Dimidi et al., 2014). Another recent meta-analysis including 21 randomized clinical trials also demonstrated that the use of probiotic *Lactobacillus* or *Bifidobacterium* strains increased stool frequency and reduced intestinal transit time by approximately 15 h in constipated adults (Miller et al., 2017). However, further studies are needed to determine the dose and treatment duration and could be effective to the use of specific probiotics to relief constipation symptoms.

16.2.3.2 Effect of probiotics on diarrhea

Probiotic formulations, in particular those containing *S. boulardii* and *L. rhamnosus*, have been suggested as prophylactic and therapeutic alternatives for prevention or treatment of acute, antibiotic-associated, and *Clostridium difficile*-associated

diarrhea (Pereg et al., 2005; Hempel et al., 2012; Suez et al., 2019). Systematic review and meta-analysis have demonstrated that probiotic administration, particularly containing *S. boulardii*, was effective against antibiotic-associated diarrhea (Szajewska and Kolodziej, 2015; Jafarnejad et al., 2016).

16.2.3.3 Effects of probiotics on lactose intolerance

The consumption of *L. acidophilus* DDS-1 at a dose of 1×10^{10} CFU/day for 4 weeks improved an array of abdominal symptoms (e.g., diarrhea, cramping, and vomiting) during an acute lactose challenge in adults with lactose intolerance (Pakdaman et al., 2016). A systematic review with 15 randomized controlled trials included studies with 8 different probiotic strains in the treatment of lactose intolerance, including, *B. longum*, *B. animalis*, *L. bulgaricus*, *L. reuteri*, *L. acidophilus*, *L. rhamnosus*, *S. boulardii*, and *Streptococcus thermophilus*. It was reported that *B. animalis* was overall the most effective species, being suggested to have a common positive benefit of probiotic consumption on lactose intolerance, although the size of the effects was variable. However, the probiotic supplementation was not always effective at reducing lactose intolerance in adults, as well as some specific species/strains and doses seemed to be more effective to alleviate the lactose intolerance symptoms than other (Oak and Jha, 2019).

16.2.3.4 Effects of probiotics on celiac disease

Few human clinical trials have been assessing the effects of a probiotic supplementation in adults with celiac disease (CD). A large prospective randomized study in 109 CD patients with irritable bowel syndrome (IBS) symptoms and strictly adherent to a gluten-free diet (GFD) showed that the intervention with multispecies probiotic (mixture of *L. casei* LMG 101/37 P-17504, *L. plantarum* CECT 4528, *B. animalis* subsp. *lactis* Bi1 LMG P-17502, *B. breve* Bbr8 LMG P-17501 and *B. breve* B110 LMG P-17500) for 6 weeks was effective to decrease the severity of IBS-type symptoms, in addition to cause a positive modulation of gut microbiota with an increase of the bifidobacteria population up to 6 weeks after the discontinuation of probiotics (Francavilla et al., 2019). Another randomized, double-blind, and placebo-controlled trial with 22 adult patients with active CD receiving *B. infantis* (NLS-SS) at a dose of 2×10^9 CFU/day for 3 weeks and a GFD improvement in gastrointestinal symptoms, such as indigestion, constipation, and gastroesophageal reflux, suggested a role for probiotics in alleviating symptoms in untreated CD (Smecuol et al., 2013).

On the contrary, a randomized, double-blind, and placebo-controlled trial conducted with 45 CD patients with persistent symptoms and on a GFD who received 5 g of a probiotic formulation (VSL#3) for 12 weeks reported improvements in clinical symptoms, with no change in fecal microbiota counts and blood safety parameters (Harnett et al., 2016).

16.2.3.5 Effects of probiotics on IBS

A meta-analysis based on 15 randomized double-blind clinical trials demonstrated the beneficial effects of probiotics on life quality and alleviation of IBS symptoms, such as abdominal pain, distension, bloating, flatulence, and IBS diagnostic scores (Didari et al., 2015). Considering the appropriate probiotic type, dose, and treatment duration for IBS, another meta-analysis, including 21 randomized controlled trials, suggested that the consumption of single probiotics at a low dose ($< 10^{10}$ CFU/day) and for a short duration (< 8 weeks) is more effective in improving overall symptom response and quality of life (Zhang et al., 2016c). On the other hand, a meta-analysis with 14 randomized controlled trials showed that protocols with suitable dose of combined probiotic *Lactobacillus* and *Bifidobacterium* strains could exert better effects in controlling a diarrhea type of IBS when compared with the use of single probiotic strains or overdose protocols, as well as that the dose of 10^9 – 10^{10} CFU/day could be a reference range (Liang et al., 2019). Still, two specific probiotics (namely, *L. plantarum* 299v and *B. infantis* 35624) were indicated as the most effective for alleviating IBS symptoms (Sniffen et al., 2018).

