Universidade de São Paulo
Faculdade de Saúde Pública

**Eventos precoces da vida, padrões alimentares e sua relação
com o perfil cardiometabólico de jovens**

Ilana Eshriqui Oliveira

**Tese apresentada ao Programa de Pós-Graduação em
Nutrição em Saúde Pública para obtenção do título de
Doutor em Ciências**

Área de concentração: Nutrição em Saúde Pública

**Orientadora: Professora Titular Sandra Roberta G.
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Banca Examinadora:

Prof. Dr.: Patrícia Helen de Carvalho Rondó

Instituição: Faculdade de Saúde Pública/ Universidade de São Paulo

Julgamento: Aprovada

Prof. Dr.: Alexandre Archanjo Ferraro

Instituição: Faculdade de Medicina/ Universidade de São Paulo

Julgamento: Aprovada

Prof. Dr.: Ana Carolina Junqueira Vasques

Instituição: Universidade Estadual de Campinas

Julgamento: Aprovada

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RESUMO

Eshriqui, I. Eventos precoces da vida, padrões alimentares e sua relação com o perfil cardiometabólico de jovens [tese]. São Paulo: Faculdade de Saúde Pública, Universidade de São Paulo; 2020.

Introdução: O índice de massa corporal (IMC) materno pré-gestacional e o aleitamento materno (AM) parecem influenciar o perfil cardiometabólico da prole no curso da vida, interferindo na composição da microbiota intestinal e formação de hábitos alimentares. **Objetivos:** Examinar associações do IMC materno pré-gestacional e AM, assim como de padrões alimentares (PA) atuais com o perfil cardiometabólico da prole no longo prazo. Objetivos específicos, descritos nos artigos desta tese, foram: **I)** avaliar associação entre o IMC materno pré-gestacional e a composição corporal da prole feminina na vida adulta; **II)** identificar PA de mulheres jovens e analisar se são influenciados pela duração do AM; **III)** avaliar a associação do AM e dos PA atuais com marcadores do metabolismo glico-lipídico; **IV)** verificar se o tipo de AM durante os primeiros 6 meses de vida está associado com a diversidade e composição da microbiota oral de adolescentes. **Métodos:** Os objetivos I, II e III foram analisados com dados da linha de base do *Nutritionists' Health Study*, no qual graduandas de nutrição e nutricionistas ≤ 45 anos foram elegíveis. Aquelas com câncer, diabetes, ou sem informação para variáveis de interesse foram excluídas, restando 587 participantes. IMC materno pré-gestacional, AM, assim como aspectos atuais sociodemográficos, de estilo de vida e estado de saúde foram obtidos via questionários *online*. Questionário de frequência alimentar validado foi aplicado e PA foram identificados por análise fatorial por componentes principais. Uma sub-amostra compareceu à clínica para coleta de sangue para avaliação do metabolismo glicídico e lipídico ($n=200$) e da composição corporal por DXA ($n=150$). Modelos múltiplos de regressão linear e logística foram construídos considerando conjunto de ajustes mínimos suficientes sugeridos por gráficos acíclicos direcionados. O objetivo IV foi avaliado com dados de 423 adolescentes finlandeses, participantes do *Finnish Health in Teens Study Cohort*. O tipo de AM (apenas AM vs. AM+formula ou apenas formula) foi obtido retrospectivamente através dos pais. A microbiota da saliva foi analisada por sequenciamento de 16S rRNA. ANCOVA e PERMANOVA foram usadas para comparar a diversidade e modelos lineares generalizados para a abundância entre os grupos. **Resultados:** Participantes do NutriHS tinham mediana de idade de 22 (IIQ 20;27) anos, IMC de 22,2 (IIQ 20,4; 25,0) kg/m^2 e parâmetros metabólicos em média dentro da normalidade. **I)** O IMC materno pré-gestacional se associou diretamente com adiposidade geral (% gordura corporal e índice de massa gorda) e central (razão gordura andróide/ginoide e volume do tecido adiposo visceral) de suas filhas, mas não com a massa magra apendicular e óssea. **II)** Quatro PA foram identificados (Processado, Prudente, Brasileiro e Lacto-vegetariano) representando 27% da variância explicada da dieta. Mulheres que receberam AM por <6 meses apresentaram menor chance de apresentar adesão moderada-a-alta ao padrão Prudente em comparação às que receberam AM por ≥ 12 meses. AM não foi associado aos demais PA. **III)** Mulheres que receberam AM por menos de 6 meses apresentaram maior chance de classificação no maior tercil de insulinemia comparadas às amamentadas por ≥ 6 meses. AM predominante <3 meses foi diretamente associado com a insulinemia e HOMA-IR. Em relação aos PA atuais, o Processado se associou diretamente com a concentração de LDL-c, enquanto o Prudente inversamente com LDL-c e razão LDL-c/HDL-c. **IV)** Média de idade e IMC dos participantes foi 11,7 anos e de 18,0 kg/m^2 . Não houve diferença de diversidade na microbiota oral segundo o tipo de AM nos primeiros 6 meses de vida. Três OTUs (do inglês, *Operational Taxonomic Unit*) pertencentes aos gêneros Eubactéria e Veillonella (filo Firmicutes) foram mais abundantes no grupo que recebeu apenas AM comparado com os que receberam fórmula. **Conclusão:** O estado nutricional materno pré-gestacional e os hábitos alimentares desde o início da vida têm potencial para influenciar o perfil cardiometabólico de longo prazo. As evidências obtidas em participantes ainda jovens e aparentemente saudáveis reforçam a necessidade de prevenção e avaliação precoce de fatores de risco cardiometabólico.

Palavras chave: Padrões alimentares, aleitamento materno, risco cardiometabólico, DOHaD, microbiota.

ABSTRACT

Eshriqui, I. Early life events, dietary patterns and its association with cardiometabolic profile in youngsters [thesis]. São Paulo: School of Public Health, University of Sao Paulo; 2020

Introduction: Maternal pre-pregnancy body mass index (BMI) and breastfeeding seem to influence offspring cardiometabolic profile throughout life, interfering on gut microbiota composition and development of eating habits. **Aim:** To examine associations of maternal pre-pregnancy BMI and breastfeeding, as well as of current dietary patterns with long-term cardiometabolic profile. Specific aims, described in thesis manuscripts, were: **I)** to evaluate association of maternal pre-pregnancy BMI with female offspring body composition at adulthood; **II)** to identify dietary patterns of young women and analyse if adherence was influenced by breastfeeding duration; **III)** to evaluate association of breastfeeding and of current dietary patterns with biomarkers of glucose and lipid metabolism; **IV)** to verify if type of feeding during the first 6 months of life was associated with oral microbiota diversity and composition of adolescents. **Methods:** The aims I, II and III were evaluated using Nutritionists' Health Study baseline data, in which nutrition students and nutritionists ≤ 45 years old were eligible. Those with cancer, diabetes, or missing information for variables of interest were excluded, resulting in 587 participants. Maternal pre-pregnancy BMI, total and predominant breastfeeding, as well as current sociodemographic, lifestyle and health aspects were obtained through online questionnaires. Validated food frequency questionnaire was applied, and dietary patterns were identified using factor analysis by principal components. A sub-sample visited the clinic for determinations of plasma glucose and lipid parameters ($n=200$) and body composition assessment by DXA ($n=150$). Multiple linear and logistic regression models were constructed considering minimum sufficient adjustments suggested by directed acyclic graphs. The aim IV was assessed with data from 423 adolescents from the Finnish Health in Teens Study Cohort. The type of breastfeeding (No Formula vs. Formula, combined or not with breastfeeding) was retrospectively obtained. Saliva microbiota was analysed by 16S rRNA sequencing. ANCOVA and PERMANOVA were used to compare diversity and generalized linear models to verify differential abundance between groups. **Results:** NutriHS participants had median age of 22 (IQR 20; 27) years, BMI of 22.2 (IQR 20.4; 25.0) kg/m^2 and metabolic parameters were in average within normal range. **I)** Maternal pre-pregnancy BMI was directly associated with general (% body fat and fat mass index) and central (android-to-gynoid ratio and visceral adipose tissue) adiposity measures of their daughters, but not with appendicular muscle mass and bone mass. **II)** Four dietary patterns were identified (Processed, Prudent, Brazilian and Lacto-vegetarian), representing 27% of diet explained variance. Women who were breastfed for <6 months had lower chance to have moderate-to-high adherence to the Prudent pattern compared to those who were breastfed for at least 12 months. Breastfeeding was not associated to other patterns. **III)** Women breastfed for <6 months had higher chance of being classified into the highest tertile of insulin compared to those breastfed for at least 6 months. Predominant breastfeeding <3 months was directly associated with insulin concentration and HOMA-IR. Regarding current dietary patterns, the Processed pattern was directly associated with LDL-c concentration, while the Prudent pattern was inversely associated with LDL-c and LDL-c/HDL-c ratio. **IV)** Mean age and BMI of participants were 11.7 and 18.0 kg/m^2 . Diversity of oral microbiota was not different according to type of feeding during the first 6 months of life. Three Operational Taxonomic Units (OTU) belonging to Eubacteria and Veillonella genera (phylum Firmicutes) were more abundant in only breastfeeding group compared to participants who received infant formula. **Conclusion:** Maternal pre-pregnancy nutritional status and dietary habits since early life have potential to influence the cardiometabolic profile in the long term. Evidence obtained in young and healthy participants reinforce necessity of prevention and early assessment of cardiometabolic risk factors.

Keywords: Dietary patterns, breastfeeding, cardiometabolic risk, DOHaD, microbiota.

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SIGLAS

ACP – Análise fatorial por componentes principais

AM – Aleitamento materno

ASM - *Appendicular skeletal muscle mass*

ASMI - *Appendicular skeletal muscle mass index*

CT – Colesterol total

DAG – Gráficos acíclicos causais (*Directed acyclic graph*)

DCNTs – Doenças crônicas não transmissíveis

DCV – Doenças cardiovasculares

DM – Diabetes mellitus

DOHaD - *Developmental Origins of Health and Disease*

DXA - *Dual-energy X-ray absorptiometry*

FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo

Fin-HIT- *The Finnish Health in Teens study cohort*

FMI - *Fat mass index*

HDL-c - Colesterol da lipoproteína de alta densidade (*high density lipoprotein cholesterol*)

HOMA-IR - *Homeostasis model assessment – insulin resistance*

IIQ – Intervalo Interquartil

IMC – Índice de massa corporal

KMO - *Kaiser-Meyer-Olkin*

LDL-c – Colesterol da lipoproteína de baixa densidade (*low density lipoprotein cholesterol*)

NHANES - *National Health and Nutrition Examination Survey*

NutriHS - *Nutritionists' Health Study*

OTU - *Operational Taxonomic Unit*

QFA - Questionário de frequência alimentar

R24h - Recordatório alimentar de 24 horas

TCLE - Termo de consentimento livre e esclarecido

TG – Triglicerídeos

VAT – *Visceral adipose tissue*

USDA - United States Department of Agriculture

APRESENTAÇÃO

A presente tese, desenvolvida com apoio financeiro da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), inclui sub-estudos derivados de dois estudos maiores intitulados *Nutritionists' Health Study* (NutriHS) e *Finnish Health in Teens study cohort* (Fin-HIT). O NutriHS trata-se de uma coorte desenvolvida com estudantes e egressos de cursos de graduação em Nutrição da cidade de São Paulo com o objetivo maior de investigar prospectivamente potenciais fatores de risco para doenças crônicas não transmissíveis. O Fin-HIT é uma coorte de crianças e adolescentes, representativa das áreas mais populosas da Finlândia, cujo objetivo principal é identificar fatores de risco precoces para o desenvolvimento de excesso de peso.

A linha de base do NutriHS ocorreu entre 2015 e 2018 (com apoio financeiro da FAPESP – processo nº 2015/10045-7), quando foram coletadas informações atuais das participantes e progressas sobre eventos precoces da vida. Encontram-se disponíveis dados referentes ao estado nutricional pré-gestacional das mães, amamentação e estilo de vida atual, particularmente no que diz respeito à dieta e atividade física. O NutriHS conta com profissionais treinados e equipamentos acurados para realização de avaliações clínicas em materiais biológicos e por métodos de imagem (composição corporal pela DXA). O fato de ser desenvolvido com indivíduos da área da nutrição – conscientes da relevância científica dos resultados advindos de pesquisa e familiarizados com os instrumentos da pesquisa – deve contribuir para adesão e fidedignidade de suas respostas. O NutriHS é promissor no sentido de explorar a relação entre eventos precoces da vida, hábitos alimentares atuais e desfechos de saúde na vida adulta. Análises deste estudo originaram os três primeiros artigos que compõem a presente tese.

Resultados da linha de base do Fin-HIT deram origem a um quarto artigo desenvolvido durante o período sanduíche (BEPE-FAPESP, processo nº 2018/24278-1) no Folkhälsan Research Center, em Helsinque, Finlândia. Na linha de base do Fin-HIT (2011–2014) foram recrutados aproximadamente 11.400 adolescentes e 9.900 pais, que responderam questões sobre eventos precoces da vida de seus filhos, como por exemplo, o tipo de aleitamento materno. Amostras de saliva foram coletadas para uma sub-amostra aleatória de adolescentes. Dados do Fin-HIT possibilitaram avaliar a relação do aleitamento materno com a microbiota oral em longo prazo, até o momento ainda não investigada na literatura.

Esta tese está estruturada nos capítulos: resumo/abstract, introdução, revisão de literatura, justificativa, objetivos, métodos, resultados, considerações finais, referências bibliográficas e anexos. O capítulo de resultados foi composto pelos quatro manuscritos científicos, frutos do desenvolvimento desta pesquisa.

1 INTRODUÇÃO

As dinâmicas de urbanização e desenvolvimento tecnológico ocorridos em diversos países, entre eles o Brasil, foram acompanhadas por processos de transição nutricional e epidemiológica (1-3). Alterações no perfil de alimentação, caracterizada por aumento da densidade energética da dieta e do consumo de alimentos ultra-processados, com alto teor de açúcares refinados, sódio, gorduras saturadas e trans e por redução de alimentos *in natura* e com elevado teor de fibras, concorreram para mudanças no estado nutricional da população (4, 5). Este cenário, combinado ao envelhecimento populacional, foi decisivo para a transição epidemiológica com redução da morbi-mortalidade devido às doenças infecciosas e aumento da prevalência de doenças crônicas não transmissíveis (DCNTs) (3, 5). DCNTs como a doença cardiovascular aterosclerótica (DCV), hipertensão arterial, diabetes mellitus (DM), câncer e outras associadas à obesidade estão entre os principais problemas mundiais de saúde pública (6). Além dos hábitos alimentares inadequados, inatividade física e outras características do moderno estilo de vida influenciam a composição corporal, contribuindo para o acúmulo de gordura corporal. A obesidade, especialmente a de distribuição central, gera estado de inflamação subclínica e resistência à insulina, presentes na fisiopatologia de doenças cardiometabólicas (7-9). Este estado se reflete em anormalidades das concentrações sanguíneas de glicose e lipídios envolvidos na patogênese destas doenças (10).

Hoje se sabe que a importância da alimentação como fator de risco para morbidades configura-se desde a vida intra-uterina. Eventos precoces da vida – tais como obesidade materna, tipo de parto e aleitamento materno – também têm sido alvos de pesquisa sobre o risco de doenças no adulto, havendo evidências de mecanismos epigenéticos (11) e participação na composição da microbiota intestinal (12, 13). Apesar das dúvidas quanto aos mecanismos, os estudos sugerem fortemente atuação do aleitamento materno como fator protetor contra o desenvolvimento de doenças cardiometabólicas (14-16). Sendo assim, é desejável aprofundar o conhecimento sobre como fatores nutricionais precoces influenciariam os clássicos fatores de risco cardiovascular na vida adulta, diretamente ou indiretamente, via desenvolvimento da microbiota e/ou formação de padrões alimentares.

Tendo em vista a complexidade do estudo de associações entre exposições no início da vida e desfechos na vida adulta e ainda a quantidade de variáveis a serem consideradas neste contexto, recursos metodológicos estratégicos têm sido testados. Dentre estes, o uso de gráficos

acíclicos causais (DAG) tem sido crescente na literatura e especialmente recomendado no campo da epidemiologia. DAG trata-se de um método analítico que possibilita estimar conjuntos mínimos de variáveis de ajuste a serem considerados durante a investigação da relação causal de interesse com base no conhecimento do pesquisador acerca da evidência científica disponível no tema lançando mão de rigorosos critérios matemáticos (17). Desse modo, ajustes insuficientes ou excessivos de modelos estatísticos são evitados, minimizando erros e vieses na interpretação de resultados dos estudos epidemiológicos (17-19).

1.1 EVENTOS PRECOCES DA VIDA E DESFECHOS DE SAÚDE NA VIDA ADULTA

É de longa data a observação de que eventos precoces da vida inclusive a intra-uterina associavam-se a morbidades nos adultos. A prole de mulheres submetidas à escassez de alimentos na Segunda Guerra Mundial nos primeiros seis meses de gestação apresentaram incidência de obesidade significativamente maior do que aqueles cujas mães sofreram desnutrição nos últimos três meses de gestação (20). Tais observações sugeriam haver adaptações metabólicas precocemente no feto que quando adulto o predisporia a doenças crônicas. O ser humano em formação seria capaz de prever o ambiente no qual cresceria, por meio de sinais hormonais maternos oriundos da placenta e da lactação. Em meados de 1990, Barker propôs que doenças crônicas seriam biologicamente programadas no útero materno e/ou no primeiro ano de vida (21). Denominou-se “programação fetal” o processo em que uma exposição em períodos críticos de desenvolvimento teria repercussão na estrutura e/ou na função de órgãos e sistemas. Estudos posteriores mostraram a associação de baixo peso ao nascer com DCNTs na vida adulta, como DM tipo 2 e DCV (22, 23). Surgia, assim, a teoria das origens da saúde e da doença no desenvolvimento fetal e infantil (do inglês, *Developmental Origins of Health and Disease* - DOHaD), defendendo que exposições durante os primeiros mil dias de vida associar-se-iam ao estado de saúde na vida adulta (24).

Este novo ramo científico ou DOHaD encontra embasamento na epigenética, na qual estudam-se alterações hereditárias na expressão gênica não decorrentes de anormalidades na sequência de nucleotídeos do DNA, mas sim de condições ambientais. Baseia-se no conceito de plasticidade, ou seja, a capacidade de um genótipo originar diferentes estados morfológicos ou fisiológicos em resposta a exposições diversas durante o desenvolvimento. Este período de exposição a certos fatores determinaria programações metabólicas no organismo que poderiam

predispor à ocorrência de doenças no adulto (25, 26). Nesse sentido, o estado nutricional da mãe no período da concepção, bem como eventos ocorridos intra-útero e após o nascimento, como o aleitamento materno ou uso de fórmulas infantis, têm sido relacionados a programações metabólicas na prole que predisporiam, no longo prazo, à ocorrência de DCNTs. Dentre as exposições adversas na gestação, cabe destacar toxinas provenientes do uso de tabaco ou álcool e oferta insuficiente ou excessiva de nutrientes ao feto, as quais impactam no peso ao nascer (14, 25, 27, 28). O tipo de alimentação (aleitamento materno ou fórmulas) nos primeiros meses de vida é apontado como importante preditor das condições de saúde pós-natal, que, combinado com a velocidade de crescimento infantil, têm sido associados ao risco de obesidade, DM e DCV no adulto (10, 14, 27). Assim, os primeiros mil dias de vida, contados a partir da concepção até o segundo ano pós-natal têm sido vistos como importante janela de oportunidades para prevenção de problemas de saúde pública mundiais.

Apesar das evidências disponíveis, os mecanismos destas associações não foram completamente elucidados. O risco aumentado de doenças cardiometabólicas no adulto é explicado pela incompatibilidade entre a disponibilidade de nutrientes nos ambientes intrauterino e pós-natal (*mismatch hypothesis*). Nesse sentido, a oferta restrita de nutrientes ao feto no intra-útero, comumente expressa por baixo peso ao nascer, induziria uma programação fetal para um fenótipo metabolicamente econômico (*thrifty pathway*). Se após o nascimento, depara-se com ambiente onde há abundância de nutrientes sua programação metabólica do período intra-útero está inadequada (*mismatched*). O uso de fórmulas infantis em substituição ao leite materno promove crescimento em velocidade superior àquela decorrente do aleitamento natural (29). O crescimento acelerado na infância, especialmente se combinado com um baixo peso ao nascimento, contribui para aumentar o risco de doenças cardiometabólicas ao longo da vida (27, 30). Modelos experimentais comprovam que restrições alimentares durante a gestação provocam alterações epigenéticas no feto e reduções nas reservas funcionais em diversos territórios orgânicos com reflexos ao longo da vida (31-34). Por outro lado, a transmissão excessiva de nutrientes para o feto, característica dos casos de obesidade materna e/ou diabetes gestacional, também se mostra associada às modificações epigenéticas e ao aumento de risco de doenças metabólicas ao longo da vida (11, 35).

Apesar de amplas evidências de que o aleitamento materno confere proteção contra exagerado ganho de peso com benefícios cardiovasculares tardios, em países de baixo e médio

desenvolvimento ainda existem controvérsias (27, 36-41). Ademais, o quanto o tipo e a duração da amamentação influenciariam o padrão alimentar do indivíduo no começo da vida adulta, influenciando sua adiposidade corporal, foi pouco estudado (25, 27, 42, 43).

Em suma, hoje se reconhece que a ocorrência e gravidade de desfechos no adulto dependem de predisposição genética, mas também da exposição a fatores ambientais e do período em que esta ocorre (44). Seu risco de doença é resultante do grau de concordância entre o ambiente previsto durante o período de alta plasticidade no desenvolvimento e o ambiente exposto no curso de sua vida.

1.2 RELAÇÃO ENTRE ESTADO NUTRICIONAL MATERNO PRÉ-GESTACIONAL E A COMPOSIÇÃO CORPORAL DA PROLE

A obesidade é um crescente problema global de saúde pública, sendo maior a prevalência entre as mulheres comparada a homens, especialmente naquelas em idade fértil (45). O excesso de peso no início da gravidez tem sido associado a diversas complicações gestacionais, contribuindo para a morbidade materna e a mortalidade infantil, bem como para o aumento do risco de doenças cardiometabólicas (46, 47).

A partir de evidências do DOHaD, outros determinantes, que não os tradicionalmente relacionados ao estilo de vida, passaram a ser estudados na tentativa de explicar o crescimento acelerado da obesidade no mundo. A literatura sugere a existência de um ciclo vicioso de transmissão intergeracional da obesidade (11, 35). Os mecanismos para tal fenômeno ainda não estão completamente elucidados e comprovação de causalidade ainda é necessária. Componentes genéticos impactam no risco desta doença (48), mas sabe-se que a programação metabólica decorrente de modificações epigenéticas ocorridas durante os primeiros 1000 dias de vida, desempenha papel relevante (11, 49, 50). Há evidências de que a obesidade materna acarrete exposição fetal a um ambiente pró-inflamatório e de resistência à insulina induzindo modificações que resultariam em aumento do risco de obesidade ao longo da vida (50, 51). Entre as modificações epigenéticas mais frequentes destacam-se a metilação do DNA, modificação de histonas e ação de RNAs não codificadores (microRNAs) (11). Na vida pós-natal, destaca-se também o papel do ambiente familiar e estilo de vida materno, principalmente relacionado aos hábitos alimentares, que, uma vez compartilhados, influenciam os comportamentos da prole (48, 52, 53).

Nesta perspectiva, alguns estudos têm encontrado a associação entre o índice de massa corporal (IMC) materno pré-gestacional e a adiposidade da prole durante a infância (54-57). Na vida adulta, esta relação foi raramente investigada (58, 59). O IMC materno pré-gestacional também já foi diretamente associado com adiposidade visceral da prole na infância (54, 56), mas tal associação não foi descrita em adultos.

Sabe-se que particularmente o tecido adiposo visceral desempenha papel relevante na geração de inflamação sistêmica e gênese de doenças metabólicas (60). Existem evidências de que mesmo em jovens adultos aparentemente saudáveis a adiposidade visceral excessiva pode prejudicar o perfil metabólico (61). Assim, a quantificação da adiposidade visceral em jovens pode ser útil para antecipar a detecção daqueles de maior risco e a prevenção de distúrbios cardiometabólicos.

Relatos de associações do IMC materno pré-gestacional com os componentes corporais livres de gordura (massa muscular apendicular e óssea) da prole são escassos e os poucos resultados são controversos (55, 57, 58). Entre os estudos conduzidos para investigar estas associações apenas um apresenta resultados da prole em idade adulta (58). Tal estudo, conduzido com famílias suecas, verificou associação direta entre o IMC materno pré-gestacional e massa magra das filhas no início da idade adulta, mas a associação não foi significativa para os descendentes do sexo masculino (58). Por outro lado, estudos conduzidos na infância (55, 57) e uma meta-análise (62) relataram associação direta entre IMC materno e massa magra da prole. As controvérsias podem ser atribuídas a diferentes características dos indivíduos e abordagens técnicas para avaliar a composição corporal. Associação direta entre o IMC materno pré-gestacional e massa óssea da prole na infância também já fora demonstrada, embora tenha sido explicada principalmente pela altura e peso da criança, desconhecendo-se se seria mantida na idade adulta (63). Até o momento, em um único estudo, não foram avaliadas associações do IMC materno pré-gestacional com os três compartimentos corporais da prole adulta, usando método acurado de quantificação do tecido adiposo visceral.

1.3 NASCIMENTO E O ALEITAMENTO MATERNO: RECOMENDAÇÕES, FREQUÊNCIA E ASSOCIAÇÃO COM DESFECHOS TARDIOS

Está bem estabelecida a relação entre baixo peso ao nascer e desfechos cardiometabólicos no longo prazo (64-66). Fatores maternos e placentários, dentre estes

redução da disponibilidade de nutrientes, podem limitar o crescimento fetal. A nutrição fetal insuficiente acarreta uma adaptação de desenvolvimento que, por sua vez, programa permanentemente a estrutura fetal, sua fisiologia e seu metabolismo em favor da sua sobrevivência, culminando em baixo peso ao nascer (27, 30, 31). Também fatores pós-natais tais como o tipo de parto e a amamentação podem influenciar as condições resultantes desta programação (42).

A associação entre parto cesárea e obesidade já foi relatada em adolescentes e na vida adulta (67-69). Uma das explicações se baseia no fato dos tipos de parto, normal ou cesárea, impactarem diferentemente na composição da microbiota intestinal do recém-nascido a qual modificará seu risco de obesidade (13, 70, 71). Além disso, menores taxas de iniciação do aleitamento materno têm sido relatadas após cesáreas em comparação com o parto vaginal (72).

Apesar da recomendação da Organização Mundial da Saúde (OMS) de aleitamento materno exclusivo até seis meses de vida e complementar até dois anos ou mais, dados do começo do século revelaram que a prevalência mundial de aleitamento materno exclusivo até quatro meses era inferior a 35% (73). Revisão sistemática verificou que em 2010 apenas 37% das crianças com menos de seis meses de países de baixo e médio desenvolvimento recebiam aleitamento materno exclusivo (74). Dados coletados em 2008 com representatividade das capitais brasileiras e do distrito federal revelaram prevalência de 41% de aleitamento materno exclusivo em menores de 6 meses (75). No sudeste do Brasil a prevalência foi similar (39,4%) (76), porém, em estudo conduzido em Santa Catarina, observou-se discrepância entre faixas etárias, de modo que 31% das crianças entre quatro e cinco meses recebiam aleitamento materno exclusivo, enquanto apenas 13% daquelas com idade entre cinco e seis meses (77). Em relação à duração do aleitamento materno, observou-se mediana de 11,2 e 9,6 meses no Brasil e em São Paulo, respectivamente, sendo que apenas 58,7% das crianças brasileiras entre 9 e 12 meses eram amamentadas em 2008, e esta prevalência foi ainda menor na região Sudeste e em São Paulo (51,4% e 48,7%, respectivamente) (76).

Considerando o importante papel do metabolismo glicídico e lipídico no risco cardiovascular, destaca-se como desfecho de interesse de estudos que investigam os efeitos do aleitamento materno na saúde em longo prazo. No entanto, poucos estudos foram conduzidos com adultos (39, 78-81). Há evidências de associação inversa entre amamentação e colesterolemia nesta fase da vida (78, 81); por outro lado, durante a infância esta associação

parece ser direta (78). Levantou-se a hipótese de que a maior concentração de colesterol no leite humano comparado às fórmulas infantis desencadearia mecanismo adaptativo de menor produção endógena de colesterol, favorecendo menores níveis sanguíneos na vida adulta (78). Em adultos da terceira geração da coorte de Framingham não foi observada associação entre amamentação e as concentrações de colesterol total (CT) e triglicerídeos (TG), mas os indivíduos amamentados apresentaram maiores valores de HDL-c comparados aos que não receberam leite materno (79). Apesar destas evidências, estudos recentemente publicados não indicaram associação entre o aleitamento materno e o perfil lipídico na vida adulta (74, 82). A literatura já sugeriu efeito protetor do aleitamento materno também sobre o DM mas de forma inconsistente e por mecanismos desconhecidos. Apesar de evidências indicarem associação inversa do aleitamento materno com a insulinemia, nenhuma relação foi observada com a glicemia de jejum em adultos (80, 81). A inconsistência nos resultados pode ser parcialmente atribuída à ausência de ajustes para variáveis de confusão relacionadas à decisão materna de amamentar, estilo de vida, status socioeconômico da família e peso ao nascer (78-81). Em metanálise recente, os autores concluíram que é possível que o aleitamento materno atue como fator protetor contra obesidade e DM em longo prazo mas que as associações ainda não são convincentes devido ao confundimento residual nas análises (74).

O aleitamento materno também vem sendo relacionado às preferências e hábitos alimentares a curto e longo prazo (43, 83-86). Acredita-se que a amamentação influencie o apetite ao longo da vida. A presença de leptina no leite humano mas não nas fórmulas infantis é um dos fatores que relacionam a amamentação à programação hipotalâmica de mecanismos de saciedade, controlando assim a ingestão alimentar (87, 88). Algumas evidências apoiam a participação da leptina na programação do sistema central de recompensa, sendo a formação das preferências alimentares (especialmente por alimentos hiperpalatáveis) influenciada pela exposição a fatores nutricionais durante o período perinatal, particularmente dietas maternas com elevado teor de açúcar e/ou gordura (89). Outra hipótese baseia-se no fato de que a exposição à diversidade de *flavors* da dieta materna durante a amamentação é importante para formação das preferências alimentares de seus filhos, capaz de aumentar a propensão de aceitação de alimentos no início da vida (89, 90).

Estudos indicam que ter recebido aleitamento materno, exclusivo ou não, associa-se ao consumo alimentar na infância (83, 84). Crianças amamentadas por mais de quatro meses

apresentaram maior probabilidade de ingerir frutas e hortaliças e menor frequência de consumo de laticínios com adição de açúcar, carne, pão branco, refrigerantes, barras de chocolate e salgadinhos fritos em comparação com as não amamentadas (84). Outro estudo confirmou que crianças que receberam aleitamento materno exclusivo consumiam frutas e vegetais com maior frequência que as que receberam fórmulas infantis em complementação ao leite materno antes da introdução dos alimentos sólidos (83). Avaliando-se o consumo alimentar de 1142 crianças, a maior duração da amamentação associou-se com preferências alimentares por carnes, peixes e ovos aos cinco anos de idade mas não por frutas e vegetais (85). Numa coorte francesa de crianças, tanto a maior duração do aleitamento materno, como introdução tardia da refeição principal e uso de alimentos preparados em casa no primeiro ano de vida associaram-se diretamente à adesão a um padrão adequado às recomendações alimentares e inversamente a um padrão caracterizado pelo consumo de alimentos processados entre 2 e 5 anos de idade (91). Concordantemente, uma coorte de nascimentos escocesa encontrou que crianças que receberam aleitamento materno comparadas com as não amamentadas ou que receberam alimentação complementar após quatro meses, apresentaram aos dois anos de idade maior chance de pertencer a um *cluster* saudável, caracterizado por maior variedade de frutas e vegetais (92). Da mesma forma, estudo australiano transversal de base populacional de crianças entre dois e oito anos de idade verificou associação direta entre amamentação e adesão a um padrão alimentar saudável (93).

Um longo seguimento de 21 anos de coorte finlandesa sugere uma estabilidade dos padrões alimentares identificados na infância, adolescência e vida adulta, apesar do aleitamento materno não ter sido avaliado (94). Estudos em adultos sobre a relação do aleitamento materno com o comportamento alimentar são escassos (43, 86, 95). Indivíduos entre 18 e 28 anos que receberam aleitamento materno exclusivo mais prolongado tiveram maior chance de realizar o desjejum >5 vezes/semana e comer lanches fritos com frequência <1 vez/semana (86). Em contrapartida, Victora et al (2003) não verificaram associação entre a amamentação e os hábitos alimentares de homens aos 18 anos de idade. Destaca-se que entre os estudos conduzidos com adultos apenas um verificou a relação entre a amamentação e o consumo alimentar avaliado por métodos estatísticos para identificação de padrões alimentares (43). Neste estudo, o tipo de alimentação de 3217 crianças nascidas no Reino Unido na década de 1930 foi avaliado no primeiro ano de vida e categorizado como: apenas aleitamento materno; aleitamento materno e

mamadeira; apenas mamadeira. Indivíduos aos 53 a 79 anos de idade que pertenceram às duas últimas categorias associaram-se inversamente com a adesão ao padrão ‘Prudente’. Naquela época, a recomendação da duração de aleitamento materno exclusivo era maior que as atuais, a composição das fórmulas infantis era diferente e a qualidade da informação sobre a duração da amamentação era bastante limitada.

Frente a este cenário, justifica-se buscar evidências mais consistentes sobre possível influência da alimentação no início da vida na definição do consumo alimentar na vida adulta. Assumindo-se que hábitos alimentares adquiridos precocemente devam ser influenciados pelo aleitamento materno (83, 84) e que estes impactam até a fase adulta (94), a confirmação destas relações no longo prazo tem implicações para saúde pública, tornando a recomendação quanto ao aleitamento ainda mais relevante. Até onde conhecemos, análise da relação entre amamentação e alimentação na vida adulta, por meio de padrões alimentares para avaliação do consumo, foi realizada em um único estudo, datado do início do século passado (43).

1.4 DESENVOLVIMENTO DA MICROBIOTA INTESTINAL E SUA RELAÇÃO COM O ESTADO DE SAÚDE

O número de microorganismos que compõem nosso organismo supera em dez vezes o número de células humanas, os quais localizam-se principalmente no trato gastrointestinal (96). Evidências indicam que a microbiota intestinal é estabelecida durante os primeiros dois anos de vida, atingindo estabilidade no terceiro ano (97, 98). Na última década, o papel da microbiota no contexto do DOHaD tem sido amplamente estudado (99, 100). Inicialmente a hipótese higienista das doenças alérgicas propunha que infecções no início da vida influenciariam o desenvolvimento do sistema imune. Com os avanços da microbiologia, este conceito foi expandido e passou a ser conhecido como teoria da microflora, na qual a exposição a fatores ambientais no início da vida teriam potencial para alterar o desenvolvimento da microbiota (99, 100).

Nesta linha, alterações na composição da microbiota no início da vida poderiam influenciar a maturação do sistema imune, resultando em estado de hipersensibilidade e/ou hiperinflamação (99). De fato, numerosas evidências indicam que exposição a fatores disruptores da microbiota nesta fase da vida se associa com doenças inflamatórias ou imunomediadas, como alergia, asma e obesidade no curso da vida (100-104). Em analogia com

a janela de oportunidade para programação metabólica prevista na teoria DOHaD, estes achados sugerem existir também um período crítico no desenvolvimento da microbiota. Neste contexto, a microbiota passa a desempenhar papel mediador importante entre eventos precoces da vida e o risco de doenças no longo prazo (53, 99).

A exposição a fatores ambientais, considerados possíveis disruptores da microbiota, pode ocorrer no período pré- e pós-natal. A modulação da microbiota intestinal durante os dois primeiros anos de vida é influenciada pela disbiose materna, tipo de parto, uso de antibióticos intraparto e durante a infância, desmame precoce ou uso de fórmulas infantis e tipo de alimentação durante introdução da alimentação complementar (12, 100).

O impacto do leite materno é um dos mais estudados, dado que além de ser uma fonte de transmissão de microbiota materna para a prole, possui oligossacarídeos com importante ação prebiótica, promovendo o crescimento de determinadas bactérias e prevenindo o crescimento de patógenos (105). A literatura dispõe evidências consistentes sobre a influência benéfica do aleitamento materno no desenvolvimento da microbiota intestinal. O próprio contato com a aréola materna parece ser importante para explicar os benefícios do aleitamento materno no desenvolvimento da microbiota (100). Crianças que recebem leite materno apresentam maior diversidade e diferente composição da microbiota intestinal quando comparadas com as alimentadas com fórmula infantil (12, 98, 106-108).

Embora mais escassos, alguns estudos também encontraram diferenças na composição da microbiota oral segundo o tipo de alimentação a que foram submetidas no início da vida (109-111). Apesar das diferenças entre as composições da microbiota intestinal e oral, há correlação entre elas de modo que os tipos de bactérias detectados em um local são capazes de prever aqueles encontrados no outro (112, 113). Observações de bactérias idênticas no leite materno e nas fezes do neonato apoiam a hipótese de que o leite humano é importante fonte de bactérias e que a microbiota oral atua como importante semeadora, contribuindo para definir a composição da microbiota intestinal na infância (113).

Já é possível aventar que a microbiota oral também se associe ao desenvolvimento de doenças no curso da vida, inclusive a cardiovascular, de grande importância para a saúde pública (114, 115). A explicação baseia-se na “hipótese inflamatória”, na qual a disbiose da cavidade oral, caracterizada por aumento de patógenos periodontais, contribuiria para inflamação sistêmica de baixo grau e, consequentemente, para aumentar o risco de aterosclerose

(115). O fato da microbiota oral ser relativamente estável em indivíduos saudáveis, a facilidade de coleta da saliva e possíveis relação com doenças no longo prazo (116, 117), motivam buscar conhecimentos sobre condições associadas ao estabelecimento da microbiota oral no início da vida vislumbrando perspectivas de futuras intervenções.

1.5 PRINCIPAIS MORBIDADES RELACIONADAS À ALIMENTAÇÃO E COMPOSIÇÃO CORPORAL

Hábitos alimentares representam importante fator de risco para componentes da síndrome metabólica e outras DCNTs (118-122), consideradas problemas de saúde pública da atualidade (10). Especialmente por estarem relacionados ao acúmulo de adiposidade visceral, desencadeante de síndrome metabólica (7, 123, 124), investigações sobre esta característica do estilo de vida têm despertado grande interesse na literatura.

Estudo conduzido com 1775 adultos afro-americanos verificou que um padrão alimentar caracterizado pelo alto consumo de *fast-food*, açúcar e salgadinhos associou-se ao maior risco de desenvolver hipertensão arterial, DM 2, HDL-c baixo e síndrome metabólica, enquanto um padrão caracterizado pelo consumo de frutas, vegetais, cereais, oleaginosas e sementes, e baixo consumo de pão branco e doces conferiu proteção para hipertensão (124). Estes resultados foram corroborados por outros que identificaram que a adesão a padrões alimentares caracterizados por alta densidade energética, elevado teor de gorduras saturadas ou baixo teor de fibras associaram-se diretamente com a síndrome metabólica ou seus componentes, mas não com a incidência de DCV (120, 121). Em acordo, estudos conduzidos com mulheres coreanas identificaram que maior adesão a um padrão alimentar baseado no consumo de arroz branco representou risco para baixas concentrações de HDL-c (125), enquanto padrões caracterizados por alimentos saudáveis, como vegetais alaranjados, peixes, frutas e laticínios, foram inversamente associados à síndrome metabólica e seus componentes (118, 119).

Apesar das associações observadas entre padrões alimentares e doenças cardiometabólicas, algumas particularidades são observadas em subgrupos de diferentes sexo (122, 126), *status* pré ou pós-menopausa (118) e estado nutricional (121). Nesse sentido, um estudo conduzido com 3090 franceses de ambos os sexos observou que o IMC atenuou a associação identificada entre um padrão alimentar com elevada densidade energética e a frequência de síndrome metabólica (121).

Estudo transversal conduzido na China verificou que padrões alimentares diretamente associados à síndrome metabólica eram diferentes segundo o sexo dos participantes. Em homens, o padrão “alimentos animais e fritos” e em mulheres o padrão “alto sal e energia” associaram-se com maior prevalência da síndrome (122). Dentro do mesmo sexo, as relações entre alimentação e doenças cardiometabólicas podem diferir segundo outras características. Em mulheres, a menopausa se apresenta como potencial modificador de efeito. Um estudo transversal de 4984 mulheres entre 30 e 74 anos de idade encontrou associação inversa entre adesão a um padrão alimentar saudável e síndrome metabólica apenas para o estrato de menopausadas (118). Sabe-se que, independentemente da idade, a prevalência da síndrome é maior após a menopausa. A deficiência de estrogênio é um dos fatores relacionados ao aumento de risco cardiovascular observadas nesta fase da vida, quando ocorre acúmulo de adiposidade visceral, predispondo a distúrbios no metabolismo glicídico e lipídico (127). Considerando que o efeito da menopausa no risco para doenças cardiometabólicas e o potencial de modificar a relação entre dieta e síndrome metabólica, é essencial que se estude mulheres jovens a fim de acrescentar conhecimento oportuno visando à prevenção precoce das alterações metabólicas próprias do envelhecimento.

Achados da pesquisa de base populacional dos Estados Unidos (*National Health and Nutrition Examination Survey* – NHANES) entre 2009 e 2012 indicaram que aproximadamente 46% das mortes por doenças cardiometabólicas associaram-se ao consumo alimentar, e que a ingestão excessiva de sal e insuficiente de sementes, frutas e vegetais estavam entre os fatores dietéticos mais relacionados à mortalidade por doenças cardiometabólicas em mulheres (128). Por ser o comportamento alimentar passível de intervenções, justifica-se investir em pesquisas que aprofundem o estudo da associação do consumo alimentar com desfechos de saúde, subsidiando o desenvolvimento de intervenções para prevenção de DCNTs e redução da sobrecarga dos serviços de assistência à saúde.

Hábitos alimentares influenciam a composição corporal e ambos elevam o risco para doenças cardiometabólicas que cursam com resistência à ação da insulina (8, 9, 129). Estudos indicam que não apenas a quantidade total de gordura corporal, mas especialmente sua distribuição está envolvida na geração da resistência à insulina, principal distúrbio fisiopatológico da síndrome metabólica (7, 129, 130). Em mulheres africanas, verificou-se que o valor médio de gordura do tronco foi maior entre aquelas com síndrome metabólica

comparadas às que não apresentavam o diagnóstico sendo, a gordura abdominal subcutânea associada ao menor risco de glicemia de jejum elevada ou síndrome metabólica, enquanto a gordura visceral se associou ao maior risco de hipertrigliceridemia. Interessantemente, a massa magra do tronco apresentou associação direta com síndrome metabólica e HDL-c baixo (129).

Há evidências de diferenças entre os sexos na relação dos componentes corporais com o risco cardiometabólico (9, 130, 131). Observou-se que indivíduos com razão cintura/estatura superior ou igual a 0,5 apresentaram maior risco para doenças cardiometabólicas que os portadores de valores abaixo deste ponto de corte. A diferença de risco foi maior no grupo de mulheres e especialmente no estrato de jovens (35-40 anos), mas não de idosos (60-70 anos) (131). Em outro estudo, o tecido adiposo visceral foi preditor melhor para a síndrome metabólica em homens, enquanto nas mulheres foi a massa de gordura total (130).

Apesar da utilidade do IMC em estudos epidemiológicos, seu uso isolado pode resultar em falsos-negativos na predição do risco de doenças, especialmente em mulheres (132-134). Em duas coortes de base populacional verificou-se que aproximadamente 30% dos participantes apresentavam-se com fenótipos metabolicamente anormais embora eutróficos segundo o IMC (133). No NHANES III, entre as mulheres com peso adequado aquelas com maior percentual de gordura apresentaram maior risco para DCV em comparação às que tinham menor percentual (135). Estudar com profundidade a composição corporal de populações jovens com elevado grau de miscigenação é relevante para analisar seu papel preditivo de doenças cardiometabólicas.

1.6 MÉTODOS DE AVALIAÇÃO DO CONSUMO ALIMENTAR E SUAS LIMITAÇÕES NA ASSOCIAÇÃO COM DESFECHOS

A avaliação do consumo alimentar representa uma ferramenta básica no campo da epidemiologia nutricional, por meio da qual é possível investigar a participação da alimentação na gênese de doenças, comparar o consumo alimentar entre indivíduos ou populações, estimar a adequação do consumo de alimentos em grupos populacionais ou ainda avaliar o impacto de medidas de intervenção. Destaca-se que os instrumentos de avaliação do consumo alimentar devem ser escolhidos com base no objetivo e no delineamento do estudo (136). Apesar da relevância desta avaliação, depara-se com certas dificuldades inerentes aos métodos empregados para este fim.

A complexidade da dieta humana que inclui nutrientes, compostos bioativos, contaminantes dentre outros fatores e a carência de um método que reúna facilidade, validade e precisão na avaliação limitam determinar relações causais com desfechos. Adicionalmente, possíveis vieses do entrevistado (por exemplo, omissão de alimentos, sub ou super-relatos) e do entrevistador (falta de padronização ou indução de respostas) aumentam as chances de relatos imprecisos. Ainda, a variedade de tabelas e *softwares* usados para avaliar a composição nutricional da dieta, bem como número de alimentos e nutrientes disponíveis, dificultam a comparação de resultados relatados nos diferentes estudos (136).

Entre os métodos diretos mais utilizados para avaliar o consumo alimentar destacam-se o diário alimentar, recordatório alimentar de 24 horas (R24h) e o questionário de frequência alimentar (QFA). O primeiro consiste em método prospectivo de elevada acurácia, entretanto, deve ser auto preenchido e demanda elevado grau de escolaridade, tempo e motivação do entrevistado. O R24h e o QFA são métodos retrospectivos que podem ser auto respondidos ou aplicados por entrevistadores treinados; demandam menor tempo e custo em comparação ao diário alimentar. O R24h e o diário estimam o consumo atual, devendo ser aplicados em mais de dois dias não consecutivos para estimar o consumo usual. O QFA possibilita a estimativa do consumo habitual de longo prazo. Uma vez que permite a classificação de indivíduos em categorias de consumo, utilizadas como *proxy* da magnitude da exposição a ser associada ao estado nutricional e/ou doenças, o QFA tem sido método de escolha em estudos epidemiológicos para avaliar determinantes de desfechos (136).

Tendo em mente que dados da ingestão de nutrientes ou alimentos isolados são insuficientes para avaliar relações com desfechos em saúde, a identificação de hábitos alimentares tem sido mais globalmente recomendada. Porém, esta análise não é isenta de limitações. Hábitos alimentares refletem uma combinação de alimentos e nutrientes de modo que a interpretação de análises estatísticas do consumo implica em limitações decorrentes da correlação entre eles. O problema de analisar alimentos ou nutrientes correlacionados no mesmo modelo estatístico resulta na redução do efeito de cada um deles em desfechos de saúde. Por outro lado, ao analisá-los separadamente, os efeitos isolados podem não ser suficientes para serem captados, embora aumente as chances de encontrar associação estatisticamente significativa (137).

Nesse contexto, a identificação de padrões alimentares – estatisticamente determinados – torna conveniente a colinearidade existente entre os itens alimentares (137, 138). Isso ocorre pois os métodos mais utilizados na identificação de padrões, como a análise fatorial, baseiam-se na redução de dados provenientes da avaliação do consumo alimentar em fatores não correlacionados, facilitando, assim, a interpretação da relação entre consumo alimentar e desfechos de saúde. Por esta razão, esta abordagem tem apresentado alta utilidade em estudos epidemiológicos. A identificação de padrões alimentares permite avaliar o consumo alimentar global de uma população, considerando efeitos cumulativos e sinergismos entre os nutrientes presentes no mesmo padrão. Possibilita abordar a alimentação de maneira mais completa quando comparada com a avaliação isolada de nutrientes ou alimentos específicos (137, 139-141). Apesar destas vantagens sobre as demais formas de avaliação do consumo, a análise de padrões alimentares é subjetiva (137), visto que requer a tomada de decisões arbitrárias, como o agrupamento dos itens alimentares, a decisão do ajuste por energia, a definição do número de padrões retidos e sua nomeação (137-139). Vale mencionar, que os padrões identificados por cada estudo são específicos para as populações em análise e não devem ser empregados em outras com diferentes características (142).

Os padrões alimentares podem ser derivados de abordagens *a priori* ou *a posteriori*. A primeira é desenvolvida quando o objetivo é verificar a aderência a recomendações alimentares pré-determinadas (por exemplo, índice de qualidade da dieta). A segunda consiste na análise estatística de dados empíricos; entre os métodos mais usados, destacam-se a análise fatorial por componentes principais (ACP), na qual os indivíduos recebem um escore dos padrões alimentares gerados, e a análise de *cluster* que agrupa os indivíduos em padrões alimentares mutuamente exclusivos. A ACP apresenta como diferencial a obtenção de escores contínuos dos padrões alimentares identificados, possibilitando avaliar o nível de adesão individual a cada um deles, representando, portanto, as práticas alimentares de forma mais fidedigna (137, 138). Existe ainda a regressão por redução de posto (RRR, do inglês *Reduced Rank Regression*), que é considerada um método híbrido, pois explica a variabilidade de nutrientes ou biomarcadores relacionados a um desfecho de interesse (conhecimento *a priori*), e do consumo alimentar (análise exploratória *a posteriori*) (142).

1.7 MÉTODOS DE AVALIAÇÃO DA COMPOSIÇÃO CORPORAL E O POTENCIAL DO DXA NA AVALIAÇÃO DOS COMPARTIMENTOS CORPORAIS

A avaliação da composição corporal apresenta ampla aplicabilidade na identificação de riscos associados à saúde (143, 144). A quantidade e distribuição de gordura corporal foram consistentemente associadas à ocorrência de doenças cardiometabólicas (8, 9, 129, 145). O monitoramento da massa muscular e óssea mostra-se relevante no processo de envelhecimento, acompanhamento do estado nutricional de atletas, entre outras indicações (146-148).

Diversos métodos estão disponíveis para avaliar a composição corporal, sendo essencial a escolha do mais adequado para garantir a acurácia dos resultados. Métodos indiretos, como a plestimografia, ultrassonografia e a dupla emissão de raios-x (DXA) são mais acurados que os “duplamente indiretos” como a antropometria e a bioimpedância elétrica (149). O objetivo da avaliação, a disponibilidade de recursos (materiais, espaço, capital e equipe) e a adesão dos indivíduos também devem ser considerados na escolha (146).

Os métodos de avaliação da composição corporal são baseados em modelos teóricos bi- ou multi-compartimentais. Os primeiros consideram a massa livre de gordura ou massa magra (inclui os lipídios essenciais distribuídos em membranas celulares fora do tecido adiposo) e a massa gorda. Os modelos multi-compartimentais permitem a avaliação de três, quatro ou mais compartimentos, segundo cinco níveis de complexidade de análise (atômico, molecular, celular, tecidual, corpo inteiro) (144, 146, 150). O método da pesagem hidrostática baseia-se em um modelo bi-compartimental, considerado como padrão ouro para estimar volume e densidade corporal; porém, apresenta baixa aceitabilidade, devido à completa submersão do corpo em água e outros requisitos para sua realização. Nesse contexto, desenvolveu-se outro método densitométrico, conhecido como plestimografia. Apesar de se tratar de um procedimento de maior praticidade, requer equipamento sofisticado (146).

A aferição de dobras cutâneas é um método antropométrico comumente utilizado para avaliar a composição corporal, que é simples e de baixo custo. Porém, possui elevada variabilidade intra e inter-observador sendo capaz de estimar apenas a gordura subcutânea, mas não a visceral (146, 151). A bioimpedância elétrica, baseada na condutividade de corrente elétrica pelo corpo humano, não depende do observador e pode ser utilizada em modelos multi-compartimentais do corpo inteiro. A necessidade de preparo do indivíduo para o exame e o fato de ser inadequada a indivíduos obesos podem ser limitações deste método (146, 152).

Métodos de imagem como a ultrassonografia, a tomografia computadorizada e a ressonância magnética podem ser empregados para avaliar a composição corporal em nível tecidual, sendo os dois últimos os mais acurados para tal função (146, 153, 154). Entretanto, limitações como a substancial exposição à radiação ionizante na tomografia e o elevado custo, especialmente da ressonância de corpo inteiro, dificultam o uso de tais métodos em larga escala (146, 154). Apesar de não ser a mais acurada e depender da experiência do operador do equipamento, a ultrassonografia mostra-se útil por ser procedimento não invasivo, de custo relativamente baixo em comparação aos dois métodos mencionados e por possibilitar a quantificação de gordura em diferentes territórios (periférico ou hepático, por exemplo) (153).

A absorciometria por dupla emissão de raios-x (DXA) também consiste em um método de imagem que possibilita a avaliação em nível molecular. A DXA apresenta-se adequada para avaliação da composição corporal em estudos longitudinais, uma vez que permite a avaliação de três compartimentos (massa gorda, magra e óssea) com elevada precisão, acurácia e reprodutibilidade. O procedimento é rápido e seguro com baixa exposição radioativa e tem menor custo para manutenção após a aquisição do equipamento se comparado a outros métodos indiretos de avaliação da composição corporal. Possíveis fontes de erro deste método referem-se ao posicionamento inadequado do indivíduo, uso de implantes de metal, desidratação de tecidos moles ou edema (146, 155). Equipamentos de DXA modernos, além de avaliarem os compartimentos de massa gorda e massa magra como os modelos anteriores, são capazes de estimar separadamente os tecidos adiposos visceral e subcutâneo (156).

2 JUSTIFICATIVA E HIPÓTESES

O DOHaD evidencia que eventos precoces da vida podem influenciar o desenvolvimento de doenças metabólicas ao longo da vida. Justifica-se estudar exposições ocorridas nos primeiros mil dias de vida, abrangendo condições desde o período pré-concepcional até eventos pós-natais, destacando-se entre estes o aleitamento materno.

Evidências indicam a existência de um ciclo vicioso de transmissão intergeracional da obesidade. Tendo em vista a elevada e crescente prevalência de obesidade no mundo e o papel da adiposidade visceral como forte fator de risco para DCV, principal causa de morte em adultos, faz-se necessário avaliar se o estado nutricional pré-gestacional materno estaria associado à composição corporal da prole. Tal associação motivaria aprofundar a investigação de relações causais e, posteriormente, estudos de intervenção em mulheres pré-concepção. Até o momento, esta associação foi investigada na infância, mas não na vida adulta empregando método acurado para avaliação dos componentes corporais capaz de diferenciar inclusive o tecido adiposo visceral.

A literatura aponta que o aleitamento materno tem potencial para influenciar a formação de hábitos alimentares e que ambos podem estar relacionados com distúrbios do metabolismo glicídico e lipídico, importantes fatores de risco cardiovascular. No entanto, informações sobre a relação entre a amamentação e a formação de hábitos alimentares em longo prazo são escassas na literatura. Identificação de padrões alimentares – método de escolha para avaliar o consumo alimentar em estudos epidemiológicos – foi usado em apenas uma publicação para análise da associação entre amamentação e consumo alimentar na vida adulta. Pouco se sabe sobre a relação entre o aleitamento materno e parâmetros cardiometabólicos em longo prazo.

Tendo em vista que hábitos alimentares, seja na infância (aleitamento materno), seja em adultos (padrões alimentares), são fatores modificáveis que podem estar envolvidos em distúrbios do metabolismo da glicose e lipídeos, justifica-se buscar novas evidências especialmente quando tais anormalidades ainda são incipientes. Sabe-se que a relação do consumo alimentar e da composição corporal com os distúrbios cardiometabólicos apresenta particularidades segundo diferentes sexos e faixas etárias (9, 122, 126, 130, 131). Estudar mulheres jovens e aparentemente saudáveis, participantes do NutriHS, tem grande potencial de acrescentar conhecimento sobre fatores predisponentes de distúrbios metabólicos, fornecendo evidências de qualidade para preencher lacunas na literatura.