16.2.3.6 Effects of probiotics on ulcerative colitis

Guidelines and recommendations from scientific experts and institutions have supported the safety use of the probiotic formulation VSL#3 containing *L. plantarum*, *L. casei*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. infantis*, *B. longum*, *B. breve*, and *S. salivarius* subsp. *thermophilus* and *E. coli* Nissle 1917, at a daily dose of 3.6×10 CFU, as a therapy strategy to achieve better response and higher remission rates in mild to moderately active ulcerative colitis (UC) in adults (Floch et al., 2015; Guarner et al., 2017). Results from a recent randomized, double-blind, and placebo-controlled trial with 81 patients with UC suggested that the intervention with the multistrains probiotic Symprove, containing *L. rhamnosus* NCIMB 30174, *L. plantarum* NCIMB 30173, *L. acidophilus* NCIMB 30175, and *Enterococcus faecium* NCIMB 30176 with each 50 mL dose containing about 10 billion live bacteria during 4 weeks, could reduce intestinal inflammation in patients with UC (Bjarnason et al., 2019). Probiotic formulations containing bifidobacteria have been overall cited as promising

strategies for UC treatment; however, more clinical trials are needed to clarify adequate doses and determine the probiotic species/strains more effective to induce the expected beneficial effects (Asto et al., 2019).

16.2.3.7 Effects of probiotics on Crohn's disease

Most of the available studies have failed to show beneficial effects of probiotics as a therapy strategy for Crohn's disease (Dong et al., 2016). Although there have been results in literature reporting that combined consumption of *S. boulardii*, *Lactobacillus* spp., and VSL#3 probiotic formulation could cause beneficial effects to individuals affected by Crohn's disease (Ganji-Arjenaki and Rafieian-Kopaei, 2018), the consumption of these microorganisms has been indicated to confer little benefits to induce clinical remission in Crohn's disease patients. Based on the available evidence, the use of probiotics in Crohn's disease should be cautiously considered (Forbes et al., 2017).

16.2.3.8 Effects of probiotics on hypertension

Clinical studies have commonly demonstrated a positive association between probiotic supplementation and blood pressure reduction. The most common species studied as prebiotics for use in hypertension condition are *L. casei*, *L. plantarum*, *L. helveticus*, *S. cerevisiae*, *L. acidophilus*, *L. fermentum*, and *L. bulgaricus* (Thushara et al., 2016). Daily supplementation with 400 mL of a drink containing *L. plantarum* 299v at a dose of 5×10^7 CFU/mL for 6 weeks in a population of smokers of both genders caused decreases in systolic blood pressure (SBP) (Naruszewicz et al., 2002). A randomized double-blind clinical trial with 40 patients with type 2 DM demonstrated that the daily administration of 200 mL of probiotic soymilk containing *L. planetarium* A7 for 8 weeks reduced both SBP and diastolic blood pressure (DBP) (Hariri et al., 2015).

Similarly, a meta-analysis with 14 randomized placebo-controlled clinical trials has shown that probiotic fermented milk reduced SBP by 3.10 mmHg and DBP by 1.09 mmHg in prehypertensive and hypertensive subjects, respectively (Dong et al., 2013). For blood pressure lowering, the only available meta-analysis considering nine randomized clinical trials demonstrated that probiotic consumption lowered SBP by 3.56 mmHg and DBP by 2.38 mmHg. These results suggested that the consumption of probiotics may potentially improve blood pressure when the daily dose is $\geq 10^{11}$ CFU, the duration of intervention is ≥ 8 weeks, and multiple probiotic strains are consumed (Khalesi et al., 2014).