A teoria da higiene aventou a existência de um período crítico de desenvolvimento da microbiota do trato gastrointestinal. Sabe-se que o aleitamento materno é fator importante para a formação da microbiota humana nos primeiros anos de vida. Maior ênfase tem sido dada ao estudo da microbiota intestinal em detrimento da oral, cujo material é mais facilmente coletado e também já foi associada com alterações cardiometabólicas. Até o momento, a associação entre o tipo de aleitamento recebido no início da vida e microbiota oral não foi estudada no longo prazo, sendo oportuno investigar se tal influência é mantida tardiamente, durante a adolescência.

Figura 1. Figura ilustrativa do ciclo da vida destacando o momento oportuno para manejo do perfil de risco cardiometabólico.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar se eventos precoces da vida influenciam variáveis relacionadas ao perfil cardiometabólico de mulheres jovens e a microbiota oral de adolescentes.

3.2 OBJETIVOS ESPECÍFICOS

3.2.1 Manuscrito 1

i) Avaliar a associação entre IMC pré-gestacional materno e a composição corporal (massa gorda – particularmente a visceral, massa óssea e massa magra) da prole feminina na vida adulta.

3.2.2 Manuscrito 2

i) Identificar padrões de consumo alimentar de mulheres adultas em idade reprodutiva, participantes do NutriHS;

ii) Comparar características maternas sociodemográficas e obstétricas, eventos precoces da vida e características atuais segundo a adesão aos padrões alimentares identificados;

iii) Investigar se a duração do aleitamento materno se associa com a adesão aos padrões alimentares.

3.2.3 Manuscrito 3

i) Investigar se há associação entre a duração do aleitamento materno e parâmetros do metabolismo glicídico e lipídico de mulheres em idade reprodutiva.

ii) Examinar associação entre padrões alimentares atuais e parâmetros do metabolismo glicídico e lipídico de mulheres em idade reprodutiva.

3.2.4 Manuscrito 4

i) Investigar se o tipo de alimentação recebida nos primeiros seis meses de vida se associa com a diversidade e composição da microbiota oral de adolescentes do Fin-HIT.

4 MÉTODOS

4.1 *Nutritionists' Health Study* (NutriHS)

4.1.1 DELINEAMENTO

Trata-se de uma coorte estabelecida em São Paulo na qual são coletadas informações referentes aos eventos precoces da vida retrospectivamente e atuais. O NutriHS teve início em março de 2014 e a coleta de dados de sua linha de base foi encerrada em 2017. Um novo centro do NutriHS na Universidade de Campinas – UNICAMP iniciou sua coleta basal em 2018 e encerrou em 2019. São previstas novas ondas de acompanhamento longitudinal dos participantes (157).

Os artigos 1, 2 e 3 desta tese foram elaborados a partir de dados provenientes da linha de base do NutriHS. Por abordar eventos precoces da vida ou características maternas, os dados foram coletados retrospectivamente, considerando-se, portanto, uma coorte retrospectiva. No caso da análise da associação entre padrões alimentares atuais e parâmetros do metabolismo glicídico e lipídico (artigo 2), o delineamento foi transversal.

4.1.2 ASPECTOS ÉTICOS

O projeto do NutriHS foi aprovado pelo Comitê de Ética da FSP/USP (Of. COEP 991.542/15 e Of. COEP 257.513/13) e a participação dos voluntários foi condicionada à assinatura digital do termo de consentimento livre e esclarecido (TCLE).

4.1.3 CAPTAÇÃO DE PARTICIPANTES

A captação de participantes ocorreu por meio de convites diretos a estudantes de graduação em nutrição ou nutricionistas formados, via palestras, cartazes em locais estratégicos e divulgação no *website* da FSP/USP, *website* do NutriHS e em redes sociais (157).

Os interessados em participar do estudo acessaram o *website* do NutriHS (*e-NutriHS*) (<http://www.fsp.usp.br/nutrihs/index.html>) onde obtiveram informações detalhadas quanto aos objetivos, etapas, procedimentos, riscos e benefícios do estudo. Aqueles que aceitaram participar assinaram eletronicamente o TCLE e foram orientados a cadastrar login e senha no

e-NutriHS para terem acesso aos questionários (157). Até outubro de 2017, 1.478 indivíduos se cadastraram no sistema.

4.1.4 CRITÉRIOS DE ELEGIBILIDADE

Para ser incluído no *NutriHS*, o participante deveria ser aluno de graduação em nutrição ou nutricionista formado em uma universidade brasileira; ter idade maior ou igual a 17 anos, não estar grávida, e ter habilidade para uso da internet. No total, 1.478 participantes foram recrutados para linha de base do *NutriHS*. Visto que esta tese tem como interesse estudar mulheres saudáveis em idade reprodutiva, homens ($n = 101$) e mulheres com idade superior a 45 anos ($n = 34$), com diagnóstico de câncer ($n = 2$) ou diabetes mellitus ($n = 10$) foram excluídas.

Para as análises que utilizam dados de consumo alimentar foram excluídas aquelas que estavam em tratamento medicamentoso para doença inflamatória intestinal ($n = 1$), reportaram consumo energético diário implausível ($n = 24$), considerado como superior a 5.000 kcal/dia, ou que apresentaram mais de dez itens faltantes no questionário de frequência alimentar (QFA) ($n = 596$). Sendo assim, 715 mulheres foram consideradas para a identificação dos padrões alimentares, das quais 587 apresentavam informação sobre o aleitamento materno e representaram a amostra final do artigo 2.

Uma sub-amostra de participantes com idade entre 20 e 45 anos e que atendiam aos limites do densitômetro (1.87 m e 201 kg) foi convidada para realizar avaliação da composição corporal. Aquelas que compareceram à entrevista e que possuíam informação retrospectiva sobre o IMC materno pré-gestacional ($n = 150$) constituíram a amostra final do artigo 1.

Por fim, para as análises envolvendo marcadores do metabolismo glico-lipídico, considerou-se uma sub-amostra de 200 participantes (artigo 3).

4.1.5 COLETA DE DADOS E VARÁVEIS DE INTERESSE

A coleta de dados foi realizada em duas etapas. A primeira consistiu no autopreenchimento de questionários disponibilizados *online* (*e-NutriHS*) e conferidos por administradores do sistema. Entre as informações coletadas, destacam-se aquelas referentes aos eventos precoces da vida (IMC materno pré-gestacional, aleitamento materno, peso ao nascer e

tipo de parto) e ao estilo de vida atual (hábitos alimentares, atividade física, socioeconômicos e saúde). A ordem para o preenchimento destes questionários era escolhida pelo participante.

Após o preenchimento, participantes foram convidados a constituir uma sub-amostra, sendo orientados a agendar via *e-NutriHS* a segunda etapa da coleta de dados, presencial. Esta etapa consistiu em duas entrevistas, nas quais foram realizadas coleta de amostras biológicas (sangue e fezes) e avaliações antropométricas, clínicas e de composição corporal. Para isso, o *NutriHS* contou com profissionais treinados, laboratório de análises clínicas e equipamento de DXA.

a) Variáveis dependentes

- **Composição corporal**

Na densitometria por dupla emissão de raios-X (equipamento *iDXA/GE Lunar®*) foram considerados os seguintes parâmetros: gordura corporal total (%) [massa gorda (kg) / peso corporal (kg) x 100], razão de gordura das regiões androide e ginóide, tecido adiposo visceral (cm³), índice de massa gorda (kg/m²) [gordura corporal / estatura²], índice de massa magra apendicular (kg/m²) [massa magra esquelética braços + pernas / estatura²], conteúdo mineral ósseo total e femoral (g) e densidade mineral óssea total e femoral (g/cm²). O equipamento utilizado dispõe de *software* que permite a diferenciação do tecido adiposo abdominal subcutâneo e visceral (156).

As participantes foram orientadas a retirar adereços de metal e permanecer imóveis durante o exame. Foram posicionadas sobre a mesa de digitalização em decúbito dorsal, com os tornozelos juntos, utilizando-se suportes de tornozelo e os braços paralelos ao corpo, com as palmas das mãos sobre a mesa. Os exames foram analisados por meio do *software* *Lunar Encore*; na avaliação do tecido adiposo visceral, a região androide é automaticamente encontrada. Todo o procedimento – que seguiu as recomendações do fabricante (158, 159) – e a análise das imagens foram realizados por profissional treinado e certificado pela Associação Brasileira de Osso e Osteometabolismo (ABRASSO). A calibração do equipamento foi verificada diariamente.

- **Glicídicas e lipídicas**

Após jejum de 12 horas, as participantes compareceram ao Centro de Saúde Escola Geraldo Horácio de Paula Souza da FSP/USP e a coleta de sangue foi realizada para determinação de biomarcadores lipídicos, glicose e insulina, utilizadas para cálculo do HOMA-IR, conforme descrito por Matthews *et al.*, 1985 [(glicemia jejum (mg/dL) x 0,0555) x insulina jejum (μ UI/mL)) / 22,5]. A glicemia foi avaliada pela hexoquinase e a insulina por método imunoenzimático (Monobind Inc., Lake Forest, CA, USA). O método enzimático colorimétrico foi empregado para determinar as concentrações de triglicerídeos, colesterol total e lipoproteína de alta densidade (HDL-c) e o ensaio imunoturbidimétrico para a determinação de apolipoproteína B. A lipoproteína de baixa densidade (LDL-c) foi calculada pela fórmula de Friedewald (160). A razão LDL-c/HDL-c foi calculada. Biomarcadores do metabolismo glicídico e lipídico foram categorizados em tercís. Considerando que a amostra estudada apresentava parâmetros, em média, dentro da normalidade, os dois primeiros tercís foram agrupados e considerados como categoria de referência para comparação com o terceiro tercil.

b) Variáveis independentes

- **IMC materno pré gestacional e aleitamento materno**

Para responder ao bloco de perguntas do *e-NutriHS* relativas a eventos precoces da vida, as participantes foram orientadas a solicitar o auxílio de suas mães (ou outro responsável em caso de falecimento da mãe) e/ou consultar seus registros de nascimento (“cartão do bebê”). Estatura materna e peso pré-gestacional foram recordados pelas mães das participantes para o cálculo do IMC materno pré-gestacional, o qual foi considerado como variável contínua e categórica (< 25 ou ≥ 25 kg/m²).

Para avaliação da duração do aleitamento materno, foram consideradas as seguintes variáveis: duração do aleitamento materno total (coletada de forma discreta de 1 a 24 meses e posteriormente categorizada como: < 6 / ≥ 6 e < 12 / ≥ 12 meses (**artigo 2**) ou < 6 / ≥ 6 meses (**artigo 3**); e duração do aleitamento materno predominante (avaliada pela idade de introdução de fórmula, leite ou outro alimento) (< 3 / ≥ 3 meses).

- **Padrões alimentares**

Os padrões alimentares foram considerados como exposição para os desfechos na vida adulta (metabolismo glico-lipídico) (**artigo 3**). Porém, em outros momentos foram considerados como desfecho, quando visava-se responder se o aleitamento materno estaria associado à adesão aos padrões alimentares na vida adulta (**artigo 2**).

O consumo alimentar foi avaliado por meio de questionário de frequência alimentar (QFA) validado (161), desenvolvido para indivíduos de ambos os sexos, com 20 anos de idade ou mais, a partir de um estudo de base populacional conduzido no município de São Paulo (162). Este QFA consiste de uma lista com 101 itens alimentares e três opções de porções (pequena, média e grande) apresentadas em gramas e medidas caseiras para cada item. Quanto à frequência de consumo, as participantes respondem o número de vezes que consomem cada alimento (0 a 10 vezes) e a unidade de tempo (por dia, semana, mês ou ano). O QFA é auto preenchido no *e-NutriHS* e refere-se ao consumo alimentar habitual no ano anterior. As respostas são imediatamente transformadas em medidas diárias, com base no banco de dados do *USDA National Nutrient Database for Standard Reference* (163), sendo assim possível estimar a ingestão diária de nutrientes e energia.

Para a análise dos padrões alimentares, os 101 itens alimentares foram agrupados em 36 grupos segundo similaridades nutricionais, frequência de consumo e hábitos alimentares regionais. A composição dos agrupamentos encontra-se no **Anexo 3**. Optou-se por utilizar a variável quantidade semanal (gramas/semana) nas análises dado que foi a frequência mais reportada ao longo do QFA. Para tanto, a variável tempo foi convertida em valores semanais, sendo: 1 (diário) = 7 x/semana; 2 (semanal) = 1 x/semana; 3 (mensal) = 0,25 x/semana e 4 (anual) = 0,02 x/semana. Em seguida, a variável tempo semanal foi multiplicada pelo número de vezes que cada alimento foi consumido (0 a 10), obtendo-se assim a variável de frequência semanal. Por fim, a frequência semanal foi multiplicada pela porção em gramas para obter a variável quantidade semanal.

As participantes com mais de dez itens com dados faltantes da variável “número de vezes” foram excluídas das análises do consumo alimentar. Para aquelas com dez ou menos itens faltantes, e que responderam a variável “número de vezes” para determinado item alimentar, mas tiveram dados faltantes para as variáveis “tempo” e/ou porção, os valores medianos das mesmas foram imputados. Caso as três variáveis (“número de vezes”, “tempo” e

“porção”) de determinado item alimentar apresentassem dados faltantes, considerou-se a ausência de consumo, ou seja, imputou-se zero para as três variáveis (164, 165).

Os padrões alimentares foram identificados por meio de análise fatorial por componentes principais – ACP (vide Análises Estatísticas). A identificação de padrões permite avaliar o consumo alimentar habitual de uma população, além de considerar efeitos cumulativos e sinergismos entre os nutrientes presentes no mesmo padrão, possibilitando uma abordagem mais completa da alimentação quando comparada à avaliação isolada de um nutriente ou alimento específico (137, 139). Desse modo, a análise de padrões alimentares é adequada a estudos epidemiológicos que investigam a relação entre consumo alimentar e desfechos de saúde (137, 138, 141). Cabe destacar que os padrões obtidos em cada estudo são específicos das populações que originaram os dados e não devem ser reproduzidos em amostras com características diferentes (140, 166).

- **Covariáveis**

Variáveis socioeconômicas, demográficas, de estilo de vida e relacionadas aos eventos precoces da vida foram obtidas por meio dos questionários estruturados, auto-preenchidos no *e-NutriHS*. As seguintes características atuais das participantes foram consideradas: idade (coletada em anos e categorizada como ≤ 19 ; 20 a 34; ≥ 35 anos), cor da pele auto-referida (branca; não branca)], renda familiar (< 6 ; 6-10; > 10 salários mínimos), tabagismo atual (não; sim), ingestão alcoólica (nunca; < 1 vez por semana; ≥ 1 vez por semana), consumo energético (kcal/dia); prática de atividade física de lazer (1º + 2º tercils vs. 3º tercil MET) e exposição ao conhecimento em nutrição [1ª metade do curso (1º ao 4º período); 2ª metade do curso (5º ao 10º período); graduação completa (nutricionistas)]. A prática de atividade física foi avaliada pela versão curta do Questionário Internacional de Atividade Física (167), previamente validado em adultos da população brasileira (168, 169). O consumo energético diário foi estimado utilizando-se a base de dados do USDA (163), a partir do QFA (162).

Além do IMC referido ao preenchimento de questionários, por ocasião da etapa presencial realizada com uma sub-amostra de participantes, o peso foi mensurado em balança digital com precisão de 100 g (Filizola®, São Paulo, Brasil) e a estatura aferida com estadiômetro fixo na parede com precisão de 0,1 cm. Adicionalmente, a pressão arterial foi avaliada após cinco minutos de repouso utilizando dispositivo automático (Omron model HEM-

712C®, Omron Health Care Inc, USA). Valores de pressão arterial sistólica e diastólica (mm/Hg) foram obtidos em triplicata e o valor médio entre as duas últimas medidas foi considerado. Como complementação à análise bioquímica padrão, foram determinadas concentrações plasmáticas de proteína C reativa (*High sensitivity PCR*) e 25-hydroxyvitamina D por meio de ensaio de quimioluminescência (Elecsys 2012, Roche Diagnostics, Rotkreuz, Switzerland).

Entre as informações referentes aos eventos precoces da vida e características maternas coletadas, destacam-se: peso ao nascer [$< 2,5$ kg (baixo peso); $\geq 2,5$ a < 4 kg (adequado); ≥ 4 kg (macrossomia)], tipo de parto (normal; cesariana); tabagismo e intercorrências gestacionais (desordens hipertensivas e DM) (não; sim), idade materna na época da gestação (< 19 ; ≥ 19 a ≤ 35 ; > 35 anos); escolaridade da mãe [< 11 (ensino fundamental completo ou menos); ≥ 11 anos (ensino médio completo)] e paridade (0; ≥ 1 filho).

Os modelos teóricos das relações entre as variáveis consideradas no presente estudo encontram-se representados em gráficos acíclicos direcionados (DAGs), construídos a partir do *software* DAGitty, disponível online em www.dagitty.net (17) [**Figura 2** (**A**- associação entre IMC materno pré-gestacional e composição corporal da prole feminina na vida adulta; **B**- associação entre aleitamento materno e padrões alimentares na vida adulta; **C**- associação entre aleitamento materno e o perfil glico-lipídico da prole; **D**- associação dos padrões alimentares com o perfil glico-lipídico)].

Figura 2- A) DAG para associação entre IMC materno pré-gestacional e composição corporal da prole na vida adulta.

(>) Exposição; (I) Desfecho; ○ Variável não observada

Figura 2- B) DAG para associação entre aleitamento materno e padrões alimentares na vida adulta.

(>) Exposição; (I) Desfecho

Figura 2- C) DAG para associação entre aleitamento materno e o perfil glico-lipídico da prole.

(>) Exposição; (I) Desfecho

Figura 2- D) DAG para associação entre padrões alimentares e o perfil glicídico e lipídico.

(>) Exposição; (I) Desfecho

4.1.6 ANÁLISE ESTATÍSTICA

a) Identificação dos padrões alimentares

Os padrões alimentares foram derivados por meio de ACP. Este método *a posteriori* consiste em uma técnica exploratória, que tem como objetivo reduzir os dados obtidos na avaliação dietética em “padrões alimentares” ou “fatores” baseando-se na correlação entre os grupos alimentares considerados na análise (170).

Os testes de Kaiser-Meyer-Olkin (KMO) e de esfericidade de Bartlett foram empregados para garantir a adequação da ACP na amostra estudada. O KMO testa a correlação entre os grupos alimentares, sendo mais adequados os coeficientes próximos de 1. Porém, para a identificação de padrões alimentares, adotou-se o ponto de corte de 0,5 (171). O teste de esfericidade de Bartlett avalia a qualidade das correlações entre as variáveis, que é aceitável quando p-valor é menor que 0,05 (170).

O número de fatores extraídos foi baseado no critério de Kaiser, que considera o percentual de variância explicada de cada fator (autovalores superiores a 1,5) e no gráfico de Cattell (*Scree Plot*) no qual o ângulo de maior declive indica o número de fatores a ser retido (170, 172). Antes e após a extração dos fatores, verificou-se as comunalidades, ou seja, os percentuais de variância explicada de cada grupo alimentar por todos os fatores juntos, sendo indicativas da confiabilidade dos grupos.

Após a extração de fatores, foi realizada a rotação ortogonal de Varimax, técnica de rotação mais utilizada na análise de padrões alimentares. Este método aumenta a diferença entre as cargas fatoriais dos grupos alimentares, originando assim fatores com estruturas mais simples, mais facilmente interpretáveis e não correlacionados (170, 172).

As cargas fatoriais representam a contribuição dos grupos alimentares em cada fator. Adotou-se o ponto de corte de $|0,20|$ para considerar contribuição significativa em dado padrão alimentar. Cargas fatoriais positivas indicam que o grupo alimentar é diretamente associado a determinado padrão, enquanto negativas indicam associação inversa (173, 174). Os fatores retidos foram nomeados segundo os grupos alimentares que o constituem e suas maiores cargas fatoriais. Ao final da análise, participantes do estudo com informação de consumo alimentar receberam um escore de cada padrão alimentar obtido (139). Quando considerados como exposição, os escores de padrões alimentares foram categorizados em tercís (**artigo 3**). No caso de os padrões alimentares representarem o desfecho do estudo (**artigo 2**), o primeiro tercil foi adotado como referência e os dois

maiores tercís foram agrupados representando adesão moderada-a-alta a cada padrão alimentar identificado.

b) Descrição da amostra

Variáveis contínuas foram descritas por meio de medidas de tendência central e dispersão, como média e desvio padrão (para variáveis com distribuição normal) ou mediana e intervalo interquartil (para variáveis sem distribuição normal), respectivamente. Gráficos do tipo histograma e o teste de Kolmogorov-Smirnov foram empregados para verificar a normalidade da distribuição das variáveis estudadas. Variáveis categóricas foram descritas por frequências absolutas (n) e relativas (%).

Características gerais da amostra (socioeconômicas, demográficas, antropométricas, estilo de vida atual e eventos precoces) foram comparadas segundo categorias de adesão aos padrões alimentares (baixa *versus* moderada-a-alta) (**artigo 2**) e variáveis de composição corporal segundo as categorias de IMC materno pré-gestacional (< 25 *versus* ≥ 25 kg/m²) (**artigo 1**). Testes t de Student e Mann-Whitney foram utilizados para comparar médias e medianas, respectivamente, enquanto o do qui-quadrado para comparar proporções.

c) Análise das associações entre exposições e desfechos

Com o objetivo de verificar a associação entre o IMC pré-gestacional materno (kg/m²) e as medidas de composição corporal (**artigo 1**) foram construídos três modelos de regressão linear para cada desfecho, sendo um modelo bruto, e dois múltiplos. As variáveis índice de massa magra apendicular e tecido adiposo visceral foram transformados em log para adquirirem distribuição normal. O primeiro modelo múltiplo (modelo 1) foi ajustado por idade materna, escolaridade e paridade materna, de acordo com o conjunto de ajustes mínimos suficientes recomendados pelo gráfico acíclico direcionado (**Figura 1-A**), construído com o *software* Daggity. O modelo 2 incluiu estatura (quando medidas de adiposidade e osso foram desfecho) ou IMC (quando o índice de massa magra apendicular foi desfecho), além das variáveis consideradas no modelo 1. Todos os modelos atenderam ao pressuposto da homocedasticidade, de modo que foram obtidos valores de $p > 0,05$ no teste White.

Para verificar a associação entre a duração do aleitamento materno e a adesão aos padrões alimentares na vida adulta (**artigo 2**) foram elaborados modelos de regressão logística brutos e múltiplos para estimar a razão de chances (e respectivo intervalo com 95% de confiança) da participante apresentar adesão moderada-a-alta a cada um dos padrões alimentares identificados. O modelo múltiplo 1 foi ajustado por escolaridade materna, IMC pré-gestacional e tipo de parto, variáveis sugeridas como conjunto mínimo de ajustes suficientes pelo gráfico acíclico direcionado (**Figura 1-B**). O modelo múltiplo 2 incluiu o ajuste por ingestão de energia além das variáveis consideradas no modelo 1.

Para avaliar a associação entre o aleitamento materno e padrões alimentares atuais com os biomarcadores do metabolismo glicídico e lipídico foram elaborados modelos de regressão logística para estimar a razão de chances de classificação no terceiro tercil dos biomarcadores. As variáveis de confusão foram selecionadas segundo os conjuntos de ajustes mínimos suficientes recomendados pelos gráficos acíclicos direcionados, apresentados nas **Figuras 1-C e 1-D**. Quando aleitamento materno foi a exposição de interesse, os modelos foram ajustados para escolaridade materna e peso ao nascer. O gráfico apresentado na **Figura 1-C** também sugeriu a inclusão de diabetes gestacional no conjunto mínimo de ajustes suficientes, mas como apenas três casos estavam presentes, optou-se por excluí-los da análise. Quando os padrões alimentares representaram a exposição de interesse, foram realizados três modelos: o modelo 1 considerou os quatro padrões alimentares e o consumo de energia; o modelo 2, incluiu ajustes para idade, atividade física moderada-a-vigorosa, hábito de fumar, frequência de ingestão de álcool, duração total do aleitamento materno e peso ao nascer, conforme sugerido pelo gráfico apresentado na **Figura 1-D**; e o modelo 3 adicionou a variável IMC atual para testar possível efeito de mediação. Para verificar tendência entre os tercis de padrões estimou-se o p-linear.

O teste de Hosmer-Lemeshow foi aplicado para verificar a qualidade do ajuste dos modelos de regressão logística e todos os atenderam ao critério de p-valor $> 0,05$. As associações foram consideradas estatisticamente significantes quando o valor de p for $< 0,05$. Todas as análises foram conduzidas no *software* estatístico STATA, versão 12.0 (Stata Corp., College Station, TX, USA).

4.2 The Finnish Health in Teens study (Fin-HIT)

4.2.1 DELINEAMENTO

Trata-se de uma coorte prospectiva de jovens entre 9 e 12 anos de idade no início do estudo e um de seus responsáveis. O Fin-HIT é coordenado pelo *Folkhälsan Research Center*, em Helsinque, Finlândia, sendo representativo das áreas mais populosas da Finlândia. Visa acompanhar os participantes por 25 anos para possibilitar o estudo de mudanças na estrutura corporal ocorridas da infância até a vida adulta. Diversos fatores de risco para o desenvolvimento de sobrepeso e obesidade estão sendo estudados, entre eles, relacionados ao estilo de vida, ambientais, marcadores genéticos e epigenéticos e características da microbiota. O estudo teve início em 2011, tendo sido realizada a primeira onda de seguimento em 2015-2016 (175).

O artigo 4 da presente tese envolve uma análise transversal de dados da linha de base do estudo Fin-HIT (2011-2014).

4.2.2 ASPECTOS ÉTICOS

O Fin-HIT foi aprovado pelo Comitê de Ética do Distrito Hospitalar de Helsinque e Uusimaa (169/13/03/00/10). Aprovação para realização da pesquisa em escolas foi obtida junto às secretarias municipais de educação e diretores das escolas incluídas. A participação no estudo foi condicionada à assinatura do TCLE (separadamente para jovens e responsáveis participantes). No caso dos participantes jovens, o TCLE também incluía a assinatura de um de seus responsáveis.

4.2.3 CAPTAÇÃO DOS PARTICIPANTES

O recrutamento dos participantes foi realizado em duas fases utilizando-se método misto. Voluntários foram recrutados de 44 municípios da Finlândia, incluindo as maiores cidades da Finlândia (Helsinque, Turku, Espoo, Oulu, Jyväskylä, Tampere e Kuopio), áreas urbanas e rurais.

A primeira fase do recrutamento teve início em 2011 (estudo piloto) por meio de uma abordagem domiciliar (11.000 domicílios foram aleatoriamente convidados por correspondência). Devido a taxa de resposta inferior ao esperado (14,5%) no estudo piloto, a segunda fase (2013-2014) foi realizada em escolas. Durante esta fase, 27.000

jovens estudantes de 496 escolas foram convidados a participar do estudo. A taxa de resposta desta fase foi de 36%. Considerando as duas fases, a taxa de resposta foi de 30%.

A abordagem de recrutamento misto não permitiu obter informações sobre características dos indivíduos que não aceitaram participar do estudo impossibilitando a comparabilidade com a amostra de participantes.

4.2.4 CRITÉRIOS DE ELEGIBILIDADE

Jovens entre 9-12 anos de idade na linha de base do estudo e um de seus responsáveis (em sua maioria mães) eram elegíveis a participar do estudo Fin-HIT. No total, foram recrutados 11.407 jovens e 9935 responsáveis para a linha de base do Fin-HIT.

Foram considerados elegíveis para o sub-estudo do Fin-HIT que originou o trabalho contido nesta tese, jovens incluídos aleatoriamente na sub-amostra de participantes que teve microbiota da saliva analisada ($n = 972$). Aqueles cujas amostras de saliva resultaram em menos de 2000 sequências lidas ($n = 83$) foram excluídos. Baseado no Registro Nacional de Seguro Social da Finlândia, aqueles que usaram antibiótico no mês anterior à coleta da saliva ($n = 46$) ou que usaram por mais de 40 vezes ao longo da vida (adotado como *proxy* de doença crônica) ($n = 1$), ou sem essas informações também foram excluídos ($n = 6$).

Entre os 836 jovens que possuíam dados de microbiota da saliva e uso de antibióticos, 423 (10-14 anos) também tiveram informação sobre o tipo de alimentação durante os primeiros seis meses de vida (obtida no questionário respondido pelos responsáveis), compreendendo o número final de participantes do sub-estudo do Fin-HIT apresentado nesta tese.

4.2.5 COLETA DE DADOS E VARIÁVEIS DE INTERESSE

Questionários disponibilizados em finlandês e sueco (duas línguas oficiais na Finlândia) foram respondidos via web por participantes jovens e responsáveis. A coleta de amostras de saliva e medidas antropométricas dos jovens foi conduzida por pesquisadores treinados nas escolas. As amostras de saliva foram obtidas sem estímulo, por meio do kit de auto-coleta OrageneVR DNA (DNA Genotek Inc., Canada). A estatura

foi aferida com estadiômetro portátil com 0,1 cm de precisão (Seca modelo 217) e o peso utilizando balança digital portátil (CAS modelo PB) com precisão de 0,01 kg. As balanças eram calibradas diariamente antes da realização de medidas.

Algumas informações também foram obtidas de Registros Nacionais de Saúde. Entre eles, o Instituto de Seguro Social da Finlândia [<http://www.kela.fi/web/492>], que inclui informações sobre prescrições médicas e compras de medicamentos; e o Instituto Nacional de Saúde e Bem Estar [<https://www.thl.fi/en/web/thlfi-en/statistics/information-for-researchers>], que gerencia o Registro Médico de Nascimento, o Registro de Assistência à Saúde e o Registro de Visitas à Atenção Primária à Saúde, assim disponibilizando informações sobre doenças, gestação, saúde do recém-nascido e diagnósticos.

a) Variáveis dependentes

As variáveis dependentes de interesse são as que expressam a composição e a diversidade da microbiota oral dos jovens.

Para avaliar tais aspectos, após a coleta, as amostras de saliva foram misturadas com reagente estabilizante e armazenadas em temperatura ambiente, segundo as instruções do fabricante do *kit* de coleta. Aplicou-se um protocolo com uma etapa de lise intensiva e ruptura mecânica das células microbianas (176). Em seguida, foi realizada extração do DNA e preparação para amplificação e o sequenciamento de acordo com um protocolo simplificado de amplificação por PCR baseado no gene 16S rRNA. A amplificação foi realizada usando os primers 16S direcionados à região V3-V4 e o protocolo de amplificação Truseq (TS) de um passo. O equipamento Illumina HiSeq1500 (Illumina Inc., San Diego, CA, EUA) foi utilizado para sequenciamento de amplicons de PCR. O MiSeq SOP no *pipeline mothur* (versão V.1.35.1) foi empregado para processar sequências (177).

O banco de dados SILVA 16S rRNA (versão V119) e a taxonomia foram usados para alinhamento e classificação das leituras de sequências de alta qualidade, as quais foram agrupadas em unidades taxonômicas operacionais (OTUs) com um valor de corte superior a 98% de similaridade de sequência. A composição da microbiota foi interpretada como abundâncias absolutas e relativas das OTUs identificadas.

A alfa-diversidade foi calculada por amostra e avaliada por meio dos índices Shannon e Inverso de Simpson. Para o cálculo destes índices são considerados o número de diferentes OTUs e a proporção das mesmas em cada amostra (abundância relativa), permitindo avaliar a riqueza e equilíbrio de abundâncias das OTUs. A beta-diversidade foi calculada entre amostras utilizando o índice de dissimilaridade Bray Curtis, permitindo, assim, analisar variações na composição da microbiota entre “diferentes territórios”.

A análise da microbiota foi realizada por bioinformata treinado e seus procedimentos foram descritos em maiores detalhes anteriormente (178).

b) Variáveis independentes

A variável independente de principal interesse deste estudo foi o tipo de alimentação (leite materno e/ou fórmula infantil) recebido durante os primeiros seis meses de vida. Este dado foi obtido retrospectivamente, por meio dos questionários administrados via web para os responsáveis participantes do estudo. O dado bruto obtido do questionário possuía as seguintes categorias: (i) Apenas leite materno; (ii) Mais leite materno do que fórmula; (iii) Ambos igualmente; (iv) Mais fórmula do que leite materno; (v) Apenas fórmula; (vi) Não sabe ou não lembra. Optou-se por agrupar as categorias ii, iii, iv e v e excluir a categoria vi, considerando o objetivo de avaliar possíveis diferenças na composição dos jovens que haviam recebido apenas leite materno em comparação aos demais que receberam fórmula. Inicialmente, era de interesse avaliar a categoria v separadamente, mas não foi possível dado ao baixo percentual de respostas. Sendo assim, a variável de interesse principal utilizada nas análises trata-se de uma variável dicotômica com as seguintes categorias: (i) Apenas leite materno; (ii) Outros.

Outras variáveis independentes foram consideradas para fins de descrição da amostra e/ou ajuste dos modelos estatísticos. Entre estas, algumas foram obtidas por meio dos questionários respondidos pelos jovens participantes, como sexo (masculino / feminino) e idade (anos) e dos questionários respondidos pelo responsável, como sexo (homem / mulher), idade (anos), idioma (finlandês, sueco / outros) e nível de escolaridade (ensino médio ou técnico / diploma universitário) do responsável. A estatura e peso dos jovens foram aferidas e utilizadas para cálculo do IMC [peso (kg) / estatura² (m)]. O tipo de parto (vaginal / cesariana) foi obtido por meio do Registro Nacional de Saúde

gerenciado pelo Instituto Nacional de Saúde e Bem-Estar. Considerou-se ainda a profundidade de sequenciamento da microbiota da saliva (número de vezes que uma base é representada dentro de um conjunto de leituras), categorizada como (i) baixa ≤ 10.000 ; ii) média > 10.000 e ≤ 100.000 ; iii) alta > 100.000 sequências.

4.2.6 ANÁLISES ESTATÍSTICAS

O teste de Kolmogorov-Smirnov foi utilizado para verificar se as variáveis do estudo aderiam à curva de distribuição normal. Médias e desvio padrão foram utilizadas para descrever variáveis contínuas e frequências absolutas e relativas para descrição de variáveis categóricas. Os testes t de Student e qui-quadrado foram empregados para comparar médias e proporções respectivamente, de acordo com as categorias da variável independente principal (apenas leite materno *versus* outros). Análise de covariância (ANCOVA) foi utilizada para comparar médias ajustadas dos índices de alfa-diversidade (Shannon e Inverso de Simpson) entre as mesmas categorias. Correlação de Person foi utilizada para testar correlação entre os índices de diversidade alfa e variáveis descritivas contínuas, enquanto teste t de Student e ANOVA foram empregados para comparar médias dos índices entre categorias de demais variáveis de caracterização da amostra. Para estas análises, adotou-se nível de significância de 5% e sendo utilizado o *software* estatístico Stata (versão 12, 2011, StataCorp LP, College Station, TX, USA).

Empregou-se análise multivariada de permutação (PERMANOVA) para testar a diferença da beta diversidade entre os grupos com diferente tipo de alimentação. A análise de coordenadas principais (PCo), baseada nas distâncias da matriz de Bray Curtis foi utilizada para visualização multidimensional da diferença da beta diversidade entre os grupos.

Modelos de regressão lineares generalizados (GLM) com distribuição binomial negativa foram empregados para comparar abundâncias das bacterianas entre os grupos de estudo, considerando OTUs e também os níveis taxonômicos de filo e gênero. Todos as OTUs com baixas contagens (< 20 unidades) foram excluídas da análise. P-valores foram corrigidos pela razão de falsas descobertas, a qual consiste na proporção esperada de falsas descobertas dentre as hipóteses H_0 rejeitadas. Esta correção é realizada com o objetivo de controlar a ocorrência de falsos-positivos (erro tipo I). Os testes

PERMANOVA e GLM foram realizados utilizando os pacotes DESeq2 (179), Vegan e phyloseq no *software* estatístico R (versão 3.4.3).

Modelos de ANCOVA, PERMANOVA e GLM foram ajustados por sexo, idade, IMC, tipo de parto, nível de escolaridade do responsável e profundidade do sequenciamento. As variáveis de ajuste foram definidas considerando-se a plausibilidade biológica e análises bivariadas.

Imputação múltipla de dados para dados faltantes de IMC ($n = 10$), tipo de parto ($n = 22$) e nível de escolaridade do responsável ($n = 15$) foi realizada por meio do procedimento “mi impute chained” no *software* Stata 12.0. A imputação foi realizada considerando as seguintes variáveis: idade, sexo e idioma do participante, tipo de alimentação durante os primeiros seis meses de vida (exposição de interesse principal), índice de diversidade Shannon e idade do responsável. Os valores imputados foram considerados nas análises múltiplas.

5 RESULTADOS

O capítulo de Resultados foi dividido em quatro artigos elaborados para esta tese de doutorado. O primeiro artigo encontra-se pronto para submissão, o segundo e o terceiro publicados, e o quarto aceito para publicação.

5.1 MANUSCRITO 1: Pre-pregnancy body mass index is associated with offspring body composition in adulthood before adiposity-related disorders: a retrospective cohort

5.2 MANUSCRITO 2: Breastfeeding duration is associated with offspring's adherence to prudent dietary pattern in adulthood: results from the Nutritionist's Health Study

(Publicado na J Dev Orig Health Dis, em 6 de junho de 2019; doi:

10.1017/S204017441900031X – **Anexo 4**)

5.3 MANUSCRITO 3: Early life feeding and current dietary patterns are associated with biomarkers of glucose and lipid metabolism in young women from the Nutritionist's Health Study

(Publicado na Eur J Clin Nutr, em 17 de outubro de 2019; doi: 10.1038/s41430-019-

0516-8 – **Anexo 5**)

5.4 MANUSCRITO 4: Breastfeeding may have a long-term effect on oral microbiota: results from the Fin-HIT cohort

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10.1186/s13006-020-00285-w – **Anexo 6**)

5.1 MANUSCRITO 1: Pre-pregnancy body mass index is associated with offspring body composition in adulthood before adiposity-related disorders: a retrospective cohort

Abstract

Background: Maternal pre-pregnancy body mass index was shown to be associated with adiposity later in life, resulting in a “vicious cycle of obesity”, but is not known if it is associated with offspring visceral adiposity and other body compartments in adulthood.

Objective: To investigate the association between maternal pre-pregnancy body mass index and offspring body composition in adulthood.

Study design: This is a retrospective cohort conducted with apparently healthy non-pregnant women (aged 20-45 years) (n=150) in São Paulo, Brazil. Participants were undergraduates of nutrition or nutritionists recruited at the baseline of the Nutritionists’ Health Study between 2014 and 2017. Maternal pre-pregnancy body mass index and current life aspects were self-reported through online questionnaires. Body composition was assessed by dual-energy X-ray absorptiometry and the following variables were obtained: body-fat (%), fat mass index (kg/m^2), android-to-gynoid fat ratio, visceral adipose tissue (cm^3), appendicular skeletal muscle mass index (kg/m^2), total bone and femur mineral content (g) and density (g/cm^2). Linear regression adjusted according to directed acyclic graphs recommendation was performed.

Results: Median age and body mass index were 22 years (IQR=20; 29) and $22.3 \text{ kg}/\text{m}^2$ (IQR=20.4; 25.3). Pre-pregnancy body mass index $\geq 25 \text{ kg}/\text{m}^2$ was reported by 14.7% of mothers. In fully adjusted models, maternal pre-pregnancy body mass index was associated with their daughters’ body-fat % ($\beta=0.31$; 95%CI=0.0004; 0.63), fat mass index ($\beta=0.17$; 95%CI=0.03; 0.30, android-to-gynoid ratio ($\beta=0.01$; 95%CI=0.004; 0.02) and visceral adipose tissue ($\beta=0.09$; 95%CI=0.02; 0.16), but not with appendicular muscle mass ($\beta=0.005$; 95%CI=-0.002; 0.01) or total bone density ($\beta=0.001$; 95%CI=-0.003; 0.006) and content ($\beta=7.13$; 95%CI=-4.19; 18.46).

Conclusions: Maternal pre-pregnancy body mass index was directly associated with offspring general and visceral adiposity but seem not to be associated with muscle or bone mass. Results reinforce importance of avoiding excess of maternal adiposity, as an attempt to break the vicious cycle of obesity transmission.

Introduction

Obesity is a worldwide public health problem and affects around 12% of adults. Prevalence rates are higher in the female sex and, especially for those at childbearing age, obesity represents a great concern (45, 46). Excessive body adiposity in early pregnancy has been associated with several gestational complications, contributing to maternal morbidity and infant mortality (46, 47), as well as to increased risk of cardiovascular disease (45).

As far as the offspring is concerned, maternal pre-pregnancy body mass index (BMI) was shown to be associated with adiposity later in life, resulting in a “vicious cycle of obesity” (54-58). Genetic components could partly explain this association (48), but it is known that epigenetic mechanisms, through metabolic programming during the first 1000 days of life, play an important role on this issue (49, 50). Maternal lifestyle, mainly related to dietary intake habits, may influence offspring behaviors and risk for outcomes (48, 180). In this context, our group found that maternal pre-pregnancy BMI was associated with offspring adherence to a processed and prudent dietary pattern in adulthood (180).

Several studies reported an association of maternal pre-pregnancy BMI and offspring adiposity in childhood (54-57), but few were conducted in adults (58, 59), and none assessed all body compartments including the visceral adipose tissue (VAT). Increased VAT generates systemic inflammation and has been considered a strong predictor of cardiovascular events, independently of BMI (60). There is some evidence that excessive visceral adiposity can deteriorate metabolic profile even in apparently healthy non-obese young adults (61), indicating that assessment of VAT in this age group could be useful to anticipate detection of those at-risk and the prevention of cardiometabolic disorders.

Associations of maternal pre-pregnancy BMI with offspring fat-free mass in childhood (55, 57) and in early adulthood (58) were less investigated and provided controversial results. The Avon Longitudinal Study of Parents and Children (ALSPAC) showed that maternal pre-gestational BMI was directly associated with bone mass in infancy, but it was mainly explained by child’s height and weight (63). It is unknown whether this association would be maintained in adulthood. To the best of our knowledge, no study has tested associations of maternal pre-pregnancy BMI with all three body compartments of the offspring in apparently healthy adults, using a reliable technique and software to identify VAT. Dual-energy x-ray absorptiometry (DXA) provides accurate

measures of body composition and advantages of low x-ray exposure, cost and scanning time against computed tomography or magnetic resonance imaging (156). We investigated whether maternal pre-pregnancy BMI was associated with offspring muscle, bone and adipose (general and visceral) body compartments assessed by DXA in early adulthood before adiposity-related disorders.

Materials and Methods

This is a retrospective cohort of women who participated at the baseline of the Nutritionists' Health Study (NutriHS). Briefly, the NutriHS, is a cohort conducted in Sao Paulo, Brazil, with non-pregnant undergraduates of Nutrition courses or nutritionists (graduates) recruited between 2014 and 2017 (157). All participants registered on the NutriHS website (www.fsp.usp.br/nutrihs), electronically signed the informed consent term and answered structured questionnaires regarding early life events and current aspects of health, socioeconomic background and lifestyle. The NutriHS was approved by the Research Ethics Committee of the School of Public Health of the University of São Paulo.

The sample of the present study was composed of female participants aged 20 to 45 years who agreed to attend a face-to-face interview and to a body composition assessment. To be eligible women should not have diabetes or cancer diagnoses nor exceed the limits of the densitometer (1.87 m and 201 kg). Those with missing information of maternal pre-pregnancy BMI (our main exposure) ($n = 51$) were excluded, resulting in a final sample of 150 apparently healthy young women for the present study.

Before answering the early life events questionnaire available on NutriHS website, participants were oriented to ask for their mothers' help, or to consult birth cards. Maternal pre-pregnancy BMI was obtained as continuous variable and further categorized as < 25 or ≥ 25 kg/m². Other maternal characteristics, such as age at conception (≤ 19 ; > 19 and < 35 ; ≥ 35 years), education (< 11 ; ≥ 11 years), parity (0; ≥ 1 pregnancy), gestational diabetes and smoking during pregnancy (no; yes) were obtained. Information of delivery (vaginal; C-section) and birthweight (< 2.5 ; 2.5 and < 4.0 ; ≥ 4.0 kg) were also collected. Participant's current life data, such as age, skin color (white; non-white); leisure physical activity practice (no; yes), smoking habit (no; yes), family income (< 6 ; ≥ 6 minimum wages) and education level (undergraduates attending the first-half of nutrition courses; undergraduates attending the second-half of nutrition courses; graduates) were also obtained.

During visits to the university health care center, weight was measured using a digital scale (Filizola®, São Paulo, Brazil) with 0.1 kg of precision, and height by a fixed stadiometer with 0.1 cm precision. A flexible and inelastic tape was used to assess waist circumference at the midpoint between the last rib and the iliac crest. Body composition was assessed using DXA by a researcher (A.M.M.V.) certified by the Brazilian Association of Bone and Osteometabolism. The equipment (GE Lunar iDXA®, Madison, WI, USA) and software provide measurements of the three body compartments (adipose, muscle and bone), differentiating the VAT. Measurements obtained were total fat (kg and %), android and gynoid fat (used to calculate Android-to-Gynoid fat ratio), VAT (cm³), appendicular skeletal muscle mass (kg) (ASM), total bone and femur mineral content (g) and density (g/cm²). Fat mass index (FMI) and ASM index (ASMI) (kg/m²) were calculated dividing total fat and ASM per squared height, respectively. According to the manufacturer's instructions, regions of interest were determined manually and the densitometer was routinely calibrated.

A random sub-sample (n = 117) was submitted to a clinical examination. Blood pressure was assessed in sitting position after 5 minutes resting using an automatic device (Omron model HEM-712C®, Omron Health Care Inc, USA). Systolic and diastolic blood pressure (mm/Hg) were obtained in triplicate and the mean value between the last two measures was considered. After overnight fasting, blood sample was collected for biomarkers determinations. Glucose (mg/dL) was determined by the glucose oxidase method; total cholesterol, HDL-c and triglycerides (mg/dL) by the enzymatic colorimetric method and LDL-c was calculated by Friedewald formula (160). High sensitivity C-reactive protein and 25-hydroxyvitamin D were measured by chemiluminescence assay (Elecsys 2012, Roche Diagnostics, Rotkreuz, Switzerland).

Statistical analyses

The Kolmogorov-Smirnov test was used to verify normal distribution of continuous variables, which were described as mean (standard deviation) or median (interquartile range). Categorical variables were described as frequencies. Student t test or Mann-Whitney was used to compared central distribution values of body composition measurements according to maternal pre-gestational BMI categories.

Linear regression models were performed to verify the association between maternal pre-pregnancy BMI (kg/m²) and body composition measurements (outcomes). ASMI and VAT were log-transformed for analyses. Model 1 was adjusted for maternal

age, maternal education and parity, according to the set of minimal sufficient adjustments recommended by the directed acyclic graph (**Figure S1**), constructed using the Dagitty software (17). Model 2 included variables considered in model 1 plus height (for measurements of adiposity and bone as outcomes) or plus BMI (for ASMI as outcome).

Statistical significance was considered at the level of 5%. Stata statistical software version 12.0 (release 12, 2011, StataCorp LP, College Station, TX) was used for analyses.

Results

One hundred and fifty participants had body composition assessed by DXA. Median age was 22 years (interquartile range 20 – 29 years) and mean values of blood pressure, plasma glucose and lipids biomarkers, as well as C-reactive protein and 25-hydroxyvitamin D were within normal ranges. Most participants were undergraduates (89.3%) and reported white skin color (73.3%) (**Table 1**). The majority had normal weight (64.0%), 6.7% were underweight, 22% overweight and 7.3% had obesity.

Regarding maternal characteristics and early life events, most mothers had at least 11 years of education (65.3%). Excessive pre-pregnancy weight was reported by 14.7%. Frequencies of gestational diabetes and smoking during pregnancy were 1.4% and 2.7%, respectively. A slight predominance (53.3%) of vaginal delivery was reported and most participants were born with adequate weight (84.5%) (**Table 1**).

Measurements of the three body compartments are shown in **Table 2**. Higher mean values of VAT, android-to-gynoid ratio and FMI were verified in participants whose mothers had excessive pre-pregnancy BMI compared to those with eutrophic mothers before pregnancy. ASMI and bone compartment measurements did not differ according to maternal pre-pregnancy BMI.

Maternal pre-pregnancy BMI was directly associated with all adiposity measurements of their daughters (**Table 3**). Both, measurements of general adiposity, such as percent of body fat ($\beta = 0.31$; $P = 0.050$) and FMI ($\beta = 0.17$; $P = 0.015$), and of central adiposity, such as VAT ($\beta = 0.09$; $P = 0.011$), as well as android-to-gynoid ratio ($\beta = 0.01$; $P = 0.005$), showed increasing values as maternal pre-pregnancy BMI increased. Results did not change after adjustment for height (model 2). Maternal pre-pregnancy BMI was also associated with ASMI ($\beta = 0.007$; $P = 0.039$), but the statistical significance was not maintained in the BMI-adjusted model. Maternal pre-pregnancy BMI was not associated with any bone compartment measurements.

Comments

Principal findings of the study

A detailed and accurate evaluation of the three body compartments was performed at early adulthood when cardiometabolic profile is still normal. In a large sample of 150 apparently healthy young women, using DXA, our findings indicated a direct association between maternal pre-pregnancy BMI and measurements of offspring adiposity, both general (FMI and percent of body fat) and central (android-to-gynoid ratio and VAT) fatness. Maternal pre-pregnancy BMI was not associated with offspring bone compartment. Regarding muscle compartment, the association detected seemed dependent on offspring BMI.

Strengths and limitations

This was the first study to evaluate the association between maternal pre-pregnancy BMI and visceral adiposity of the young adult offspring. Several clinical data confirmed that our sample had not adiposity-related disorders, representing a unique opportunity to investigate usefulness of body composition measurements even before the occurrence of metabolic diseases. The use of a DXA equipment able to identify VAT is a strength of our study, considering that accumulation of this particular tissue is involved in the pathophysiology of the most important public health problem nowadays.

Another strength was related to statistical analyses including adjustments oriented by directed acyclic graph. This approach has been valued due to its adequacy to test associations of interest minimizing confounding bias and avoiding overadjustments (17, 18).

The recall of maternal pre-pregnancy BMI implies recall bias and should be mentioned as a limitation. Nonetheless, it was reported that women are able to self-report pre-pregnancy weight with reasonable accuracy almost 30 years later (181). Our homogenous sample, composed of young, apparently healthy and educated women, implies limitation regarding external validity of our study; i.e. generalization of our results to different samples with different characteristics is not appropriate.

Results in the context of what is known

The direct association between maternal pre-pregnancy BMI and offspring adiposity agreed with previous studies that also assessed body composition using indirect methods, such as DXA and air displacement plethysmography in childhood (54-57, 62). We found three studies assessing offspring adiposity in adulthood, but only one considered maternal pre-pregnancy BMI (58), while the others assessed maternal weight at early or

late pregnancy (59, 182). One of them was conducted with 276 adults in Scotland whose skinfold-estimated body fatness was predicted by maternal early pregnancy BMI (59). Similar to our findings, a Swedish study assessed body composition of 226 participants aged 19-21 years using DXA and identified that maternal pre-pregnancy BMI was directly associated with fat mass of daughters, but not sons. However, VAT measurement was not available (58).

The association between maternal pre-pregnancy BMI and offspring central adiposity in young adult women, expressed by the VAT volume and the android-to-gynoid ratio in our study, had not been reported, but agrees with studies conducted with children in the USA (54, 56). The latter studies verified that offspring of overweight or obese mothers had higher visceral adiposity compared to children born to women with pre-pregnancy BMI lower than 25 kg/m².

Clinical Implications

In contrast to others, we highlight that our sample included a low proportion of participants with overweight or obese mothers before pregnancy, and still such associations were present, suggesting being maintained until adulthood. It could be argued that the epigenetic impact of mothers' adiposity on their offspring occurs in a continuum during the life cycle of their daughters.

We reinforce that participants of our study had on average normal BMI and were apparently healthy, i.e. without disturbances of glucose and lipid metabolism, low-grade inflammation or vitamin D deficiency. It is known that vitamin D contributes to reduce inflammation, which has an important role for cardiometabolic disease development (183). Normal mean values of C-reactive protein and 25-hydroxyvitamin D support that our sample is at low cardiometabolic risk. Even so, findings suggest that general and visceral fat contents could be impacted by maternal adiposity. Thus, our study calls attention to the importance of nutritional care during the pre-gestational period. Avoiding maternal weight excess at conception should contribute to preventing increase of offspring visceral adiposity, which is known to be an important trigger of insulin resistance and cardiometabolic diseases (60, 61). We emphasize these aspects against the current scenario of worldwide prevalence of overweight women of 38% and even higher in Brazil (184), favoring a vicious cycle of obesity transmission.

Intergenerational transmission of body adiposity has been explained by three main mechanisms. First, by a genetic component, considering a report that around 60% of fat mass may be heritable (52). Second, by epigenetic mechanisms, i.e. maternal adiposity

could induce adaptive response during pregnancy that is a period of plasticity. According to the Developmental Origins of Health and Disease (DOHaD), this period comprises the first thousand days of life, starting at conception. DOHaD proposes that insults during this period (e.g. fetal overnutrition) might trigger epigenetic changes, such as DNA methylation, resulting in phenotypic alterations that could increase the risk of disease development later in life (25, 49, 185). The third mechanism refers to common environmental factors, transmitted from mother to offspring, such as dietary habits (48, 52). Our previous study (180) showed that daughters of overweight mothers had lower chance to adhere to a prudent diet, characterized by healthy foods such as fruits, vegetables and whole grains, and higher chance to adhere to a processed diet, composed of high energy density foods, such as fast-foods, sweets and fried food, which could favor body fat accumulation (186).

Our study also showed that maternal pre-pregnancy BMI seemed to impact offspring ASM, but this association lost statistical significance after adjusting for offspring BMI, which is a metric with good specificity to detect excess of body adiposity in populations worldwide despite poor sensitivity at the individual level (187). We could speculate that the impact of maternal adiposity on offspring muscle mass occurs simultaneously with accumulation of body fat, i.e. the increase of muscle mass seems to be dependent on the increase of adiposity, but our study design impedes inference of causality. Different from ours, the Swedish study (58) verified that maternal pre-pregnancy BMI was inversely associated with daughters' lean mass in early adulthood, but the association was not significant for sons. On the other hand, studies in childhood using air-displacement plethysmography have reported direct association between maternal BMI and offspring lean mass (55, 57). A meta-analysis of studies that assessed body composition at childhood using indirect methods also reported that maternal pre-pregnancy BMI seem to be directly associated with offspring lean mass, although high heterogeneity between studies included (62). Controversies could be attributed to different approaches to assess body composition and subjects' characteristics. We highlight that, as composed by Brazilians, our study sample is characterized by high miscegenation, which could also limit comparability with other studies.

Our results do not support an impact of maternal pre-gestational BMI on offspring bone mass in adulthood. In contrast, results from ALSPAC indicated a direct association between maternal BMI and offspring bone size and density in childhood; however, the association was similar when considering paternal BMI instead of maternal. Also, this

relation was mainly explained by child's height, thus suggesting that the influence of maternal pre-pregnancy BMI on offspring bone mass was not explained by intrauterine environment but seemed to be driven by family shared environmental and genetic factors (63). We speculate that these common environmental characteristics become weaker during adolescence and adulthood, when subjects are more independent and acquire their individuality.

Research Implications

The hypothesis that maternal pre-pregnancy BMI impacts her daughters' visceral adiposity in early adulthood which could contribute to the development of cardiometabolic diseases needs to be investigated in the follow-up of NutriHS participants and in populations with different characteristics.