16.2.3.9 Effects of probiotics on type 2 diabetes mellitus

The probiotics most frequently used in clinical trials to investigate the effects of intervention on the metabolism of type 2 diabetes mellitus (T2DM) subjects have been *L. acidophilus*, *L. sporogenes*, *B. bifidum*, and *B. lactis* used alone or in combination, with intervention duration between 4 and 6 weeks (Bordalo Tonucci et al., 2017). A meta-analysis including seven randomized clinical trials with T2DM patients demonstrated that probiotics consumption could reduce FPG by 15.92 mg/dL and HbA1c by 0.53%, with a potentially greater effect when the duration of intervention is ≥ 8 weeks or when multiple probiotic strains are consumed (Zhang et al., 2016d). A randomized, double-blind, and controlled clinical trial reported that the consumption of 120 g/day of probiotic fermented goat milk containing *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* BB-12 (10^9 CFU each for 6 weeks) decreased antiinflammatory cytokines (TNF- α and resistin) and prevented an increase in TC and LDL-c in T2DM patients (Tonucci et al., 2017). Other randomized clinical trials demonstrated that the consumption of yogurt containing *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* BB-12 decreased FPG, TC, LDL-c, and HbA1c levels, in addition to increase erythrocyte superoxide dismutase, glutathione peroxidase activity, and total antioxidant status. These results suggest that the tested probiotic yogurt could be a promising tool for T2DM management (Ejtahed et al., 2011, 2012).

16.2.3.10 Effects of probiotics on overweight/obesity

The current evidence regarding overweight and obese patients who received probiotics supplementation has consistently showed a reduction in waist circumference (WC) and body mass index (BMI) and improvements in plasma lipid profile and glucose metabolic parameters (Wang et al., 2019). A meta-analysis including 25 randomized clinical trials demonstrated that the beneficial effects on anthropometric parameters were overall greater when multiple probiotic strains were consumed and intervention duration was ≥ 8 weeks (Zhang et al., 2015). The probiotics most studied in clinical trials with overweight/obese individuals belong to *Lactobacillus* and *Bifidobacterium* genera, including *L. curvatus*, *L. plantarum*, *L. rhamnosus*, and *B. animalis* subsp. *lactis* (Thushara et al., 2016). The consumption of different *Lactobacillus* strains (2.7×10^{10} CFU/day of probiotic administration for 2–3 months) was associated with weight loss in adults (Brusaferro et al., 2018). However, data regarding the impact of probiotic dose, duration of administration, and long-term effects of the consumption of different species/strains are still conflicting.

16.2.3.11 Effects of probiotics on dyslipidemias

Studies have investigated the hypocholesterolemic effects of probiotic strains of *Lactobacillus* and *Bifidobacterium*, such as *S. thermophilus*, *E. faecium*, *L. acidophilus*, *B. longum*, *L. casei*, *S. boulardii*, *L. curvatus*, *L. plantarum*, and *L. reuteri* (Thushara et al., 2016). A meta-analysis including 11 randomized clinical trials showed that *L. acidophilus* reduced TC and LDL-c levels to a greater extent than other bacterial species, as well as that probiotic intervention duration of > 4 weeks could be more effective to improve these parameters (Shimizu et al., 2015). Another meta-analysis including 15 randomized clinical trials demonstrated that probiotic supplementation was effective in reducing TC, LDL-c, BMI, WC, and inflammatory markers. Additionally, it was concluded that probiotics consumption was more effective when delivered by fermented milk or yogurt, consumed for at least 8 weeks, used in multiple strains, as well as that *L. acidophilus* was the most effective species to reduce LDL-c levels (Sun and Buys, 2015).

16.2.3.12 Effects of probiotics on chronic kidney disease

Growing evidence has suggested that supplementation with probiotics may be a promising therapeutic strategy to attenuate chronic kidney disease (CKD) (Koppe et al., 2015). In a randomized double-blind controlled clinical trial with 33 patients undergoing hemodialysis, the intervention with a probiotic mix containing *S. thermophilus*, *L. acidophilus*, and *B. longum* at a dose of 9×10^{10} CFU/day for 3 months failed to reduce uremic toxins and inflammatory markers (Borges et al., 2018). In addition, a randomized double-blind controlled clinical trial with 22 nondialysis CKD patients, which evaluated the effects of the consumption of these same strains, dose, and intervention period, showed that uremic toxins were not influenced by the probiotics and the IL-6 plasma levels were increased after probiotic supplementation (de Faria Barros et al., 2018).