Conclusion

We conclude that maternal pre-pregnancy BMI seems to be long-termly associated with female offspring general and visceral adiposity, but not with muscle or bone components, even among apparently healthy young women. These findings reinforce the relevance of nutritional care for women at childbearing age (especially when planning pregnancy) as an attempt to prevent excess of maternal adiposity during pregnancy and the propagation of the vicious cycle of obesity to offspring. Interventions in early life must be prioritized once it shows potential to drive favorable health trajectories with less effort, compared to actions aiming change of habits in later life.

Details of Ethics Approval

The NutriHS was approved by the Research Ethics Committee of the School of Public Health of the University of São Paulo (CAAE 12455313.8.0000.5421) in February, 2013.

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Table 1. Current and early life characteristics of NutriHS participants included in the present study (n = 150) and their mothers' data.

	Mean (SD) or N (%)
Current Life Characteristics	
Age (years) ^a	22 (20; 29)
Body mass index (kg/m ²) ^a	22.3 (20.4; 25.3)
Waist circumference (cm)	77.4 (9.3)
Systolic blood pressure (mmHg) ^b	107 (11)
Diastolic blood pressure (mmHg) ^b	71 (8)
Fasting plasma glucose (mg/dL) ^b	83 (9)
LDL-c (mg/dL) ^b	98 (27)
HDL-c (mg/dL) ^b	55 (12)
Triglycerides (mg/dL) ^{a, b}	82 (55; 117)
C-reactive protein (mg/L) ^c	3.0 (1.7)
25-hydroxyvitamin D (nmol/L) ^b	26.5 (11.5)
Skin color	
White	110 (73.3)
Non-white	40 (26.7)
Leisure physical activity practice	
No	79 (57.7)
Yes	58 (42.3)
Current smoking habit	
No	129 (92.8)
Yes	10 (7.2)
Graduation in nutrition	
First-half undergraduate	65 (43.3)
Second-half undergraduate	69 (46.0)
Graduate	16 (10.7)
Family income (number of minimum wages) ^d	
< 6	83 (59.7)
≥ 6	56 (40.3)
Early life and Mother's Characteristics	
Maternal age (years)	
≤ 19	8 (5.3)
>19 and < 35	123 (82.0)
≥ 35	19 (12.7)
Maternal education (years)	
< 11	52 (34.7)
≥ 11	98 (65.3)
Maternal parity	
0	73 (48.7)
≥ 1	77 (51.3)
Maternal pre-pregnancy body mass index (kg/m ²)	
< 25.0	128 (85.3)
≥ 25.0	22 (14.7)
Gestational diabetes	
No	145 (98.6)
Yes	2 (1.4)
Smoking during pregnancy	
No	143 (97.3)
Yes	4 (2.7)
Birthweight (kg)	
< 2.5	16 (10.8)
2.5 and < 4.0	125 (84.5)
≥ 4.0	7 (4.7)
Type of delivery	
Vaginal	80 (53.3)
C-section	70 (46.7)

^a Variables described as median (interquartile range); ^b n = 117; ^c n = 111;

^d One minimum wage is equivalent to 724.0 Brazilian real (or US\$ 212.9)

Table 2. Means and standard deviations of body composition measurements according to maternal pre-pregnancy body mass index (n = 150).

Variables	Total	Maternal pre-pregnancy BMI (kg/m ²)		<i>P</i> ^a
		< 25	≥ 25	
Visceral adiposity tissue (cm ³) ^b	104.00 (39.00; 255.00)	85.00 (34.00; 253.00)	164.50 (81.00; 393.00)	0.041
Android-to-gynoid fat ratio	0.78 (0.18)	0.77 (0.18)	0.87 (0.13)	0.005
Body fat (%)	33.77 (6.37)	33.41 (6.59)	35.90 (4.46)	0.090
Fat mass index (kg/m ²)	7.93 (2.70)	7.73 (2.64)	9.06 (2.81)	0.033
Appendicular skeletal muscle mass index (kg/m ²) ^b	6.23 (5.79; 6.72)	6.25 (5.72; 6.68)	6.18 (5.99; 7.38)	0.253
Total bone mineral content (g)	2280.99 (265.80)	2272.64 (262.24)	2329.18 (287.11)	0.359
Femur mineral content (g)	29.41 (3.75)	29.39 (3.85)	29.56 (3.20)	0.840
Total bone mineral density (g/cm ²)	1.13 (0.09)	1.13 (0.09)	1.14 (0.10)	0.666
Femur mineral density (g/cm ²)	1.01 (0.13)	1.02 (0.13)	1.01 (0.09)	0.729

BMI, body mass index

^a Refers to Mann-Whitney test or Student t test^b Variables described as median (interquartile range)

Table 3. Linear regression analysis for the association between maternal pre-pregnancy body mass index and offspring's DXA-determined body composition measurements (n = 150).

	Crude			Model 1 ^a			Model 2 ^b		
	β (95% CI)	<i>P</i>	R ²	β (95% CI)	<i>P</i>	R ²	β (95% CI)	<i>P</i>	R ²
Visceral adiposity tissue ^c (cm ³)	0.09 (0.02; 0.15)	0.012	0.04	0.09 (0.02; 0.16)	0.010	0.05	0.09 (0.02; 0.16)	0.011	0.05
Android-to-gynoid fat ratio	0.01 (0.003; 0.02)	0.006	0.05	0.01 (0.004; 0.02)	0.004	0.06	0.01 (0.004; 0.02)	0.005	0.07
Body fat (%)	0.34 (0.04; 0.65)	0.028	0.03	0.34 (0.02; 0.65)	0.038	0.04	0.31 (0.0004; 0.63)	0.050	0.07
Fat mass index (kg/m ²)	0.16 (0.03; 0.29)	0.013	0.04	0.17 (0.03; 0.30)	0.015	0.05	-	-	-
Appendicular skeletal muscle mass index ^c (kg/m ²)	0.007 (0.001; 0.01)	0.034	0.03	0.007 (0.0003; 0.01)	0.039	0.05	0.005 (-0.002; 0.01)	0.149	0.15
Total bone mineral content (g)	4.62 (-8.32; 17.56)	0.481	0.003	4.44 (-8.82; 17.69)	0.509	0.04	7.13 (-4.19; 18.46)	0.215	0.31
Femur mineral content (g)	0.07 (-0.12; 0.25)	0.480	0.003	0.08 (-0.11; 0.27)	0.396	0.03	0.10 (-0.08; 0.28)	0.277	0.12
Total bone mineral density (g/cm ²)	0.001 (-0.003; 0.006)	0.541	0.003	0.001 (-0.003; 0.006)	0.631	0.01	0.001 (-0.003; 0.006)	0.577	0.02
Femur mineral density (g/cm ²)	0.002 (-0.004; 0.008)	0.573	0.002	0.003 (-0.004; 0.009)	0.424	0.02	0.002 (-0.004; 0.009)	0.459	0.02

CI, confidence interval

^a Adjusted by maternal age (≤ 19 ; >19 and < 35 ; ≥ 35 years), maternal education (<11 ; ≥ 11 years) and parity (0; ≥ 1), as recommended by DAG.

^b Adjusted by variables included in Model 1 plus height (cm); except for appendicular skeletal muscle index model 2, which included body mass index (kg/m²).

^c Variables were log transformed

Figure S1. Directed Acyclic Graph (DAG) for the association between maternal pre-pregnancy BMI and offspring body composition.

Notes: Minimal sufficient adjustment: Maternal age, maternal education and parity.

Legend= (>) exposure; (I) outcome; ○ unobserved variable.

BMI, Body mass index

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5.2 MANUSCRITO 2: Breastfeeding duration is associated with offspring's adherence to prudent dietary pattern in adulthood: results from the Nutritionist's Health Study

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Abstract

Little is known about the long-term effect of breastfeeding on dietary habits. We examined the association between breastfeeding duration and adherence to current dietary patterns of young women. This was a cross-sectional analysis of 587 healthy women aged ≤ 45 years, undergraduates or nutrition graduates. Maternal characteristics and breastfeeding duration [<6 , 6 to <12 ; ≥ 12 months (reference)] were recalled. Diet was assessed using a food frequency questionnaire and patterns were identified using factor analysis by principal component. Adherence to patterns was categorized in tertiles; the first (T1=reference) was compared to T2+T3 (moderate-to-high adherence). Logistic regression was performed considering the minimal sufficient adjustment recommended by the Directed Acyclic Graph. Median age was 22 (IQR 20; 27) years and BMI 22.2 (IQR 20.4; 25.0) kg/m^2 . The 4 dietary patterns identified (*Processed*, *Prudent*, *Brazilian* and *Lacto-vegetarian*) explained 27% of diet variance. Women breastfed for <6 months showed lower chance of moderate-to-high adherence to the *Prudent* pattern (OR=0.53, P=0.04). Breastfeeding was not associated with the other patterns. Maternal pre-pregnancy BMI was directly associated with moderate-to-high adherence to the *Processed* pattern (OR=2.01, P=0.03) and inversely to the *Prudent* pattern (OR=0.52, P=0.02). Higher adherence to the *Brazilian* pattern was associated with proxies of low socioeconomic status and the *Lacto-vegetarian* pattern with the opposite. **Conclusion:** Confirmation in prospective studies of the association found in this study between breastfeeding with the *Prudent* pattern in adult offspring could suggest that early feeding practices influence long-term dietary habits, which could then affect the risk of nutrition-related diseases.

Keywords: Dietary patterns; breastfeeding; DOHaD

1. Introduction

The first 1000 days of life have been shown to be important for adult health. According to the Development Origins of Health and Disease theory (DOHaD), exposure to certain conditions in this period may trigger metabolic programming that could increase susceptibility to chronic diseases, such as obesity, diabetes and cardiovascular disease later in life (23, 30, 188). In this context, type of early feeding (breast milk or formula) is potentially associated with long-term health outcomes (14, 29). The World Health Organization's breastfeeding recommendation until 24 months of life (73) coincides with the end of the 1000-day period. In Brazil, approximately 40% of children aged 9 to 12 months are no longer breastfed (76), and this scenario seems to be even worse in high-income countries, where more than 70% of 12 month old children are not breastfed (74).

The development of food preferences and dietary habits in the short and long-run could be influenced by breastfeeding (43, 83-86). Proposed underlying mechanisms are related to the presence of leptin in human milk but not in infant formulas, which plays a role in hypothalamic programming of satiety (87, 88), and to the central reward systems (89). Additionally, being exposed to the flavors from the maternal diet during breastfeeding or even earlier during gestation via amniotic liquid may be important for the development of food preferences and propensity of accepting new foods in early life (89, 90).

Longer breastfeeding has been associated with lower consumption of processed foods (84), preference for natural sources of animal protein (85), and higher adherence to healthy dietary patterns (91) in 2- to 8-year old children. Although these findings probably reflect the mother's lifestyle, a Finnish cohort suggested reasonable stability of dietary patterns identified throughout infancy, adolescence and adulthood (94). In adult life, this association is scarcely reported and controversial (43, 86, 95). As far as we know, only one study used the dietary patterns approach to assess the relationship between former habits and diet during adulthood (43). Among 3,217 adults, those who were bottle-fed compared to those only breastfed during the first year of life had lower adherence to the prudent pattern. Despite limitations (different composition of infant formula in the 1930s, lack of information about breastfeeding duration and possible residual confounding), these findings raised the need to deepen knowledge in this issue.

Since dietary patterns analysis allows a broad evaluation considering synergism and cumulative effect of nutrients in the same pattern, this has been preferred in nutritional

epidemiology. This approach is more appropriate than evaluating isolated nutrients or food groups when the objective is to identify associations between diet and health outcomes. Dietary patterns are population-specific, although similar food groups may be used to characterize patterns across different populations (137, 139).

The Nutritionist's Health Study (NutriHS) is a web-based study conducted on nutrition undergraduates and nutritionists that represents a unique opportunity to obtain good quality dietary information and to test association with early life events. We aimed to identify *a posteriori* dietary patterns of adult women from the NutriHS and examine if adherence to the identified patterns is influenced by breastfeeding duration, independent of maternal socioeconomic characteristics.

2. Methods

2.1 Design and participants

This is a cross-sectional analysis of NutriHS baseline data. Briefly, the NutriHS is a cohort conducted with the main objective of assessing predictors of cardiometabolic outcomes, retrospectively and prospectively, in non-pregnant undergraduates or nutrition graduates (157). Those who met the eligibility criteria and signed electronically the informed consent were invited to fill out structured questionnaires using the *e*-NutriHS system (<http://www.fsp.usp.br/nutrihs/index.html>). Questionnaires were presented in several blocks of questions whose filling was time-consuming. For the purpose of the present study, sociodemographic data, early life events, health aspects, physical activity and dietary intake questionnaires were considered. Participants were also invited to a face-to-face visit for physical examination and collection of biological samples at the São Paulo University health care center.

A total of 1,478 participants were recruited between March 2014 and October 2017. Inclusion criteria for the present analysis were female sex, age ≤ 45 years, without cancer, diabetes, or use of medication for inflammatory bowel disease. Only women were included in our analyses due to the scarcity of men in the NutriHS (23). Daily energy intake $> 5,000$ kcal/day (percentile 97 of energy intake distribution) or < 500 kcal/day (189), or missing information for more than ten items in the food frequency questionnaire (FFQ) were exclusion criteria. Therefore, 587 women composed the final sample (**Figure 1**).

2.2 Dietary intake assessment

Dietary intake was assessed using a validated FFQ for adult population living in São Paulo (161). The FFQ had the previous year as the time-frame and was composed of 101 food items with frequency options (0 to 10 times), and their respective time units (day, week, month or year) and portion options (described in three household measures and grams or milliliters). For statistical analysis, we standardized frequency in week times (since it was the time unit of frequency most reported), multiplied by quantitative portion, then we considered the weekly amount consumed (in grams or milliliters) to identify dietary patterns. Daily energy intake was estimated considering the USDA National Nutrient Database for Standard Reference (163).

Participants with up to 10 food items with missing information regarding frequency but having fulfilled at least one of the three FFQ variables had median values inputted. If the three variables of a given food item were missing, absence of consumption was interpreted and zero was attributed (164, 165).

Items were grouped into the following 36 food groups according to nutritional similarities, frequency of consumption and regional eating habits: bean; white rice; brown rice; lentil (lentil/dry pea/chickpeas); noodles or pasta; roots, tubers and soups (cooked, baked or mashed potato /cassava /yam, cassava or maize flour, mayonnaise salad, vegetable soup, cream soup); fried fish and chicken; roasted fish and chicken; eggs; beef; processed meat (ham/mortadella/salami; nuggets/meatball, dried meat, sausage, bacon); pork meat (loin/steak); offal (chicken or bovine heart/liver); vegetables; olive oil or oil for salad dressing; salt; baked salted pastries; fast food (French fried potato or cassava, fried salted pastries, pizza, hot-dog/hamburger); snacks (salty snacks, popcorn); industrialized sauces (shoyu, ketchup/mustard); sweets/ desserts (biscuit without filling, stuffed/wafer/buttery, simple or stuffed cake, morning cereal, candy/lollipop, whipped cream/condensed milk, chocolate/bonbon, fruit jam, jelly, confectionery pie, pudding or mousse, ice cream); fruits; natural juice; oats; nuts (peanut/cashew/Brazil nuts/walnuts); white bread; whole grain bread; yogurt and fruit vitamin; whole milk; skimmed milk; spreading fat (mayonnaise, butter/margarine); white and yellow cheese; tea; coffee; industrialized beverages (industrial juice, soda, chocolate milk, soy-based drink); and alcoholic beverages (beer, wine, distilled ones).

2.3 Early life events and current life characteristics

Participants were instructed to request help of their mothers/guardians or to consult their birth cards before answering the early life events questionnaire. Breastfeeding duration (main exposure) was categorized in: < 6 ; ≥ 6 to < 12 ; ≥ 12 months, the last being considered as the reference. We also obtained information about maternal education (< 11 ; ≥ 11 years of education); pre-pregnancy body mass index (BMI) (< 18.5 ; ≥ 18.5 to < 25 ; ≥ 25 kg/m²); mode of delivery (vaginal; C-section); and birthweight (< 2.5 ; ≥ 2.5 to < 4.0 ; ≥ 4.0 kg).

Selected data regarding participants' current life were employed in the analyses of this sub-study. Age (continuous and categorized as: ≤ 19 ; 20 to 34; ≥ 35 years); skin color (white; non-white); alcohol intake (never; < 1 time/week; ≥ 1 time/week); graduation in nutrition (first half of the course; second half of the course; or graduates); family income (< 6 ; 6 to 10; > 10 minimum wages) and self-reported BMI (continuous and categorized as: < 18.5 ; ≥ 18.5 and < 25 ; ≥ 25 kg/m²) were described according to dietary patterns adherence.

2.4 Statistical analysis

- **Dietary pattern analysis**

Factor analysis by principal component analysis (PCA) was employed to identify participant's dietary patterns, which were the outcomes of main interest. Based on the correlation of the food groups, food data were reduced to factors or "dietary patterns", facilitating the interpretation of dietary intake (137, 139). The number of factors identified was defined according to Kaiser criteria and Scree Plot (**Supplementary Figure 1**), in which eigenvalues ≥ 1.5 were considered, as well as personal interpretability. Before applying PCA, Kaiser–Meyer–Olkin (KMO) test and Bartlett's test of sphericity were used to verify the sample adequacy (KMO > 0.5 and p-value of Bartlett's test < 0.05) (171). After factors extraction, orthogonal varimax rotation was applied. Although all factor loadings contributed to the dietary patterns scores, only food groups with factor loadings higher than $|0.2|$ in a given pattern were considered relevant to characterize it; positive loadings indicated direct associations between food group and dietary pattern, while negative loadings meant inverse associations (173). Each participant received a score of each dietary pattern. Dietary pattern scores were categorized into tertiles; the first was considered as the reference (low adherence) and the two highest tertiles were grouped as moderate-to-high adherence.

- **Descriptive and association analyses**

Absolute and relative frequencies were described and chi-squared test used to compare dietary patterns adherence (low *versus* moderate-to-high adherence) according to current life characteristics. The Kolmogorov-Smirnov test was used to evaluate normal distribution of continuous variables. Medians and interquartile ranges of energy intake (kcal/day), age and BMI were described and compared using the Mann-Whitney test, since these variables were not normally distributed.

Logistic regression models were employed to verify the association between breastfeeding duration and dietary patterns adherence during adult life. Confounding variables were selected according to the minimal sufficient adjustment recommended by the Directed Acyclic Graph (DAG), created in the online software Dagitty (17) (**Supplementary Figure 2**). Model 1 was adjusted for maternal education, pre-pregnancy BMI and mode of delivery. Considering energy intake as a competing exposure (i.e. it predicts the outcome but not related with any other variable in the model), a second model was adjusted for variables in model 1 plus daily energy intake in order to improve the precision. Hosmer–Lemeshow test was applied to verify the goodness of fit and all models met the criterium of $p > 0.05$.

All analyses were conducted using Stata Statistical Software (release 12, 2011, StataCorp LP, College Station, TX) and statistical significance considered at the level of 5%.

3. Results

In a total of 715 participants, four dietary patterns were identified, which explained 26.7% of the total variance of food intake. Participants who were excluded from dietary pattern analysis due to missing dietary data were similar to those who were included, except for a higher percentage of graduate participants among the excluded ones (**Supplementary Table 1**). The first pattern, labeled *Processed*, was characterized by a high intake of baked salted pastries, noodles, roots/soups, fried white meat, processed and pork meat, fast food, snacks, industrialized sauces and beverages, alcoholic beverages and sweets. The second pattern, named *Prudent*, was characterized by a high intake of fruits (whole and juice), yogurt, oats, nuts, vegetables, oil for salad, salt, roots/soups, roasted white meat, beef, eggs, brown rice, lentil, whole grain bread and tea. The third pattern was named *Brazilian* since it was directly associated with the consumption of traditional foods such as white rice, bean, processed meat, beef, salt, white bread,

spreading fat, whole milk and coffee, and inversely associated with brown rice, lentil and nuts. These three patterns showed the major contribution for the diet explained variance. The fourth pattern, *Lacto-vegetarian*, was associated with a high intake of alcoholic beverages, brown rice, whole grain bread, oats, spreading fat, cheese and skimmed milk, and a low intake of animal protein sources (fish, chicken, pork, beef, offal), oil for salad dressing, salt and white rice (**Table 1**).

Among the 587 participants with information of food intake and breastfeeding, the majority was aged 20 to 34 years (median age 22; interquartile range 20 - 27 years) and reported white skin color and monthly family income lower than 6 minimum wages. Approximately 93% were undergraduates (49% of total sample had completed less than half of the course). A quarter of the sample was overweight or obese (median BMI 22.2; interquartile range 20.4 - 25.0 kg/m²). Regarding early life events, more than 90% of participants had been breastfed and, among these, 35% for at least 12 months. Almost 82% had adequate birthweight, 41% were born by vaginal delivery and 23% had mothers with excessive pre-pregnancy BMI (**Table 2**).

Median BMI was higher among women who presented moderate-to-high adherence to the *Processed* pattern compared to those with low adherence to this pattern. Women with higher adherence to the *Lacto-vegetarian* pattern had higher median age compared to those with lower adherence to this pattern. White women had higher probability of having moderate-to-high adherence to *Lacto-vegetarian* and lower to *Brazilian* patterns compared with non-white women. Participants who reported regular alcohol intake had higher probability of being classified into the two highest tertiles of adherence to *Processed* and *Lacto-vegetarian* pattern, and lower probability of moderate-to-high adherence to the *Brazilian* pattern. The subgroup of graduates was more likely to adhere to the *Lacto-vegetarian* pattern and less likely to the *Brazilian* one. Family income was directly associated with adherence to *Prudent* and *Lacto-vegetarian* patterns and inversely associated with adherence to the *Brazilian* one (**Table 3**).

Participants who were breastfed for < 6 months had approximately 47% lower chance of having moderate-to-high adherence to the *Prudent* pattern (OR 0.53; 95%CI 0.29; 0.97) during adult life compared to those breastfed for ≥ 12 months. Breastfeeding duration was not associated with adherence to *Processed*, *Brazilian* or *Lacto-vegetarian* patterns. Additionally, low maternal education was directly associated with adherence to the *Brazilian* pattern (OR 2.40, 95%CI 1.44; 4.02), and inversely associated with the *Lacto-vegetarian* one. The latter association showed to be dependent of energy intake. Offspring

of mothers with excessive weight at conception had a higher chance of moderate-to-high adherence to the *Processed* (OR 2.01, 95%CI 1.09; 3.71) and a lower chance to the *Prudent* (OR 0.52, 95%CI 0.30; 0.92) pattern (**Table 4**).

4. Discussion

Using dietary pattern analysis, our study investigated the hypothesis that early feeding could be associated with food consumption later in life. This approach, compared to the traditional ones, has contributed to the knowledge about the relation between nutrition and diseases (137). We identified four dietary patterns – *Processed*, *Prudent*, *Brazilian* and *Lacto-vegetarian* – in a special sample of young women with knowledge in nutrition. Only 35% of our sample was breastfed for at least 12 months, and compared to these, those who have received maternal milk for less than 6 months were less likely to adhere to the *Prudent* pattern in adult life. Offspring of mothers with pre-conception excessive weight had a higher chance of moderate-to-high adherence to the *Processed* dietary pattern, and a lower to the *Prudent*, while low maternal education seems to be directly associated with adopting traditional Brazilian dietary habits, and inversely associated with the *Lacto-vegetarian* pattern. Our findings are relevant since they reinforce the need of encouraging long breastfeeding which may have beneficial impact in further dietary habits, potentially conferring protection or risk for multiple diseases.

The *Processed* pattern was the one that most explained the diet variance, and was similar to previously reported in Brazilian adults (190-192). Such patterns have been associated with unfavorable health outcomes, such as type 2 diabetes and cardiovascular disease (193). Our *Prudent* pattern resembles those pre-pregnancy pattern named ‘seasoned vegetables and lean meats’ (191), or adult’s patterns named ‘vegetable/fruit’ (190), or ‘healthy’ (192). In addition to fruits, vegetables and lean meats, commonly present in these patterns, ours also included oats, nuts, olive oil for salad dressing, yogurt, brown rice and lentils, thus combining characteristics of a Mediterranean-style diet (except for the absence of wine intake) and the Dietary Approach to Stop Hypertension, both associated with metabolic health benefits (194-196).

Adoption of certain dietary patterns has been associated with sociodemographic data both in high (197-200) and low-middle income settings (201, 202). Commonly, healthier dietary habits imply higher income and access to healthcare information (203). Despite the homogenous education level of our sample, we observed that women with a higher family income were more likely to adhere to the *Prudent* pattern, which is in line

with the positive association between education (as a proxy of economic status) and adherence to healthy dietary patterns found in other studies (191, 192). In contrast, participants with a lower socioeconomic status (lower family income, lower maternal education, non-White and undergraduates) had a higher probability to adhere to the *Brazilian* pattern. These findings corroborated studies that had identified inverse associations of the education (190) and family income (192), and direct associations of mulatto skin-color (192) with adherence to previously described ‘Traditional/Common-Brazilian’ patterns. In a probabilistic sample from the National Survey of Households, the traditional pattern was inversely associated with excessive body weight (204). We speculate that populations with Brazilian-like diets should have advantages in preserving their typical dietary habits.

Our less explanatory pattern was the *Lacto-vegetarian*, which we believe to be specific of the sample studied including women with knowledge in nutrition. The name was attributed considering that this pattern was inversely associated with animal protein sources. We speculate that adherence to this pattern was related to more conscious food choices such as, oats, whole bread and brown rice instead of refined versions and low-fat milk rather than whole milk. Curiously, this pattern was also directly associated with alcoholic beverages intake; we believe this could be a particularity of our young sample. Having a bachelor’s degree in nutrition, a higher educated mother, white skin color and higher family income increased the probability of being classified into the two highest tertiles of adherence to this pattern.

Our most interesting finding was related to the association of early life feeding and current dietary patterns of participants. Thirty-five percent of our sample was breastfed for at least 12 months, which is lower than seen in 2008 in São Paulo city (49% of 9-12-month children were breastfed) (76), but higher than that observed in most high-income countries where breastfeeding prevalence was lower than 20% (74). The education level of NutriHS participants might be indicative of a better socioeconomic condition than the general sample from São Paulo city. Based on data from 153 countries, an inverse association between the gross domestic product per head and breastfeeding prevalence was reported (74).

Participants breastfed for less than 6 months, compared to those breastfed for 12 months or longer, had a lower chance of moderate-to-high adherence to the *Prudent* pattern in adulthood. Our finding is in agreement with other studies conducted in children (91-93) and with the only one in which the association was evaluated in adults (43). These authors

observed an association between type of feeding and a 'Prudent' score in adulthood, i.e. having been exposed only to breast milk instead of having received bottle feeding, combined or not with breast milk, was associated with greater intake of fruit, vegetables, whole meal cereals and oily fish, and lower intake of refined cereals, sugar and full-fat dairy products. A study, in which eating behavior was evaluated, also supports our findings since longer exclusive breastfeeding was associated with healthier behaviors later in life (86). On the other hand, in a Brazilian study conducted in adult men no association between breastfeeding and a score of dietary fat content was detected (95).

We found that maternal excessive pre-pregnancy BMI was also associated with dietary patterns in early adulthood, increasing chance of adherence to the *Processed* and decreasing adherence to the *Prudent* pattern. However, this finding needs to be confirmed in studies with appropriate theoretical model to infer causality regarding these variables. Literature has indicated that maternal BMI might be a proxy of intergeneration transmission of healthier lifestyle, in terms of diet, physical activity, smoking or adiposity (57, 205, 206). At this context, associations between parental and offspring's BMI could be explained by shared familial genetic and environmental factors (207). Among these factors, food availability could represent an environmental issue, while changes in leptin gene, which is related with satiety programming, might be one of the genetic pathways. A study in mice verified that a high-fat diet before and during pregnancy, which we could speculate to be usual among overweight and obese mothers, resulted in epigenetic changes in leptin gene expression (208).

Flavors present in breastmilk represent a potential mechanism to explain the association between breastfeeding and adherence to the *Prudent* pattern. Longer exposure of newborns to flavors from maternal diet through breast milk could enhance the acceptance of new foods during the weaning period, and it could be translated into lower food neophobia throughout infancy and adolescence (209, 210). In fact, our *Prudent* pattern is the one characterized by the largest number of food groups with positive factor loadings, suggesting a non-monotonous diet. Such a finding reinforces the hypothesis that food choices established early in life could be associated with later food habits (94). In addition, the presence of leptin in human milk could play an important role in food intake regulation (87). Previous studies conducted on normal weight mothers have shown that breast milk leptin was directly correlated to maternal leptin concentrations in blood and both with the maternal BMI. Also, breast milk leptin level during early lactation phase was associated with infant BMI and weight gain (211, 212). Our findings of inverse association of

adherence to the *Prudent* pattern and direct association of the *Processed* pattern with excessive maternal BMI support a role for maternal adiposity in the relation between breastfeeding and offspring dietary habits.

Our study has limitations regarding the retrospective design that implies recall bias. Breastfeeding duration and pre-pregnancy BMI were long-termed recall by participants with mother's help. However, previous studies pointed out that these variables, as well as birthweight and gestational age, were reported accurately 20 years after birth (181, 213). The FFQ used to assess dietary intake of the previous year could also involve recall bias although this tool has been used widely in nutrition epidemiology, allowing classification according to diet exposure level (124, 214, 215). The dichotomisation of the dietary pattern scores and the consequent reduction of statistical power should be another limitation. This statistical approach was used due to the lack of a linear relation between breastfeeding and the scores.

This study has methodological strengths such as the dietary pattern analysis (137, 139) and the use of DAG to perform regression models avoiding under or over adjustments (17). The possibility of residual confounding exists since direct measures of mother's lifestyle and health consciousness, such as the mothers' diet and child feeding practices, were not available. However, it was minimized since we included the pre-pregnancy BMI taken as proxy and employed an *a priori* theoretical model to account for major confounders in our analysis. The fact that the sample had been composed of female undergraduates or nutrition graduates, who were aware of the relevance of providing reliable data and had familiarity with tools for dietary assessment, is a strength of our study. However, such homogeneity does not permit generalization of our results to male sex and other samples. Despite providing reliable data which could ensure internal validity, external validity is still a concern in our study.

In conclusion, association of longer breastfeeding with moderate-to-high adherence to the *Prudent* pattern in adult life suggest that early life feeding practices may have long-term impact on offspring dietary habits. The associations found need to be confirmed in prospective studies, which would be of interest to reinforce the importance of looking at early feeding practices to improve interventions against nutrition-related diseases in adulthood.

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Ethical Standards

The authors assert that all procedures contributing to this work comply with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional research ethics committee of the School of Public Health from University of São Paulo.

Figure 1. Flow chart of NutriHS participants included in the present study, São Paulo, Brazil.

Table 1. Factor loadings distribution for four dietary patterns identified in women from the NutriHS (n = 715), São Paulo, Brazil^a.

Food groups	Factor loadings ^b			
	Processed	Prudent	Brazilian	Lacto-vegetarian
Baked salted pastries	0.51	-0.10	0.02	-0.08
Noodles/ pasta	0.58	0.09	0.06	0.06
Fish and chicken (fried)	0.32	0.02	0.09	-0.24
Processed meat	0.48	0.03	0.20	-0.18
Pork meat	0.21	0.15	0.06	-0.38
Fast food	0.78	-0.13	-0.02	-0.001
Snacks	0.34	-0.02	0.08	-0.02
Industrialized sauce	0.32	-0.02	0.05	0.17
Industrialized beverages	0.49	-0.12	0.16	-0.02
Alcoholic beverages	0.29	0.02	-0.02	0.22
Sweets/ desserts	0.59	0.08	0.19	-0.08
Roots, tubers and soups	0.30	0.39	-0.06	0.03
Vegetables	-0.10	0.68	-0.06	0.001
Fruits	-0.02	0.50	0.03	-0.13
Natural juice	0.09	0.32	-0.07	-0.10
Oats	-0.19	0.45	-0.18	0.20
Tea	-0.03	0.25	-0.09	0.05
Whole grain bread	0.01	0.28	0.03	0.51
Yogurt and fruit vitamin	-0.04	0.50	0.01	0.17
Olive oil or oil for salad dressing	0.02	0.39	0.08	-0.23
Fish and chicken (roasted)	-0.01	0.45	-0.05	0.09
Eggs	-0.06	0.36	-0.19	0.15
Beef	0.08	0.27	0.23	-0.30
Brown rice	-0.07	0.24	-0.29	0.32
Lentil	0.01	0.32	-0.22	0.05
Salt	0.19	0.27	0.25	-0.33
Nuts	-0.12	0.37	-0.29	0.16
White bread	0.08	-0.17	0.67	0.06
Coffee	-0.08	0.07	0.24	0.19
Whole milk	-0.01	-0.06	0.39	-0.01
Bean	-0.04	0.18	0.55	-0.08
White rice	0.08	-0.01	0.66	-0.26
Spreading fat (mayonnaise / butter)	0.11	-0.07	0.67	0.23
Cheese	0.16	0.19	-0.05	0.37
Skimmed milk	-0.02	0.18	0.01	0.41
Offal	0.14	0.16	-0.12	-0.42
Eigenvalues	3.63	2.67	1.90	1.50
Explained variance (%)	7.68	7.63	6.75	4.90
Cumulative explained variance (%)	7.68	15.32	22.06	26.96

^a Kaiser Mayer Olkin test = 0.727; Bartlett's test of sphericity, p < 0.001.^b Dietary pattern labels were informed by the food groups/items with factor loadings higher than |0.20| and in bold, all factor loadings contributed to the dietary patterns scores.

Table 2. Current and early life characteristics of women from the NutriHS, São Paulo, Brazil.

Variables	N (%)
Age (years)	
≤ 19	131 (22.3)
20 to 34	420 (71.6)
≥ 35	36 (6.1)
Skin color	
White	411 (70.3)
Non-white	174 (29.7)
Alcohol intake	
Never	414 (70.5)
<1 time/week	97 (16.5)
≥ 1 time/ week	76 (13.0)
Graduation in nutrition	
First half	285 (48.5)
Second half	260 (44.3)
Graduate	42 (7.2)
Family income (number of minimum wages) ^a	
< 6	367 (66.8)
6-10	112 (20.4)
> 10	70 (12.8)
Body mass index (kg/m²)	
< 18.5	42 (7.2)
18.5 to < 25.0	398 (67.8)
≥ 25.0	147 (25.0)
Breastfeeding	
Yes	619 (90.8)
Never	63 (9.2)
Breastfeeding duration (months)	
< 6	178 (30.3)
6 to <12	206 (35.1)
≥ 12	203 (34.6)
Birthweight (kg)	
< 2.5	66 (12.3)
2.5 to 3.9	440 (81.6)
≥ 4.0	33 (6.1)
Mode of delivery	
Vaginal	240 (41.0)
C-section	347 (59.0)
Maternal pre-pregnancy body mass index (kg/m²)	
< 18.5	25 (6.6)
18.5 to < 25.0	266 (70.6)
≥ 25.0	86 (22.8)
Maternal education (years)^c	
< 11	255 (43.7)
≥ 11	329 (56.3)

^a One minimum wage is equivalent to 724.0 Brazilian real (or US\$ 212.9).

Table 3. Frequencies of dietary patterns adherence levels according to current lifestyle characteristics in women from the NutriHS, São Paulo, Brazil.

	Adherence level to dietary patterns ^a											
	Processed			Prudent			Brazilian			Lacto-Vegetarian		
	Low n = 197	Moderate- to-high n = 390	<i>P</i> ^b	Low n = 196	Moderate- to-high n = 391	<i>P</i> ^b	Low n = 189	Moderate- to-high n = 398	<i>P</i> ^b	Low n = 196	Moderate-to- high n = 391	<i>P</i> ^b
Energy intake (kcal/day) ^c	1815 (1483; 2318)	2482 (2004; 3049)	<0.001	1813 (1512; 2251)	2427 (2004; 3048)	<0.001	1781 (1461; 2235)	2462 (1986; 3054)	<0.001	2490 (1887; 3164)	2118 (1703; 2684)	<0.001
Age (years) ^c	22 (20; 28)	22 (20; 26)	0.627	22 (19; 27)	22 (20; 27)	0.458	22 (20; 28)	22 (20; 26)	0.584	21 (19; 24)	22 (20; 28)	0.003
Body mass index (kg/m ²) ^c	21.6 (20.1; 23.3)	22.7 (20.6; 25.4)	0.001	22.1 (20.1; 25.1)	22.3 (20.5; 25.0)	0.482	22.0 (20.5;23.7)	22.3 (20.3; 25.4)	0.107	22.1 (20.2; 25.0)	22.2 (20.4; 25.0)	0.492
Skin color ^d												
White	132 (32.1)	279 (67.9)	0.275	129 (31.4)	282 (68.6)	0.095	144 (35.0)	267 (65.0)	0.030	119 (28.9)	292 (71.1)	<0.001
Non-white	64 (36.8)	110 (63.2)		67 (38.5)	107 (61.5)		45 (25.9)	129 (74.1)		77 (44.3)	97 (55.7)	
Alcohol intake												
Never	169 (40.8)	245 (59.2)	<0.001	145 (35.0)	269 (65.0)	0.155	118 (28.5)	296 (71.5)	0.008	159 (38.4)	255 (61.6)	<0.001
<1 time/week	19 (19.6)	78 (80.4)		33 (34.0)	64 (66.0)		37 (38.1)	60 (61.9)		21 (21.7)	76 (78.3)	
≥ 1 time/ week	9 (11.8)	67 (88.2)		18 (23.7)	58 (76.3)		34 (44.7)	42 (55.3)		16 (21.1)	60 (78.9)	
Graduation in nutrition												
First half	90 (31.6)	195 (68.4)	0.568	97 (34.0)	188 (66.0)	0.915	100 (35.1)	185 (64.9)	0.003	105 (36.8)	180 (63.2)	0.031
Second half	91 (35.0)	169 (65.0)		86 (33.1)	174 (66.9)		68 (26.2)	192 (73.8)		84 (32.3)	176 (67.7)	
Graduate	16 (38.1)	26 (61.9)		13 (30.9)	29 (69.1)		21 (50.0)	21 (50.0)		7 (16.7)	35 (83.3)	
Family income (number of minimum wages) ^{d,e}												
< 6	127 (34.6)	240 (65.4)	0.358	140 (38.2)	227 (61.8)	<0.001	88 (24.0)	279 (76.0)	<0.001	143 (39.0)	224 (61.0)	0.001
6-10	31 (27.7)	81 (72.3)		33 (29.5)	79 (70.5)		41 (36.6)	71 (63.4)		27 (24.1)	85 (75.9)	
> 10	25 (35.7)	45 (64.3)		11 (15.7)	59 (84.3)		47 (67.1)	23 (32.9)		15 (21.4)	55 (78.6)	

^a Dietary patterns adherence classified according to score tertiles (Low adherence= 1st tertile; moderate-to-high adherence= 2nd + 3rd tertiles);^b p-value refers to chi-squared test;^c Continuous variables described as median (interquartile range); p-value refers to Mann-Whitney test;^d Sample reduction due to missing information (skin color: n= 585; family income: n= 549);^e One minimum wage is equivalent to 724.0 Brazilian real (or US\$ 212.9).

Table 4. Odds ratio (OR) of being classified into moderate-to-high adherence to dietary patterns according to breastfeeding duration in women from the NutriHS, São Paulo, Brazil.

	Crude ^a		Model 1 ^b		Model 2 ^c	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Processed pattern						
Breastfeeding duration (months)						
< 6	0.86 (0.56; 1.32)	0.487	1.03 (0.60; 1.78)	0.914	1.03 (0.57; 1.87)	0.921
6 to < 12	0.94 (0.62; 1.41)	0.751	0.96 (0.57; 1.63)	0.886	1.09 (0.61; 1.93)	0.769
≥ 12 (reference)	1		1		1	
Maternal pre-pregnancy BMI (kg/m ²)						
< 18.5			0.99 (0.42; 2.38)	0.996	1.18 (0.47; 2.96)	0.729
≥ 25.0			1.82 (1.04; 3.21)	0.037	2.01 (1.09; 3.71)	0.026
18.5 to < 25.0 (reference)	1		1		1	
Maternal education (< 11 years)			0.80 (0.51; 1.25)	0.320	0.62 (0.38; 1.01)	0.055
≥ 11 years (reference)	1		1		1	
Mode of delivery (C-section)			1.04 (0.67; 1.63)	0.851	1.13 (0.69; 1.83)	0.631
Vaginal (reference)	1		1		1	
Prudent pattern						
Breastfeeding duration (months)						
< 6	0.79 (0.52; 1.21)	0.281	0.57 (0.33; 0.99)	0.045	0.53 (0.29; 0.97)	0.039
6 to <12	1.35 (0.88; 2.05)	0.167	0.78 (0.45; 1.37)	0.395	0.86 (0.47; 1.55)	0.605
≥ 12	1		1		1	
Maternal pre-pregnancy BMI (kg/m ²)						
< 18.5			1.30 (0.49; 3.45)	0.592	1.57 (0.57; 4.33)	0.383
≥ 25.0			0.57 (0.34; 0.96)	0.033	0.52 (0.30; 0.92)	0.024
18.5 to < 25.0 (reference)	1		1		1	
Maternal education < 11 years			0.75 (0.48; 1.19)	0.222	0.60 (0.37; 0.98)	0.043
≥ 11 years (reference)	1		1		1	
Mode of delivery (C-section)			0.95 (0.60; 1.51)	0.843	1.05 (0.64; 1.71)	0.857
Vaginal (reference)	1		1		1	
Brazilian pattern						
Breastfeeding duration (months)						
< 6	0.78 (0.51; 1.20)	0.267	0.92 (0.53; 1.59)	0.765	0.95 (0.52; 1.73)	0.860
6 to < 12	0.93 (0.61; 1.42)	0.742	1.06 (0.62; 1.81)	0.845	1.22 (0.67; 2.20)	0.510
≥ 12	1		1		1	
Maternal pre-pregnancy BMI (kg/m ²)						
< 18.5			1.18 (0.48; 2.94)	0.716	1.50 (0.56; 4.06)	0.422
≥ 25.0			0.90 (0.54; 1.52)	0.705	0.89 (0.50; 1.59)	0.694
18.5 to < 25.0 (reference)	1		1		1	
Maternal education < 11 years			2.49 (1.56; 3.98)	<0.001	2.40 (1.44; 4.02)	0.001
≥ 11 years (reference)	1		1		1	
Mode of delivery (C-section)			0.68 (0.43; 1.07)	0.098	0.69 (0.42; 1.14)	0.147
Vaginal (reference)	1		1		1	
Lacto-vegetarian pattern						
Breastfeeding duration (months)						
< 6	1.32 (0.86; 2.02)	0.208	1.19 (0.70; 2.05)	0.519	1.20 (0.70; 2.08)	0.505
6 to <12	1.16 (0.77; 1.75)	0.465	1.39 (0.82; 2.39)	0.224	1.36 (0.79; 2.33)	0.273
≥ 12	1		1		1	
Maternal pre-pregnancy BMI (kg/m ²)						
< 18.5			1.00 (0.39; 2.53)	0.999	0.92 (0.36; 2.36)	0.868
≥ 25.0			0.70 (0.42; 1.18)	0.178	0.71 (0.42; 1.20)	0.199
18.5 to < 25.0 (reference)	1		1		1	
Maternal education < 11 years			0.62 (0.40; 0.97)	0.036	0.66 (0.42; 1.03)	0.070
≥ 11 years (reference)	1		1		1	
Mode of delivery (C-section)			0.94 (0.60; 1.48)	0.797	0.92 (0.58; 1.46)	0.721
Vaginal (reference)	1		1		1	

^a n = 587;^b Model 1: adjusted for pre-pregnancy BMI, maternal education and mode of delivery (n = 376);^c Model 2: adjusted for variables from model 1 plus energy intake (n = 376);

BMI, body mass index.

Supplementary Table 1. Characteristics of participants included in dietary pattern analysis and excluded due to missing information in the food frequency questionnaire.

	Women excluded	Women included	P- value
Continuous variables (median and interquartile range)	N = 596	N = 715	
Age (years)	23 (20; 27)	22 (20; 27)	0.054
Body mass index (kg/m ²)	22.1 (20.5; 25.0)	22.2 (20.4; 25.2)	0.650
Categorical variables (n and %)			
Skin color			
White	394 (70.2)	499 (70.3)	0.985
Non-white	167 (29.8)	211 (29.7)	
Alcohol intake			
Never	373 (65.7)	494 (69.1)	0.423
<1 time/week	111 (19.5)	124 (17.3)	
≥ 1 time/ week	84 (14.8)	97 (13.6)	
Graduation in nutrition			
First half	277 (48.8)	355 (49.6)	0.010
Second half	219 (38.5)	305 (42.7)	
Graduate	72 (12.7)	55 (7.7)	
Family income (number of minimum wages ^a)			
< 6	373 (73.0)	440 (66.6)	0.059
6-10	86 (16.8)	135 (20.4)	
> 10	52 (10.2)	86 (13.0)	
Breastfeeding duration (months)			
< 6	35 (31.8)	178 (30.3)	0.694
6 to <12	34 (30.9)	206 (35.1)	
≥ 12	41 (37.3)	203 (34.6)	

^a One minimum wage is equivalent to 724.0 Brazilian real (or US\$ 212.9).

Supplementary Figure 1. Scree Plot of Eigenvalues according to number of factors retained in factor analysis by principal component.

Supplementary Figure 2. Directed Acyclic Graph (DAG) for the association between breastfeeding and dietary patterns adherence.

Notes: Minimal sufficient adjustment: Maternal pre-pregnancy BMI, maternal schooling and mode of delivery.

Legend= exposure; outcome; ancestor of outcome; ancestor of exposure; ancestor of exposure and outcome; other variable; → causal path; → biasing path. BMI, Body mass index

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5.3 MANUSCRITO 3: Early life feeding and current dietary patterns are associated with biomarkers of glucose and lipid metabolism in young women from the Nutritionist's Health Study

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Abstract

Background/ Objectives: We investigated if breastfeeding duration and current dietary patterns (DP) were associated with glucose and lipid metabolism biomarkers in women from the Nutritionist's Health Study. **Subjects/ Methods:** This is a cross-sectional analysis of 200 healthy undergraduates and nutrition graduates aged ≤ 45 years. Total [<6 ; ≥ 6 months] and predominant [<3 ; ≥ 3 months] breastfeeding was recalled using questionnaires. Diet was assessed using a food frequency questionnaire. DP obtained by factor analysis by principal component were categorized into tertiles of adherence (T1=reference). Glucose and lipid biomarkers were categorized into tertiles (T1+T2=reference). Logistic regression was applied considering minimal sufficient adjustment recommended by directed acyclic graphs. **Results:** Median (interquartile range) age and BMI were 23.0 (20.0; 28.5) years and 22.6 (20.7; 25.4) kg/m², respectively. Mean $\pm$ SD values of glucose, LDL-c and HDL-c were 82.0 $\pm$ 9.0, 101.1 $\pm$ 29.6 and 54.4 $\pm$ 12.4 mg/dL, respectively. Women breastfed for <6 months had higher chance of being classified into T3 of insulin (OR=2.87; 95%CI=1.28-6.40). Predominant breastfeeding <3 months was associated with insulin levels (OR=2.27; 95%CI=1.02-5.02) and HOMA-IR (OR=2.36; 95%CI=1.06-5.26). Breastfeeding was not associated with lipids. The *Processed* pattern was directly associated with LDL-c (T3: OR 6.08; 95%CI 1.80-20.58; P-trend=0.004), while the *Prudent* pattern was inversely associated with LDL-c (T3: OR 0.26; 95%CI 0.08-0.87; P-trend=0.029) and LDL-c/HDL-c ratio (T3: OR 0.28; 95%CI 0.08-0.97; P-trend=0.046). **Conclusion:** Early feeding could be a protective factor against insulin resistance development, while current DP were associated with lipid profile. This evidence indicates that from early life until early adulthood, dietary habits might influence women's cardiometabolic risk profile. **Abbreviations:** NutriHS, Nutritionists' Health Study; DAG, Direct Acyclic Graphs

1. Introduction

Glucose and lipid metabolism disturbances are important risk factors for cardiovascular disease, a major public health problem worldwide (10). Insulin resistance and increased LDL-c should be monitored to prevent cardiovascular events (216, 217). In studies of risk factors for cardiovascular diseases, the female sex has been underrepresented, despite being recognized biological and psychosocial gender differences (218).

The impact of diet on glucose and lipid metabolism and cardiometabolic outcomes may also differ between sexes (122, 219-222). Once these abnormalities are more common after menopause (218), most studies have assessed its impact in middle-aged women (215, 221, 223, 224). Considering that menopausal hormonal changes could modify the effect of diet on metabolic outcomes (118), assessment of dietary patterns at a younger age could be more appropriate to investigate whether modifiable dietary habits are associated with predictors of cardiometabolic disease, such as glucose and lipid metabolism biomarkers.

Few studies used dietary patterns approaches to evaluate this association in women during early adulthood (220, 225). Dietary patterns analysis considers interaction and synergic effects of food groups and nutrients and has been recommended in nutritional epidemiology to evaluate associations with health outcomes (137, 139).

Cardiometabolic diseases programming starts early in life, especially during the first 1,000 days of life (26). A potential protective effect of breastfeeding against these diseases has been investigated. Few studies on the association of early life feeding with glucose and lipid metabolism biomarkers in adults have reported inconsistent results (14, 39, 79-82, 226-228). Evidence of breastfeeding protection against type 2 diabetes is consistent in high-income countries (40, 228, 229), but not in low- and middle-income countries (80).

Once early life diet may affect glucose and lipid metabolism, investigations in young adults are desirable to prevent cardiovascular outcomes. Our hypothesis was that longer breastfeeding as well as current adherence to healthier dietary patterns were associated with a favorable metabolic profile. By including young women, the Nutritionist's Health Study (NutriHS) offers a unique opportunity to examine associations of breastfeeding duration and current dietary patterns with glucose and lipid biomarkers.

2. Subjects and Methods

This is a sub-study of the NutriHS, which is a cohort conducted with non-pregnant undergraduates and nutrition graduates, approved by the Research Ethics Committee of the School of Public Health of the University of São Paulo, Brazil (157). Eligibility criteria were met by 1,478 participants recruited between 2014 and 2017. For the purpose of this sub-study, 101 men, 34 women >45 years, 2 with diagnosis of cancer and 10 with diabetes were excluded,

resulting in 1,331 women. Self-reported data were collected at baseline using electronic structured questionnaires (<http://www.fsp.usp.br/nutrihs/index.html>) (**Figure 1**).

To detect differences in proportions of glucose and lipid biomarkers categories according to exposures, with a power of 80% and a two-tailed level of significance of 5%, 124 participants were needed. A random subset of 200 participants had biomarkers levels determined. For seven participants, there was insufficient plasma sample for insulin determination (**Figure 1**).

Retrospective cohort design was considered when breastfeeding was the main exposure and a cross-sectional analysis when current dietary patterns were exposures.

Before answering questionnaires about early life events, participants consulted their mothers or birth cards. Early life feeding was assessed considering total breastfeeding [<6 ; ≥ 6 months (reference)] and predominant breastfeeding, which refers to the age of introduction of formula, animal milk or solid food [<3 ; ≥ 3 months (reference)]. Birthweight and maternal characteristics, such as education level, pre-pregnancy body mass index (BMI) and gestational diabetes were also obtained.

Age, family income, graduation level (undergraduates attending the first half or the second half of the nutrition course; graduates/nutritionists), smoking habit and alcohol intake were assessed. Physical activity was evaluated using the International Physical Activity Questionnaire (167) validated in Brazilians (168) and expressed as tertiles of METs from moderate to vigorous-intensity activities (First + second *versus* third tertile).

Dietary intake regarding the previous 12 months was assessed using a validated food frequency questionnaire (161). Daily energy intake was estimated according to the USDA National Nutrient Database for Standard Reference (163). Dietary patterns were identified by factor analysis by principal component as previously described (180). The four dietary patterns (*Processed*, *Prudent*, *Brazilian* and *Lacto-vegetarian*) together explained 27% of diet variance. The *Processed* pattern was characterized by high intake of salted pastries, noodles, roots/soups, fried white meat, processed and pork meat, fast-food, snacks, industrialized sauces and beverages, alcoholic beverages and sweets. The *Prudent* pattern was characterized by high intake of fruits, yogurt, oats, nuts, vegetables, salad oil, salt, roots/soups, roasted white meat, beef, eggs, brown rice, lentil, whole grain bread and tea. The *Brazilian* pattern was directly associated with intake of white rice, bean, processed meat, beef, salt, white bread, spreading fat, whole milk and coffee, and inversely associated with brown rice, lentil and nuts. The *Lacto-vegetarian* pattern was associated with high intake of alcoholic beverages, brown rice, whole grain bread, oats, spreading fat, cheese and skimmed milk, and low intake of animal protein sources, salad oil, salt and white rice. Dietary pattern scores were categorized into tertiles (First = low; second = moderate; third = high adherence), having the first as reference.

A random sub-sample of participants was invited to a face-to-face interview. Weight was measured using a digital scale with accuracy to the nearest 100 g and height using a stadiometer with 0.1 cm of precision. Fasting plasma glucose was determined by the glucose oxidase method and insulin by immunofluorimetric assay (Monobind Inc., Lake Forest, CA, USA). Insulin resistance was evaluated using homeostasis model assessment (HOMA-IR), (230). Total cholesterol, HDL-c and triglycerides were measured using enzymatic colorimetric method and LDL-c estimated by Friedewald formula (160). LDL-c/HDL-c ratio was calculated. Biomarkers (outcomes) were categorized into tertiles and the first two were grouped (reference), while the highest was isolated (First + second *versus* third tertile) considering that our sample was within the near-normal ranges.

Statistical analyses

Categorical variables were described as absolute and relative frequencies. Kolmogorov-Smirnov test was used to verify normal distribution of continuous variables, described as mean (standard deviation) or median (interquartile range).

Logistic regression was performed to estimate odds ratios (OR) and 95% confidence interval of being classified into the third tertile of biomarkers. Confounding variables were selected according to minimal sufficient adjustment sets recommended by Direct Acyclic Graphs (DAG), constructed using the Daggity software (17) (**Supplementary Figures 1 and 2**). When breastfeeding was the main exposure, models were adjusted for maternal education and birthweight. Adjustment for gestational diabetes was indicated, but since only three cases were present, these were excluded (**Supplementary figure 1**). When dietary pattern was the main exposure, three models were performed: model 1 considered the four dietary patterns and energy intake; model 2 adding adjustments for age, moderate-to-vigorous physical activity, smoking habit, frequency of alcohol intake, total breastfeeding duration and birthweight as suggested by the DAG (**Supplementary figure 2**); model 3 adding BMI to test a possible mediation effect (data not shown). Linear P-trend across tertiles of patterns was estimated. All models showed goodness-of-fit according to Hosmer-and-Lemeshow test (p-value >0.05).

Significance was considered at a level of 5%. Analyses were performed using Stata Statistical Software (release 12, 2011, StataCorp LP, College Station, TX).

3. Results

The sub-sample of 200 women had median values (interquartile range) of age of 23.0 (20.0; 28.5) years, BMI of 22.6 (20.7; 25.4) kg/m² and energy intake of 9,188 (7,447; 11,943) kJ/day [2,195 (1,779; 2,853) kcal/day]. Except for lower frequencies of graduates and alcohol intake in the sub-sample, other characteristics did not differ from the entire NutriHS sample

(**Table 1**), assuring reasonable representativeness. Most participants of the sub-sample were undergraduates (96%), did not smoke nor consume alcohol and had normal BMI. Approximately, 81% were born with adequate weight and a quarter was breastfed for <6 months or had predominant breastfeeding for <3 months.

Mean or median values of glucose and lipid metabolism biomarkers of the entire sample were within the normal ranges. Looking at the highest tertiles, only mean value of LDL-c (133.8 ± 21.2 mg/dL) was slightly above the recommendation (**Table 2**).