On the other hand, a randomized double-blind controlled clinical trial performed with 42 patients undergoing hemodialysis demonstrated that probiotic supplementation with *L. rhamnosus* at a dose of 1.6×10^7 CFU/day decreased uremic toxins (*p*-cresol and phenol) after 4 weeks of intervention (Eidi et al., 2018). A systematic review including 60 studies totalizing 645 adults with CKD showed conflicting findings on the available literature concerning the effects of probiotics on uremic toxins (Martin et al., 2019). Still, there is no consensus on the optimal dose, intervention time period, and specific probiotic strains to improve CKD condition, suggesting that probiotic therapy should be chosen with caution in CKD patients (Cavalcanti Neto et al., 2018).

16.2.3.13 Effects of probiotics on HIV infection

An open-label study with 30 HIV + men on antiretroviral therapy who received probiotic fermented milk drink containing *L. casei* Shirota (LcS) at a dose of 6.5×10^9 CFU/day for 4 weeks reported an increase of T lymphocytes and CD56+ cells and decreases in cytokine levels, tumor growth factor- β , and inflammatory marker cystatin-C (Falasca et al., 2015). Additionally, a double-blind, randomized, and placebo-controlled trial performed with 44 HIV + patients who received treatment with *S. boulardii* at a dose of 6×10^7 CFU/day for 12 weeks reported that LPS-binding protein (a marker of translocation) and IL-6 (a marker of inflammation) were decreased after the intervention (Vijaya Kumar et al., 2015).

A meta-analysis suggested that probiotic use could exert positive effects on increased CD4 counts, recurrence or management of bacterial vaginosis, and diarrhea management in human with immunodeficiency virus infection (Carter et al., 2016). However, there has not been enough evidence to recommend the use of probiotics by HIV-infected patients. Single-strain probiotics or multistrain probiotic mixtures and their respective dose to optimize outcomes have not yet been identified and longer duration studies are needed to select the best practices (Van den Nieuwboer et al., 2015).

Taken together, the data of available literature have overall demonstrated that probiotic administration may be a safe strategy for improvement of host health in several clinical conditions from pregnancy up to adult life. Schematic drawing showing the main clinical condition in humans who can benefit with probiotic therapy has been represented in Fig. 16.1.

16.3 Conclusion and future prospects

In our understanding, not all probiotic strains have the same efficacy for all clinical indications, thus only strains with confirmed efficacy and safety use should be recommended. In addition, more strict specific regulations addressing the medical benefit of probiotic strain should be developed to ensure an appropriate protection and rational use of probiotics to promote human health, as well as to serve consumers and market interests. The probiotics most studied to promote improvements in human health have been isolated from dairy products and human gastrointestinal tract. However, in the last decades,

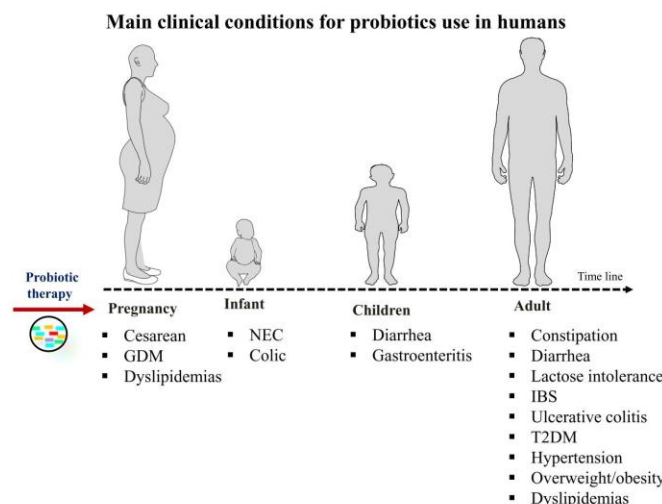


FIG. 16.1 Schematic drawing showing the main clinical condition in humans that can benefit with probiotic therapy. Gestational diabetes mellitus (GDM); necrotizing enterocolitis (NEC); irritable bowel syndrome (IBS); type 2 diabetes mellitus (T2DM).

potentially probiotic strains from plant sources have been also isolated, which should be the focus of studies considering their effects on human health. Lastly, an international bank of well-cataloged probiotic strains with well-defined clinical studies and information about the qualification of the deposited strains to a specific use based on their performance in clinical trials could assist the development of more effective products and success application for human health promotion.