Women breastfed for <6 months had a higher chance to be classified into the third tertile of insulin (OR=2.87; 95%CI 1.28; 6.40). Predominant breastfeeding <3 months was also associated with insulin levels (OR=2.27; 95%CI 1.02; 5.02) and HOMA-IR (OR=2.36; 95%CI 1.06; 5.26). Birthweight showed to be a confounder on these associations, since macrosomia was directly associated with insulin levels and HOMA-IR. Breastfeeding was not associated with lipid biomarkers. Maternal education <11 years was inversely associated with the offspring's HDL-c during adult life (**Table 3**).

Table 4 shows associations between three major dietary patterns identified and metabolic biomarkers, considering adjustment for variables of current and early life (model 2). Significant association of total breastfeeding duration with insulin was maintained independently of current dietary patterns (OR=4.18; $P = 0.003$). Considering predominant breastfeeding duration instead of total breastfeeding, its association with HOMA-IR was also maintained (OR=2.57; $P = 0.038$). The *Processed* pattern was directly associated with LDL-c (P -trend=0.004) and participants with moderate adherence to this pattern had a higher chance to be classified into the third tertile of plasma glucose ($P=0.007$) and LDL-c/HDL-c ratio ($P=0.032$), although a linear trend was not found. The *Prudent* pattern was associated with a lower chance of classification into the highest tertiles of LDL-c (P -trend=0.029) and LDL-c/HDL-c ratio (P -trend=0.046). Participants with moderate adherence to the *Prudent* pattern had a lower chance to be classified into the third tertile of triglyceridemia ($P=0.043$) and plasma glucose ($P=0.033$), but such associations were not dose-response since were not significant for those with the highest adherence. Participants with moderate adherence to the *Brazilian* pattern had a lower chance to be classified into the highest tertile of LDL-c ($P=0.003$; P -trend=0.326), while high adherence to this pattern seemed to be directly associated with insulin levels (P -trend=0.005) and HOMA-IR (P -trend=0.003) (**Table 4**). The *Lacto-vegetarian* pattern had the lowest explained variance (not shown in Table); this was associated with HDL-c (T3: OR=0.36; 95%CI=0.13; 0.97; P -trend=0.044) and LDL-c/HDL-c ratio (T3: OR=3.82; 95%CI=1.27; 11.49; P -trend=0.017). BMI was not considered a mediating factor, since its inclusion in the model 2 did not change any effect size (OR changed <10%) and the statistical significance was maintained (except for the association between moderate adherence to the *Prudent* pattern and triglycerides).

4. Discussion

In this study of young women, both early feeding and current dietary patterns were associated with glucose and lipid metabolism biomarkers. A relevant aspect was to focus on the female sex that has been less investigated in literature. The inverse association of longer breastfeeding with insulin levels and HOMA-IR suggests that breastfeeding could exert a protective effect against insulin resistance later in life. High adherences to the *Prudent* and *Processed* patterns in adulthood were inversely and directly associated with an unfavorable lipid profile, respectively. This indicates that dietary habits since early life until present time might influence the cardiometabolic risk profile of young women even when their biomarkers are still within the near normal ranges.

Women who had shorter breastfeeding exhibited a higher chance of higher insulin levels and HOMA-IR, which could be interpreted as decreased insulin sensitivity. Plasma glucose of our participants was unable to differentiate subsets according to the breastfeeding duration. These findings are in line with a meta-analysis which reported that breastfed infants showed lower insulin levels and lower chance of type 2 diabetes later in life, but mean glucose was not different from bottle-fed subjects (40). Our study was also in agreement with others involving adolescents, in which exclusive breastfeeding in the first three months of life was inversely associated with insulin levels and HOMA-IR but not with plasma glucose (231), or adults from developed (79) and middle-income countries (80). Middle-aged participants of the Dutch Famine Cohort, who were exclusively breastfed before leaving the maternity hospital, showed lower fasting glucose, insulin and proinsulin levels compared to those who were bottle-fed (226). As far as we know, few studies have investigated associations of breastfeeding duration with biomarkers of glucose metabolism or diabetes incidence in healthy adults, and inconsistent results have been reported (227, 229).

Our suggestion of a protective role of longer breastfeeding duration against insulin resistance has biological plausibility. Human milk has lower energy and protein content than conventional formulas, which could contribute to lower insulin like growth factor-1 concentration and prevent accelerated growth during childhood, known to be associated with metabolic diseases later in life (29, 232). Also, human milk contains hormones involved in energy balance and insulin sensitivity, such as leptin and adiponectin (233). In addition to breastfeeding, intra-utero conditions – reflected by the birthweight – may be also important for associations of glucose metabolism disturbances in adulthood.

The lack of association between breastfeeding and lipid biomarkers in our study agrees with previous studies (82, 227, 228), but differs from others (39, 226). Another possible link between breastfeeding and metabolic disturbances in adulthood could be dietary habits (43, 180), which in turn were associated with glucose and lipid biomarkers (119, 122, 219-221). Our group

previously reported an association of breastfeeding duration with adherence to the *Prudent* pattern (180). We verified that although both breastfeeding and current dietary patterns were associated with glucose and lipid biomarkers, it seemed to affect preferentially one or another metabolic pathway. We speculate that early feeding could be associated with the development of glucose metabolism disturbance, while current diet could be associated with abnormal lipid profile.

It is important to emphasize that although the Brazilian pattern has been directly associated with insulin levels and HOMA-IR in our fully adjusted models (Model 2), confidence intervals seemed to be affected considering all the confounders. Thus, these results should be interpreted with caution, considering that not seem to be accurate; and that the Brazilian diet was previously inversely associated with cardiometabolic risk factors (219).

We verified that our *Prudent* pattern was inversely associated with LDL-c and LDL-c/HDL-c ratio, thus indicating a favorable lipid profile. On the other hand, adherence to the *Processed* pattern was directly associated with LDL-c. Although our cross-section design does not allow inferring causality, these significant associations in young women, without cardiometabolic diseases, are suggestive of a role of diet in lipid profile. As the FFQ used refers to the previous year consumption, bias of reversal causality is less likely. Considering that dietary habits are modifiable in face of nutritional counselling, we highlight the relevance of monitoring lipid biomarkers before middle age, when preventive measure could be more effective.

In our study, most participants and their mothers before pregnancy had normal BMI and the association of dietary patterns with glucose and lipid biomarkers did not seem to be BMI-mediated. We speculate that the role of BMI as a mediating factor could be different in samples composed of older participants and with higher prevalence of obesity and comorbidities. Evidences suggest that maternal adiposity could contribute to explain the inter-generational transmission of obesity (56, 234). Previous findings of the NutriHS showed positive association between maternal pre-pregnancy BMI and DXA-determined general and central adiposity measures of their daughters during adulthood (unpublished data), which could deteriorate the cardiometabolic profile later in life. Without disregarding a genetic component, we suggest that environmental factors should be contributing to explain these associations. We previously reported that maternal pre-pregnancy BMI was also associated with offspring's adherence to the *Prudent* and *Processed* patterns (180). Thus, we reinforce the importance of obesity prevention before conception in attempt to minimize a vicious cycle of body weight accumulation and its comorbidities.

In agreement with our results, in young Finnish women, a health-conscious pattern similar to our *Prudent* pattern, was inversely associated with total cholesterol and LDL-c and this was unaffected by BMI adjustment (235). In the same study, a pattern characterized by traditional

foods (sausage, pork meat and potatoes) also present in our *Processed* pattern, was directly associated with LDL-c, as in ours, and with insulin levels. It is important highlight that dietary patterns differ according to socioeconomic and cultural characteristics and might not be reproducible among different populations (139). To the best of our knowledge, in young Brazilian women, only one study previously examined dietary patterns and its association with metabolic biomarkers. In line with our results, the Common-Brazilian pattern was inversely associated with LDL-c, and also with other lipid particles (219).

Our *Prudent* pattern resembles the Dietary Approach to Stop Hypertension, which has been inversely associated with cardiovascular risk (195, 225). High intakes of fruits, vegetables, nuts and whole grains in these patterns should have contributed to high dietary fiber and phytochemicals contents, known to be beneficial to risk factors such as LDL-c (236). On the other hand, a combination of unhealthier dietary habits in our *Processed* pattern (high intake of processed meats, simple carbohydrates, fried foods and alcoholic beverages) could explain to the deleterious association found (237).

A strength of our study refers to peculiarities of participants, who had nutrition knowledge to manage tools to assess diet and may be conscious about the relevance of providing good quality data, enhancing chances of reliable answers. On the other hand, the specificity of our sample of highly educated young women does not allow us to generalize our findings to samples with different characteristics. The use of DAGs was also strength, since it minimizes insufficient or over adjustments (17). As far as we know, no previous study used DAG to investigate associations of early and current dietary habits with metabolic outcomes.

A limitation of this study refers to the cross-sectional design, which makes it impossible to assess temporality and causality. Regarding the association between early feeding and biomarkers, the retrospective cohort design could imply in recall bias of breastfeeding duration, which could represent a limitation of our study. Nonetheless, it was reported that breastfeeding duration can be accurately recalled 20 years later (213).

Conclusions

Both early feeding and current dietary habits were associated with biomarkers of glucose and lipid metabolism of young women, suggesting that they could influence risk of metabolic disturbances. It is possible that longer breastfeeding may confer protection against insulin resistance in adult life. Eating habits were associated with lipid profile even in young and healthy women. Further investigations with prospective design in early adulthood are needed.

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Table 1. Main characteristics of the entire eligible NutriHS sample and of the sub-sample for whom biomarkers were determined.

Current life characteristics n (%)	Entire sample n = 1131	Sub-sample with biomarkers n = 200	P-value ^a
Age (years)			
≤ 19	252 (22.3)	34 (17.0)	0.170
> 19 to 34	792 (70.0)	146 (73.0)	
≥ 35	87 (7.7)	20 (10.0)	
Graduation level			
First-half undergraduates	547 (49.3)	104 (52.0)	0.011
Second-half undergraduates	442 (39.9)	88 (44.0)	
Graduates	120 (10.8)	8 (4.0)	
Smoking habit			
No	1069 (96.4)	189 (94.5)	0.203
Yes	40 (3.6)	11 (5.5)	
Alcohol intake			
Never	761 (68.6)	126 (63.0)	0.045
<1 time/week	190 (17.1)	49 (24.5)	
≥ 1 time/week	158 (14.3)	25 (12.5)	
Family income (number of minimum wages) ^b			
< 6	714 (70.7)	120 (64.8)	0.271
6-10	181 (17.9)	41(22.2)	
> 10	115 (11.4)	24 (13.0)	
Self-reported body mass index (kg/m ²)			
< 18.5	66 (6.0)	14 (7.0)	0.738
≥ 18.5 and < 25.0	760 (68.5)	132 (66.0)	
≥ 25.0	283 (25.5)	54 (27.0)	
Early life events			
Maternal education (years)			
< 11	296 (44.8)	81 (41.1)	0.354
≥ 11	364 (55.2)	116 (58.9)	
Pre-pregnancy body mass index (kg/m ²)			
< 18.5	25 (6.9)	10 (6.9)	0.209
≥ 18.5 and < 25.0	245 (68.1)	110 (75.3)	
≥ 25.0	90 (25.0)	26 (17.8)	
Gestational diabetes			
No	663 (99.2)	195 (98.5)	0.322
Yes	5 (0.8)	3 (1.5)	
Birthweight (kg)			
< 2.5	68 (12.0)	22 (12.0)	0.982
≥ 2.5 and < 4.0	461 (81.3)	148 (80.9)	
≥ 4.0	38 (6.7)	13 (7.1)	
Breastfeeding (months)			
< 6	170 (31.2)	45 (26.5)	0.241
≥ 6	375 (68.8)	125 (73.5)	
Predominant breastfeeding (months)			
< 3	146 (27.0)	44 (23.9)	0.413
> 3	395 (73.0)	140 (76.1)	

^a P-value refers to chi-squared test.^b One minimum wage is equivalent to 724.0 Brazilian real (or US\$ 212.9).

Table 2. Mean (standard deviation) or median (interquartile range) values of glucose and lipid metabolism biomarkers of the entire sample of NutriHS participants and those classified in the third tertile.

	Entire sample		3 rd tertile		
	n	Mean (SD)	n	Mean (SD)	minimum - maximum
Glucose (mg/dL) ^a	200	82.0 (9.0)	70	91.8 (5.5)	86.0-116.0
Insulin (UI/L) ^b	193	8.7 (6.3 – 12.2)	65	13.5 (12.2 – 15.8)	10.4-82.5
Homa-IR ^b	192	1.7 (1.2 – 2.5)	64	2.8 (2.5 – 3.5)	2.1-17.3
Total cholesterol (mg/dL) ^c	200	173.9 (34.8)	67	212.6 (21.4)	186.0-281.0
HDL-c (mg/dL) ^c	200	54.4 (12.4)	70	68.0 (8.6)	58.0-94.0
LDL-c (mg/dL) ^c	199	101.1 (29.6)	68	133.8 (21.2)	109.0-207.0
Triglycerides (mg/dL) ^{b,d}	200	82.0 (58.0 – 110.0)	67	128.0 (110.0 – 154.0)	100.0-495.0

^a To convert mg/dL glucose to mmol/L, multiply mg/dL by 0.056.

^b Values described as median (interquartile range).

^c To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.0259.

^d To convert mg/dL triglycerides to mmol/L, multiply mg/dL by 0.0113.

Table 3. Odds ratios (OR 95% CI) of being classified into the third tertile of glucose and lipid biomarkers according to breastfeeding duration in women from the NutriHS, São Paulo, Brazil.

	Glycemic biomarkers					
	Plasma glucose		Insulin		HOMA-IR	
	Crude	Multiple ^a	Crude	Multiple ^a	Crude	Multiple ^a
Model 1						
Total Breastfeeding^b < 6 months	1.03 (0.50; 2.14)	0.91 (0.42; 2.00)	2.33 (1.11; 4.88)	2.87 (1.28; 6.40)	1.43 (0.68; 3.02)	1.58 (0.71; 3.52)
Low birthweight	1.85 (0.75; 4.60)	2.42 (0.83; 7.07)	1.31 (0.49; 3.53)	0.56 (0.14; 2.24)	1.30 (0.48; 3.49)	0.96 (0.28; 3.31)
Macrosomia	1.39 (0.43; 4.48)	2.26 (0.63; 8.11)	2.85 (0.90; 8.98)	5.13 (1.36; 19.37)	2.81 (0.89; 8.89)	4.91 (1.32; 18.26)
Maternal education <11 years	0.60 (0.32; 1.12)	0.52 (0.25; 1.05)	1.00 (0.54; 1.86)	1.07 (0.51; 2.26)	0.85 (0.45; 1.58)	0.86 (0.41; 1.79)
Model 2						
Predominant breastfeeding^c < 3 months	1.27 (0.62; 2.59)	1.03 (0.47; 2.25)	1.89 (0.92; 3.88)	2.27 (1.02; 5.02)	2.00 (0.97; 4.15)	2.36 (1.06; 5.26)
Low birthweight	-	1.97 (0.76; 5.11)	-	1.01 (0.35; 2.88)	-	1.02 (0.36; 2.93)
Macrosomia	-	2.27 (0.63; 8.12)	-	5.27 (1.41; 19.71)	-	5.63 (1.49; 21.19)
Maternal education <11 years	-	0.57 (0.29; 1.15)	-	0.76 (0.37; 1.55)	-	0.69 (0.33; 1.41)
	Lipid biomarkers					
	Triglycerides		HDL-c		LDL-c	
	Crude	Multiple ^a	Crude	Multiple ^a	Crude	Multiple ^a
Model 1						
Total Breastfeeding^b < 6 months	0.84 (0.40; 1.75)	0.94 (0.43; 2.05)	1.03 (0.50; 2.10)	1.07 (0.50; 2.29)	1.36 (0.66; 2.77)	1.17 (0.55; 2.52)
Low birthweight	0.29 (0.08; 1.03)	0.25 (0.05; 1.15)	1.49 (0.60; 3.68)	1.44 (0.49; 4.22)	1.34 (0.54; 3.36)	1.98 (0.69; 5.70)
Macrosomia	1.58 (0.50; 4.95)	1.47 (0.42; 5.13)	0.54 (0.14; 2.04)	0.80 (0.20; 3.27)	0.58 (0.15; 2.21)	0.41 (0.08; 2.02)
Maternal education <11 years	0.93 (0.50; 1.70)	0.91 (0.46; 1.81)	0.51 (0.27; 0.95)	0.47 (0.23; 0.94)	1.17 (0.64; 2.13)	1.52 (0.77; 3.00)
Model 2						
Predominant breastfeeding^c < 3 months	0.74 (0.35; 1.57)	0.98 (0.44; 2.17)	1.21 (0.60; 2.43)	1.08 (0.50; 2.31)	1.30 (0.63; 2.65)	1.23 (0.57; 2.63)
Low birthweight	-	0.30 (0.08; 1.10)	-	1.50 (0.58; 3.87)	-	1.29 (0.50; 3.35)
Macrosomia	-	1.68 (0.48; 5.92)	-	0.81 (0.20; 3.28)	-	0.42 (0.08; 2.05)
Maternal education <11 years	-	0.79 (0.40; 1.56)	-	0.48 (0.25; 0.95)	-	1.39 (0.71; 2.70)

^a Multiple Model 1 (Total breastfeeding as main exposure) and 2 (Predominant breastfeeding as main exposure) were adjusted by birthweight (reference category ≥ 2.5 and < 4.0 kg) and maternal education (reference category ≥ 11 years).

^b Reference category total breastfeeding ≥ 6 months.

^c Reference category predominant breastfeeding ≥ 3 months.

Table 4. Odds ratio (OR 95% CI) of being classified into the third tertile of glucose and lipid metabolism biomarkers according to dietary patterns adherence in women from the NutriHS, São Paulo, Brazil.

Dietary Patterns (T1 = reference)		Glycemic biomarkers					
		Plasma glucose		Insulin		HOMA-IR	
Processed	Model 1 ^a	Model 2 ^b	Model 1 ^a	Model 2 ^b	Model 1 ^a	Model 2 ^b	
T2	3.01 (1.32; 6.87)	4.18 (1.49; 11.72)	0.79 (0.36; 1.75)	0.92 (0.32; 2.61)	1.41 (0.63; 3.16)	2.04 (0.70; 5.94)	
T3	2.21 (0.84; 5.81)	3.14 (0.90; 10.90)	1.07 (0.45; 2.58)	1.86 (0.55; 6.25)	1.44 (0.58; 3.57)	2.21 (0.60; 8.09)	
P-trend ^c	0.106	0.072	0.874	0.316	0.434	0.231	
Prudent							
T2	0.49 (0.21; 1.15)	0.32 (0.11; 0.91)	0.97 (0.42; 2.24)	2.58 (0.85; 7.84)	0.86 (0.37; 2.00)	1.67 (0.55; 5.04)	
T3	0.68 (0.26; 1.78)	0.53 (0.16; 1.72)	1.16 (0.46; 2.89)	2.91 (0.85; 9.94)	1.17 (0.46; 2.95)	3.19 (0.90; 11.32)	
P-trend ^c	0.435	0.293	0.753	0.088	0.745	0.073	
Brazilian							
T2	0.56 (0.24; 1.31)	0.45 (0.17; 1.20)	1.05 (0.47; 2.37)	2.30 (0.79; 6.74)	0.95 (0.42; 2.18)	1.84 (0.63; 5.41)	
T3	1.83 (0.68; 4.88)	2.20 (0.68; 7.13)	1.42 (0.56; 3.60)	6.70 (1.79; 25.12)	1.81 (0.70; 4.67)	7.78 (2.01; 30.13)	
P-trend ^c	0.229	0.188	0.459	0.005	0.221	0.003	

Lipid biomarkers								
LDL-c			HDL-c		LDL-c/HDL-c		Triglycerides	
Processed	Model 1 ^a	Model 2 ^b	Model 1 ^a	Model 2 ^b	Model 1 ^a	Model 2 ^b	Model 1 ^a	Model 2 ^b
T2	1.68 (0.71; 3.96)	2.11 (0.75; 5.93)	0.89 (0.41; 1.93)	0.66 (0.26; 1.69)	1.63 (0.72; 3.68)	3.12 (1.10; 8.82)	0.93 (0.42; 2.07)	0.89 (0.35; 2.27)
T3	4.93 (1.86; 13.06)	6.08 (1.80; 20.58)	1.42 (0.59; 3.43)	1.48 (0.50; 4.39)	1.59 (0.62; 4.05)	2.05 (0.59; 7.20)	1.39 (0.57; 3.42)	1.24 (0.41; 3.76)
P-trend ^c	0.001	0.004	0.439	0.478	0.333	0.261	0.470	0.700
Prudent								
T2	0.71 (0.30; 1.65)	0.18 (0.06; 0.58)	0.81 (0.36; 1.87)	1.00 (0.36; 2.74)	0.59 (0.26; 1.37)	0.21 (0.07; 0.64)	0.36 (0.15; 0.87)	0.35 (0.13; 0.97)
T3	0.33 (0.12; 0.89)	0.26 (0.08; 0.87)	1.71 (0.68; 4.30)	2.30 (0.74; 7.16)	0.37 (0.14; 0.97)	0.28 (0.08; 0.97)	1.04 (0.42; 2.55)	0.85 (0.29; 2.47)
P-trend ^c	0.029	0.029	0.253	0.151	0.044	0.046	0.933	0.768
Brazilian								
T2	0.32 (0.13; 0.76)	0.21 (0.07; 0.58)	0.84 (0.39; 1.80)	0.93 (0.38; 2.31)	0.58 (0.25; 1.34)	0.44 (0.16; 1.19)	1.16 (0.52; 2.60)	1.08 (0.43; 2.69)
T3	0.72 (0.28; 1.89)	0.56 (0.18; 1.77)	0.60 (0.23; 1.52)	0.74 (0.24; 2.27)	0.92 (0.36; 2.35)	0.58 (0.18; 1.89)	1.30 (0.50; 3.38)	1.19 (0.39; 3.62)
P-trend ^c	0.508	0.326	0.280	0.599	0.863	0.367	0.592	0.753

^a Model 1 was adjusted for energy intake (kcal/day).

^b Model 2 was adjusted for energy intake (kcal/day); physical activity (1st+2nd/ 3rd MET tertiles), age (< 19/ ≥19 and <35/ ≥35 years), smoking habit (no/ yes), alcohol intake (never/ < 1 x/week/ ≥ 1 x/week), total breastfeeding duration (<6 months/ ≥ 6 months) and birthweight (<2 kg/ ≥ 2.5 and < 4.0 kg/ ≥4kg).

^c Refers to *P* for linear trend across dietary pattern tertiles.

Supplementary Figure 1. Directed Acyclic Graph (DAG) for the association between breastfeeding and glucose and lipid metabolism biomarkers.

Notes: Minimal sufficient adjustment: maternal education, birthweight and gestational diabetes .

Legend= (>) exposure; (I) outcome.

BMI, Body mass index

Supplementary Figure 2. Directed Acyclic Graph (DAG) for the association between dietary patterns and glucose and lipid metabolism biomarkers.

Notes: Minimal sufficient adjustment: Age, physical activity, alcohol intake, smoking, total breastfeeding duration and birthweight.

Legend= (>) exposure; (I) outcome.

BMI, Body mass index

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5.4 MANUSCRITO 4: Breastfeeding may have a long-term effect on oral microbiota: results from the Fin-HIT cohort

Abstract

Background: Breastfeeding contributes to gastrointestinal microbiota colonization in early life, but its long-term impact is inconclusive. We aimed to evaluate whether the type of feeding during the first six months of life was associated with oral microbiota in adolescence. **Methods:** This is a cross-sectional sub-study using baseline information of 423 adolescents from the Finnish Health in Teens (Fin-HIT) cohort. Type of feeding was recalled by parents and dichotomized as (i) No formula; (ii) Formula (breastmilk + formula or only formula). Saliva microbiota was analysed using 16S rRNA (V3–V4) sequencing. Alpha diversity and beta diversity were compared between feeding type groups using ANCOVA and PERMANOVA, respectively. Differential bacteria abundance was tested using appropriate general linear models. **Results:** Mean age and body mass index were 11.7 years and 18.0 kg/m², respectively. The No formula group contained 41% of the participants. *Firmicutes* (51.0%), *Bacteroidetes* (19.1%), and *Proteobacteria* (16.3%) were the most abundant phyla among all participants. Alpha and beta diversity indices did not differ between the two feeding groups. Three Operational Taxonomic Units (OTUs) belonging to *Eubacteria* and *Veillonella* genera (phylum *Firmicutes*) were more abundant in the No formula than in the Formula group (log2fold changes/ p-values: -0.920/ <0.001, -0.328/ 0.001, -0.577/ 0.004). **Conclusion:** Differences exist in abundances of some OTUs in adolescence according to feeding type during the first six months of life, but our findings do not support diversity and overall oral microbiota composition in adolescents being affected by early feeding type.

Keywords: Microbiota; saliva; breastfeeding; adolescent

1. Background

The Developmental Origins of Health and Disease (DOHaD) theory has been a target of many studies to explain the global epidemic of non-communicable diseases. DOHaD theory proposes that exposures during critical development periods, such as the first 1000 days of life, could unleash metabolic programming that is able to modify structure and function of organs and systems, impacting health status later in life (24-26).

Recently, the role of microbiota in the context of DOHaD has been evaluated (99). Gut microbiota is known to be established during the first two years of life, reaching stability by the third year (97, 98). Perturbations of microbiota during early life have been associated with later inflammatory or immune-mediated diseases, such as allergy, asthma, and obesity (101-104), indicating that microbiota also has a critical period of development.

Evidence of possible protection of breastfeeding against obesity and related outcomes in adulthood supports the DOHaD theory (14, 27, 74). An explanation for the protective effect is its role during the gut microbiota establishment period (101, 238). Relative to formula-fed infants, breastfed infants seem to have a lower abundance of *Clostridium* and a predominance of *Bifidobacteria* and *Lactobacilli* in the gut (12, 13, 98, 102, 106-108).

Studies have suggested that also the infant oral microbiota is influenced by the type of feeding (109-111). *Proteobacteria* and *Actinobacteria* phyla were more abundant in the oral cheek of breastfed neonates, while formula-fed infants had a higher predominance of *Bacterioidetes* (110). In examining saliva culture data of 3-month-old infants, vital *Lactobacillus* species were found in breastfed but not formula-fed ones (109). A longer term effect, assessed at 4 and 12 months after birth, reinforced differences in oral microbiota composition between breast- and formula-fed infants (111).

Although differences exist between gut and oral microbiota compositions, some similarities have been described (112, 113, 117). Community types present in these sites seem to be associated, i.e. types of bacteria detected in one site are able to predict those found in the other (112, 113). It has been suggested that oral microbiota seeds gut microbiota in infancy. Observations that both mother's milk and infant's faeces are colonized by some identical bacteria support the hypothesis that human milk is an important source of bacteria, contributing to define gut microbiota composition (113).

Considering that oral microbiota is relatively stable over time in healthy individuals, that perturbations could favour non-oral diseases such as type 2 diabetes (116, 117), and that saliva is easily collected, the use of saliva samples represents a unique opportunity to investigate factors associated with microbial composition. As far as we know, no study has evaluated whether the association between breastfeeding and oral microbiota composition is maintained until adolescence, which is a critical period in the life course for the prevention of chronic diseases. We aimed to evaluate whether the type of feeding during the first six months of life is associated with oral microbiota diversity and composition in Finnish adolescents. Our hypothesis is that those who received formula at early life (combined or not with breastmilk) had a lower exposure to breastmilk (in quantitative terms) compared to those who were fed only with breastmilk, which could influence the microbiota development.

2. Methods

2.1 Design and study population

This is a cross-sectional sub-study using baseline data from the Finnish Health in Teens (Fin-HIT) cohort ⁽¹⁷⁵⁾. Briefly, Fin-HIT is a representative study of the most populated areas of Finland, for which approximately 11 400 adolescents and 9900 parents (one per adolescent, mostly mothers) were recruited between 2011 and 2014. The Fin-HIT study protocol was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa (169/13/03/00/10), and all participants provided informed consent.