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252 New and future developments in microbial biotechnology and bioengineering

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254 New and future developments in microbial biotechnology and bioengineering

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ANEXO A – CERTIFICADO DE APROVAÇÃO DO PROJETO PELA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DE LABORATÓRIO (CEUA)



Universidade
Federal da
Paraíba

Comissão de Ética no
Uso de Animais

Reitoria



CERTIFICADO

Certificamos que a proposta intitulada "Intervenção com mix de *Lactobacillus fermentum*: Uma nova terapêutica para prevenir o desenvolvimento de hipertensão arterial na prole de ratos submetidos à dislipidemia materna?", protocolada sob o CEUA nº 9492260418 (ID 000302), sob a responsabilidade de **José Luiz de Brito Alves e equipe; Yohanna de Oliveira** - 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 17/07/2018.

We certify that the proposal "Intervention with a mix of *Lactobacillus fermentum*: A new therapy to prevent the development of arterial hypertension in the offspring of rats submitted to maternal dyslipidemia?", utilizing 130 Heterogenics rats (60 males and 70 females), protocol number CEUA 9492260418 (ID 000302), under the responsibility of **José Luiz de Brito Alves and team; Yohanna de Oliveira** - 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 07/17/2018.

Finalidade da Proposta: [Pesquisa \(Acadêmica\)](#)

Vigência da Proposta: de [08/2018](#) a [08/2022](#) Área: [Nutrição](#)

Origem:	Unidade de Produção Animal - IPeFarM			
Espécie:	Ratos heterogênicos	sexo:	Fêmeas	idade: 80 a 100 dias N: 14
Linhagem:	Rattus Norvegicus - Wistar			Peso: 200 a 240 g
Origem:	Unidade de Produção Animal - IPeFarM			
Espécie:	Ratos heterogênicos	sexo:	Machos	idade: 90 a 150 dias N: 04
Linhagem:	Rattus Norvegicus - Wistar			Peso: 300 a 400 g
Origem:	Unidade de Produção Animal - IPeFarM			
Espécie:	Ratos heterogênicos	sexo:	Machos	idade: 85 a 95 dias N: 56
Linhagem:	Rattus Norvegicus - Wistar			Peso: 300 a 350 g
Origem:	Unidade de Produção Animal - IPeFarM			
Espécie:	Ratos heterogênicos	sexo:	Fêmeas	idade: 85 a 95 dias N: 56
Linhagem:	Rattus Norvegicus - Wistar			Peso: 190 a 230 g

Local do experimento: Os procedimentos experimentais apresentados no referido projeto serão realizados no Laboratório de Controle Neural da Circulação e Hipertensão Arterial (Lacncha) do Centro de Biotecnologia da UFPB, bem como na sala de experimentação em animais hipertensos do biotério Prof. Dr. Thomas George. O referido laboratório conta com uma área útil de 80 m², contendo ambientes para os registros da pressão arterial e frequência cardíaca equipados com mesas cirúrgicas para roedores, 3 sistemas para registro de pressão arterial, instrumental cirúrgico 2 aparelhos estereotáticos, balanças, 1 micrótomo criostato, 1 máquina de gelo, 1 equipamento de PCR em tempo real, 1 fotodocumentador de gel, 1 ultrassom portátil, 1 luminômetro, 1 sistema para registro da ventilação, 1 caixa pletismográfica, 1 sistema de cubas para banho de órgãos, 1 sistema de telemetria para registro da pressão arterial e frequência cardíaca, 1 sistema de registro de nervos, 1 microscópio estereoscópico, 1 microscópio invertido de fluorescência, 1 máquina de ventilação artificial, 1 sistema de anestesia inalatória, 1 bomba peristáltica, 4 computadores e 1 impressora multifuncional. A estrutura laboratorial apresentada possibilitará a execução de todos os procedimentos experimentais propostos neste projeto.

João Pessoa, 25 de julho de 2018



**Universidade
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Uso de Animais**

Reitoria



Islandia Gisela A. Gonçalves

Profa. Dra. Islandia Gisela Albuquerque Gonçalves
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal da Paraíba

Ricardo Romão Guerra

Prof. Dr. Ricardo Romão Guerra
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal da Paraíba