Saliva samples from all adolescents were collected and a random subsample ($n = 972$) had the oral microbiota analysed, maintaining representativeness of the cohort population. Participants whose samples generated less than 2000 sequence reads ($n = 83$) were excluded. Based on register data from the Social Insurance Institution of Finland, those who had used antimicrobials in the last month ($n = 46$) or had used them for more than 40 times throughout life (taken as a proxy of chronic disease) ($n = 1$) or without this information were also excluded ($n = 6$). Thus, 836 adolescents had saliva microbiota data, 423 (aged 10-14 years) of whom also had information on feeding type during the first six months of life, comprising the final number of participants in this study (**Figure 1**).

Figure 1. Flow chart of Fin-HIT participants included in this study.

2.2 Variables

The type of feeding during the first six months of life was the main exposure of this analysis. At baseline, these data were retrospectively collected using a web questionnaire answered by parents. This variable was dichotomized as follows: (i) No formula and (ii) Formula (referring to those who received formula, combined or not with breastmilk).

Other variables of interest were adolescent's gender (male/female) and age (years); and parent's gender (male/female), age (years), language (Finnish, Swedish/others) and education level (high school or technical level/university degree). Adolescent's height (nearest 0.1 cm) and weight (nearest 0.01kg) were measured by trained fieldworkers using portable stadiometers (Seca model 217) and portable digital scales (CAS model PB). Body mass index (BMI) was calculated as weight (kg)/height² (m). Type of delivery (vaginal/ C-section) was obtained from the national health register managed by the National Institute for Health and Welfare [<https://www.thl.fi/en/web/thlfi-en/statistics/information-for-researchers>].

2.3 Saliva collection and oral microbiome analysis

Adolescents' unstimulated saliva samples were collected using the Oragene[®] DNA (OG-500) Self-Collection Kit (DNA Genotek Inc., Ottawa, ON, Canada), mixed with stabilizing reagent and stored at ambient temperature, according to the manufacturer's instructions. A protocol with an intensive lysis step and mechanical disruption of microbial cells was employed (176). Afterwards, total DNA was extracted; sample amplification and sequencing were prepared according to a simplified in-house 16S rRNA gene-based PCR amplification protocol. Amplification was performed using 16S primers targeting V3-V4 region and the Truseq (TS)-tailed 1-step amplification protocol. The Illumina HiSeq1500 instrument (Illumina Inc., San Diego, CA, USA) was used for PCR amplicons sequencing. MiSeq SOP in the mothur pipeline (version V.1.35.1) was used to process sequences (177). The SILVA 16S rRNA database (version V119) and taxonomy were used for alignment and classification of the high-quality sequence reads, which were clustered into operational taxonomic units (OTUs) at a cut-off value >98% sequence similarity. Detailed procedures of saliva collection and microbial analysis were previously described (178).

Alpha diversity (Shannon and Inverse Simpson Indices) was calculated per sample and beta diversity between the samples using Bray Curtis dissimilarity indices. Sequencing depths were categorized as (i) low $\leq 10\,000$; (ii) medium $> 10\,000$ and $\leq 100\,000$; (iii) high $> 100\,000$ sequences.

2.4 Statistical analysis

Kolmogorov-Smirnov test was used to verify normal distribution of variables, which were described with means (standard deviation) or frequency (%). Student t-test and Chi-squared test were used to compare continuous and categorical variables according to feeding type groups. ANCOVA was used to compare alpha diversity between groups. Pearson correlation was employed to test correlations between alpha diversity indices and continuous variables, while Student t-test and ANOVA were used to compare these indices between categories of the other variables. These analyses were performed using Stata Statistical Software (release 12, 2011, StataCorp LP, College Station, TX, USA), and statistical significance was set at the level of 5%. Permutational analysis of variance (PERMANOVA) was used to test difference in beta diversity between feeding type groups. Principal coordinate analysis based on Bray Curtis distances was used to illustrate beta diversity between groups.

General linear models (GLMs) with negative binomial distribution were employed for comparisons of bacteria abundance between feeding type groups as OTU and considering phylum and genus levels. All OTUs with low counts (< 20) were excluded. P-values were corrected by false discovery rate. PERMANOVA and GLM were carried out using DESeq2 (179), Vegan, and phyloseq in R (version 3.4.3). Gender, age, BMI, type of delivery, parent's education, and sequence reads were considered confounders in all adjusted models.

Multiple imputation for missing values of BMI ($n = 10$), type of delivery ($n = 22$), and parent's education ($n = 15$) was performed with “mi impute chained” procedure in Stata 12.0. Imputed values were considered in the multivariate analysis.

3. Results

Of the 423 adolescents at baseline of Fin-HIT, 52% were female; their mean age and BMI were 11.7 years and 18.0 kg/m² (73% were normal weight), respectively. Most participants were born by vaginal delivery (81.1%) and received infant formula (solely or combined with breastmilk) (58.6%) during their first six months of life. The majority of participating parents were female (87.4%), Finnish speakers (90.3%), and had a university degree (59.6%) (**Table 1**).

Mean (standard deviation) values of alpha diversity indices were 2.9 (0.3) and 10.1 (3.1) for Shannon and Inverse Simpson, respectively, and these did not differ between the No formula

and Formula groups (**Table 1**). Beta diversity was also similar between groups (P-value = 0.881) (**Figure 2**). In the No formula group, vaginal delivery was more frequent than in the Formula group (88.3% vs. 76.1%, P-value = 0.002) (**Table 1**). Alpha diversity was not associated with adolescents' or parents' characteristics (**Table 2**).

In 423 samples of adolescents' saliva, 22 924 455 raw sequences reads were obtained and 1,049 OTUs were evaluated. In total, 11 phyla, 16 classes, 24 orders, 44 families, and 76 genera were identified. As depicted in **Figure 3**, a great predominance of phylum *Firmicutes* was found, followed by *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* (panel A), while the predominant genera were *Veillonella*, *Prevotella*, and *Streptococcus* (panel B).

Comparing groups of feeding type according to abundance of bacteria, no difference was observed at phylum level. Three OTUs (#019, #232, and #158) were more abundant in No formula than in Formula group (log2fold changes/ adjusted p-values: -0.920/ <0.001, -0.328/ 0.001, and -0.577/ 0.004, respectively) (**Figure 4; and see table at Additional file 1 for detailed information**). These three OTUs belonged to phylum Firmicutes, class *Clostridia*, and order *Clostridiales*; OTU #019 belonged to genus *Eubacteria* and #232 and #158 to genus *Veillonella*. Considering the entire genera, genus *Eubacteria* (log2fold change -0.742, adjusted p-value <0.0003), but not genus *Veillonella*, was more abundant in the No formula group.

Table 1. Main data of participants at Fin-HIT baseline and comparison according to type of feeding.

	All n = 423	No formula n = 175	Formula n = 248	P-value
Continuous variables	Mean (SD)			
Adolescent's age (y)	11.7 (0.3)	11.7 (0.3)	11.7 (0.3)	0.074
Parent's age (y)	44.0 (5.7)	44.3 (5.4)	43.8 (5.8)	0.389
Adolescent's body mass index (kg/m ²) ^a	18.0 (2.9)	17.7 (2.8)	18.2 (2.9)	0.077
Alpha diversity ^b				
Shannon Index	2.9 (0.3)	2.9 (0.3)	2.9 (0.3)	0.877
Inverse Simpson	10.1 (3.1)	10.1 (3.2)	10.1 (2.3)	0.949
Categorical variables	Frequency (%)			
Adolescent's gender				
Male	204 (48.2)	82 (46.9)	122 (49.2)	0.636
Female	219 (51.8)	93 (53.1)	126 (50.8)	
Type of delivery ^c				
Vaginal	325 (81.1)	144 (88.3)	181 (76.1)	0.002
C-section	76 (18.9)	19 (11.7)	57 (23.9)	
Parent's language				
Finnish	382 (90.3)	156 (89.1)	226 (91.1)	0.638
Swedish	36 (8.5)	16 (9.2)	20 (8.1)	
Other	5 (1.2)	3 (1.7)	2 (0.8)	
Parent's education ^d				
High school/ technical level	165 (40.4)	72 (43.4)	93 (38.4)	0.318
University degree	243 (59.6)	94 (56.6)	149 (61.6)	

^a n = 413 due to missing data^b Adjusted for adolescent's gender, age, and body mass index, type of delivery, parent's education, and sequence reads^c n = 401 due to missing data^d n = 408 due to missing data

Table 2. Correlations and comparison of mean values of diversity indices by participants' characteristics.

Continuous variables	Shannon		Inverse Simpson	
	r	P-value	r	P-value
Adolescent's age	0.015	0.763	-0.003	0.949
Adolescent's body mass index	-0.047	0.344	-0.037	0.456
Categorical variables	Mean (SD)	P-value	Mean (SD)	P-value
Adolescent's gender				
Male	2.9 (0.3)	0.338	10.2 (3.0)	0.412
Female	2.9 (0.3)		9.9 (3.2)	
Type of delivery				
Vaginal	2.9 (0.3)	0.579	10.1 (3.1)	0.579
C-section	2.9 (0.3)		9.9 (3.0)	
Parent's language				
Finnish	2.9 (0.3)	0.776	10.0 (3.1)	0.802
Swedish	2.9 (0.2)		10.2 (3.0)	
Other	3.0 (0.4)		10.9 (4.1)	
Parent's education				
High school/ technical level	2.9 (0.3)	0.544	9.8 (3.0)	0.190
University degree	2.9 (0.3)		10.2 (3.2)	
Sequence reads				
Low	2.8 (0.2)	0.274	9.3 (2.1)	0.360
Medium	2.9 (0.3)		10.0 (2.9)	
High	2.9 (0.4)		10.3 (3.4)

Figure 2. Principal coordinate analysis (beta-diversity) for the saliva microbiota according to type of feeding (P-value=0.881).

Figure 3. Relative abundances of phyla (A) and genera (B) in saliva microbiota.

Figure 4. Means and standard errors (SEs) of abundance for significantly different OTUs between feeding type groups.

4. Discussion

We investigated the long-term association of early life feeding type with oral microbiota composition in adolescence. A few differences emerged in abundances of some specific genera according to early feeding type, but our data do not suggest a significant impact on the diversity of microbiota. Abundances of commensal OTUs belonging to genera *Veillonella* and *Eubacteria* were higher in adolescents who had not been fed with formula than in others who had received infant formula, combined or not with breastmilk.

To the best of our knowledge this is the first study to explore associations of early feeding type with oral microbiota diversity and composition at this stage of life. Such relationships have been investigated mainly during the first year of life (109-111). Despite higher abundance of certain OTUs, the overall composition (beta diversity) or the alpha diversity of oral microbiota did not differ between adolescents with different early life feeding type. In general, high phylogenetic diversity in gut microbiota has been associated with several health outcomes (239), but long-term health implications of this picture are unclear yet. We speculate that our negative results regarding overall microbiota diversity and composition could result from a stronger impact of other exposures during childhood such as weaning age, moment of introduction of solid foods, Westernized eating pattern and use of antimicrobials (108, 239-241).

As far as the gut is concerned, it was reported that microbiota composition in breastfed babies tended to be more stable and has less diverse bacterial community compared with formula-fed ones (242, 243). Since 3 years of age, their composition converge to resemble that of an adult (97). A multicentre cohort of 903 infants (including Finns) followed from 3 to 46 months of age showed that breastfeeding explained the greatest part of microbiome establishment during the first year of life. Also, breastfed infants had lower diversity than those weaned at an earlier stage. As age increased and breastmilk exposure decreased, diversity became more similar between groups, supporting the hypothesis that weaning is an important event affecting maturation of the gut microbiome (98). Some studies have evaluated the long-term effect of breastfeeding on gut microbiota (98, 108). A prospective birth cohort study of 281 Dutch children reported that breastfeeding duration was associated with gut microbiota composition in children aged 6-9 years (108).

Little is known how these changes occur in saliva, driven by diet, physical activity and antibiotics administration, which could alter the host health status. Although gut and oral microbiota had distinct compositions, community types of these sites seem to be associated (113, 117). A study conducted with 20-day neonates found a positive correlation of subdominant family *Lactobacillaceae* in saliva and faeces samples of the same infant, and showed that *Staphylococcus spp.* and *Streptococcus spp.* were shared between the two ecosystems and also with human milk (113). Comparable results have been reported in studies assessing gut and oral microbiota; i.e. early feeding seems to impact on microbiota profile (12, 98, 109, 110).

Comparisons with our findings are limited because we were the first to evaluate the long-term effect of early feeding on oral microbiota. Furthermore, exposures occurring during infancy – particularly related to antibiotics use – may affect microbiota composition mainly at the gut level (244, 245). The protective role of breastfeeding against increased body adiposity and antibiotic use in childhood, especially by promoting beneficial microbiota, was eliminated by the use of antibiotics in early life (246). Additionally, differences in microbiota composition from one population to another are recognized (247-249).

According to unpublished data of the Fin-HIT, most of the participants used antimicrobial drugs during their first years of life, which could contribute to a lasting impact on oral microbiota diversity and composition at adolescence. Regarding diet, it is suggested that especially the high and frequent intake of sucrose and other fermentable carbohydrates result in the accumulation of acidogenic and aciduric microorganisms, driving a pathogenic biofilm community formation. Particularly, high frequency of sugar intake could disrupt the homeostasis between commensals and pathogens resulting in dysbiosis, which could increase chances of caries, inflammation and periodontitis in more susceptible individuals (43). Thus, we suppose that sugar intake during childhood could be an important factor affecting diversity and composition of oral microbiota. A previous study, conducted in public child health service in Finland, showed that sugar introduction occurs early in life. Almost half of 6-month old children was receiving sugar-sweetened beverages and more than 90% of those older than 16 months were receiving sweets (44). Further, another Finnish study showed that 95% of 1-year-old babies consume mass-produced baby foods, of which some are sweetened with juice-concentrates (70-80% of sugar) (45).

Our finding of a major predominance of phylum *Firmicutes* in the human oral cavity, followed by *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* is consistent with previous studies in 1- to 2-month-old (110) and 12-month-old children (111). A study conducted in 38 healthy, full-term, vaginally delivered neonates from Australia found similar alpha diversity and proportion of *Firmicutes* between breastfed and formula-fed groups (96.3 vs. 95.3%) (110), in accord with our findings in an older age group. A higher relative abundance of *Bacteroidetes* and a lower abundance of *Actinobacteria* and *Proteobacteria* were also observed in formula-fed infants than in breastfed infant.

Holgerson *et al.* (109) showed that 3-month-old exclusively breastfed infants clustered separately from formula-fed infants, according to their oral microbiota. In total, 14 probes differed significantly between these two feeding types. Among these probes, cultivable *Lactobacilli* and *Eubacterium yurii* were more abundant in breastfed than exclusively formula-fed infants, in whom the mean number of species per child was higher. *Lactobacillus*, but not *Eubacterium*, has been consistently reported in breastmilk, and thus, the presence of the former in infants' oral microflora was expected. We do not know whether the higher abundance of *Eubacteria* observed in our breastfed adolescents could be detected earlier, during their infancy. Another study reported higher species richness in 4-month-old formula-fed infants and marked differences in saliva microbiota composition between feeding type subgroups (111). However, at 12 months of age these differences were no longer significant.

We found that *Eubacteria* and *Veillonella* – both genera of the normal oral microbiota – were more abundant in adolescents of No formula group compared with those who received infant formula in early life. *Eubacteria* is an atypical genus in samples of breastmilk, but *Veillonella*, *Gemella*, *Rothia*, *Lactobacillus*, *Streptococcus*, and *Staphylococcus* have been previously described as the most abundant genera in breastmilk (250). The association of No formula with higher *Veillonella* abundance seems favourable, considering that this genus has a beneficial effect on dental plaques by metabolizing lactate to weaker acids such as acetate and propionate (251). Since lactate is a cariogenic factor produced by *Streptococcus mutans*, increased abundance of *Veillonella* could be interpreted as a compensatory effect to protect against dental injuries. Higher abundance of the species *Veillonella parvula* was previously described in subgingival biofilm samples from healthy subjects compared with those with chronic periodontitis,

and its presence was inversely correlated with inflammatory biomarkers from gingival crevicular fluid (114). Periodontal disease is considered a risk factor for cardiovascular disease, and the proposed underlying mechanism was based on increased circulating pro-inflammatory cytokines (115).

Strict anaerobic bacteria from the genus *Eubacteria* are chemoheterotrophs, i.e. are unable to synthesize their own organic molecules, requiring mixed organic acids from the host. Thus, *Eubacteria* energy is obtained from carbohydrates or protein metabolism, resulting in end-products such as butyrate and acetate (252). These short-chain fatty acids have been associated with beneficial effects, such as cardiovascular and colonic disease prevention (253). The presence of *Eubacteria* in our sample of healthy adolescents was expected since this a member of normal saliva microflora and plaques. Our methods were unable to identify species of *Eubacteria* that would be desirable since periodontal pathogenic *Eubacteria* species were previously described (114, 254). A study of patients undergoing haemodialysis identified higher taxa of *Eubacterium nucleatum* in sulcular fluid from the periodontal pocket of non-diabetics than diabetics. Despite being a pathogen, the prevalence of periodontitis was similar between the groups (254).

Although evidence has suggested beneficial roles of *Veillonella* and *Eubacteria*, it was not possible to extrapolate these in our sample since the study design does not allow comparing health outcomes according to abundances of specific bacteria. Prospective studies are needed to investigate whether individuals with distinct abundances of specific bacteria have different chances of developing cardiovascular disease or other outcomes.

A strength of this study was the large sample size in which next-generation sequencing technology was used to analyse microbiota. This is a sub-study of a well-designed prospective cohort, including reliable information obtained from standardized questionnaires and national health registers (175). Statistical analyses were duly adjusted, minimizing confounding bias. The main limitations of our study comprised a design impeding establishment of causality and retrospective data collection (feeding type answered by parents), which could generate recall bias. Considering that we evaluated associations of an event occurred long time ago (type of feeding) and another occurring in adolescence, there is a possibility of recall error, especially among parents who had multiple children. However, it has been reported that information about breastfeeding recalled by mothers after 20 years still shows reasonable accuracy (213). We could not consider

“exclusive breastfeeding” since no information regarding water, tea, and food intake in early life was available, or “exclusive formula-fed” (not combined with breastmilk) since its prevalence in our study was relatively low (2.4%). Previously, these categories of “exclusive” feeding type were clearly separated by microbiota composition, but partially breastfed infants were interspersed between these groups (109). Despite this limitation that could have attenuated differences, we still detected some differences according to feeding type. We speculate that differences identified are explained by a higher exposure to breastmilk at early life by participants of the No formula group compared to those of the Formula group.

Finally, the lack of oral health status could be regarded as a limitation. It is known that diet, especially in combination with poor oral health, salivary dysfunction, scarce fluoride exposure and poor oral hygiene, can modulate oral microbiota resulting in dysbiosis (255). Since Finland public oral health care services are freely available for individuals younger than 18 years, most children and adolescents have relatively good oral health, although differences exist depending on maternal educational level and region. Not having information on oral health could contribute to confounding bias and thus is a limitation in this study.

Conclusion

The type of feeding in early life is not associated with overall changes in composition or diversity but with some alterations in adolescents’ oral microbiota (higher abundance of *Veillonella* and *Eubacteria*). The clinical relevance of these findings and their health implications need to be explored in future studies. Other exposures during childhood (e.g. antimicrobial use, sugar consumption and oral health) could play a stronger role in adolescents’ oral microbiota diversity and composition than the early feeding. Further prospective studies, considering the type of feeding in early life, age of weaning, and other factors during childhood, are needed to confirm our findings.

Additional file 1. Differentially abundant bacteria at OTU-level by type of feeding (No formula vs. Formula) during the first six months of life.

OTU (nearest taxa)	Base Mean	log2FoldChange ^a	P adjusted
OTU 019 (Eubacterium)	517.592	-0.920	4.49E-05
OTU 158 (Veillonella)	2.820	-0.328	0.004
OTU 232 (Veillonella)	1.103	-0.577	0.001

^a Negative value means lower abundance among “Formula” group compared to “No formula”.

List of abbreviations

DOHaD: Developmental Origins of Health and Disease;

Fin-HIT: Finnish Health in Teens cohort;

BMI: Body Mass Index;

OTU: Operational Taxonomic Unit;

GLM: General Linear Model

Declarations

Ethics approval and consent to participate: The Fin-HIT study was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa (169/13/03/00/10), and all participants provided informed consent.

Consent for publication: Not applicable

Availability of data and materials: The data that support the findings of this study are available from Heli T. Viljakainen but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Heli T. Viljakainen.

Competing interests: The authors declare that they have no competing interests. Where authors are identified as personnel of the International Agency for Research on Cancer / World Health

Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer / World Health Organization.

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Authors' contributions: IE designed the study, carried out the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript. HTV and SRG designed the study and critically reviewed and revised the manuscript. SCR handled the bioinformatics data and reviewed and revised the manuscript. EW conceptualized and designed the Fin-HIT cohort, coordinated data collection, designed the study, and reviewed and revised the manuscript. RAOF designed the study, carried out the statistical analyses, and critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

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6 CONSIDERAÇÕES FINAIS

A presente tese apresenta resultados inéditos ao explorar a relação de eventos precoces da vida, como o estado nutricional no período pré-concepcional e o aleitamento materno (ou uso de fórmulas infantis), com desfechos cardiometabólicos no início da vida adulta. Estudar parâmetros do metabolismo glicídico, lipídico, da composição corporal, e até mesmo da microbiota, nesta fase da vida, representa oportunidade única para investigar sua utilidade antes do desenvolvimento de distúrbios metabólicos e eventualmente para propor marcadores de risco precoce.

A partir do NutriHS, estudo conduzido com nutricionistas ou estudantes de cursos de graduação em nutrição do sexo feminino e aparentemente saudáveis, foram obtidos resultados com relevante contribuição científica neste campo. Vale levantar que a homogeneidade da amostra, por um lado pode ser compreendida como ponto forte de nosso estudo por aumentar as chances de respostas fidedignas. Por outro lado, a generalização dos resultados para amostras com diferentes características não é indicada, devendo ser considerada limitação do estudo.

Verificou-se que adiposidade corporal excessiva no período pré-gestacional, evidenciada pelo IMC materno, se associou com maior massa adiposa total e visceral da prole na vida adulta. No entanto, o estado nutricional pré-gestacional das mães das participantes do NutriHS não influenciou de forma independente sua massa óssea e muscular. Ainda que se pesem fatores genéticos contribuindo para a associação com adiposidade da prole, o conhecimento atual da epigenética permite supor a participação do estilo de vida materno antes da concepção para o desfecho (massa adiposa, total e visceral), estilo esse que pode ter se mantido durante a gestação e mesmo ter influenciado o desenvolvimento destas participantes na infância.

Quatro padrões alimentares foram identificados na população estudada – Processado, Prudente, Brasileiro e Lacto-vegetariano. O excesso de peso no período pré-gestacional também parece estar relacionado com os hábitos alimentares da prole durante a vida adulta, considerando que se associou com a maior adesão ao padrão Processado, e menor adesão ao padrão Prudente. Apesar deste estudo não permitir inferir causalidade nesta associação, este achado poderia indicar um mecanismo explicativo da associação entre excesso de peso materno e a adiposidade da prole, dado que a literatura sugeriu ser o IMC materno um *proxy* da transmissão intergeracional de estilo de vida.

Tanto o aleitamento materno quanto hábitos alimentares atuais foram associados ao perfil cardiometabólico da prole no início da vida adulta, ainda com parâmetros metabólicos, em média,

dentro das faixas de normalidade. Apesar de não apresentar associação com o perfil lipídico, a maior duração do aleitamento materno esteve inversamente associada à concentração de insulina e ao índice HOMA-IR parecendo ser um fator protetor contra o desenvolvimento de resistência à insulina. Por outro lado, os hábitos alimentares atuais associaram-se ao perfil lipídico, sendo a maior adesão ao padrão Prudente e menor adesão ao padrão Processado associadas a um perfil lipídico desfavorável, ou seja, mais propenso à aterogênese.

Interessantemente, a maior duração do aleitamento materno se associou com certos hábitos alimentares das participantes do NutriHS no longo prazo. Apenas 35% de nossa amostra foi amamentada por 12 meses ou mais e, comparadas a este grupo, participantes amamentadas por menos de seis meses apresentaram menor adesão ao padrão Prudente. Sugerimos que a prática mais duradoura de amamentar no peito possa estar relacionada ao fato da prole ter cultivado hábitos mais saudáveis no curso da vida, conferindo, consequentemente, proteção contra alterações cardiometabólicas.

A amostra de jovens avaliada na Finlândia apresenta características sociodemográficas bastante distintas da população do Brasil. Independente disso, análises rigorosas testaram a associação a alimentação precoce de adolescentes com a microbiota oral, baseadas em dados metagenômicos de alta fidedignidade. O tipo de alimentação nos primeiros seis meses de vida não parece ter influenciado a composição e diversidade da sua microbiota oral. No entanto, diferenças pontuais (maior abundância de bactérias dos gêneros *Veillonella* e *Eubacteria*) foram identificadas na microbiota daqueles que não receberam fórmula infantil nos primeiros seis meses de vida, em comparação com os alimentados por fórmula (combinada ou não com o aleitamento materno). Sugere-se que tais diferenças possam em parte ser explicadas pela maior exposição ao leite materno no grupo que não recebeu fórmula. Especula-se que outras exposições ocorridas nesta janela de oportunidade para o desenvolvimento da microbiota, como por exemplo, uso de antibióticos, poderiam contribuir de forma mais significativa que o tipo de alimentação para o estabelecimento da microbiota.

Em suma, nossos achados reforçam a teoria DOHaD, apoiando a relevância da relação entre eventos precoces da vida e o desenvolvimento de alterações cardiometabólicas na vida adulta. Vale destacar, que as associações aqui descritas foram verificadas em indivíduos ainda aparentemente saudáveis, as quais poderão ser acentuadas em fases posteriores de suas vidas. Considerando que a coleta de dados referentes aos eventos precoces da vida foi retrospectiva, viés de memória deve

ser considerado, sendo necessários estudos prospectivos para comprovar resultados obtidos e possibilitar inferências causais. Uma vez confirmados, cresce a relevância do cuidado nutricional para mulheres em idade reprodutiva (especialmente para aquelas que planejam engravidar) como estratégia de combate ao ciclo vicioso de obesidade para prole.

Hábitos alimentares são potencialmente modificáveis em qualquer fase da vida e exercem papel fundamental para definir o estado nutricional e riscos de doenças. Esta pesquisa corrobora com o importante papel do aleitamento materno para saúde no decorrer da vida, podendo influenciar na adesão a padrões alimentares saudáveis que refletirão em adequado perfil cardiometabólico. Espera-se que nossos resultados possam estimular mais estratégias de prevenção de doenças cardiometabólicas com vistas aos eventos precoces da vida.

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ANEXOS

ANEXO 1. TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO DO NUTRIHS

Termo de Consentimento Livre e Esclarecimento

Prezado aluno,

Este termo de consentimento livre e esclarecido (TCLE) tem como finalidade esclarecê-lo a respeito dos objetivos, riscos e benefícios do presente estudo. Além disso, destaca que sua participação é livre e que sua privacidade será garantida em qualquer circunstância.

Ao clicar no botão "Consinto em participar" certifico que, após convenientemente esclarecido pelo pesquisador e ter entendido o que me foi explicado, aceito participar e concordo com os termos descritos no TCLE.

Título: Estudo de Saúde de Nutricionistas - Fase 1 (ESNutri 1) da FSP-USP

Este Termo descreve a finalidade, os procedimentos, benefícios, riscos, desconfortos e advertências deste estudo. É importante para sua decisão sobre a participação no estudo que leia e compreenda as explicações dos procedimentos propostos abaixo.

Objetivo: Avaliar a associação de variáveis de composição corporal com biomarcadores de risco cardiovascular; analisar a composição da microbiota intestinal e sua associação com hábitos alimentares e com biomarcadores de risco cardiovascular.

Benefício: Para indivíduos incluídos no estudo será a investigação de fatores de risco e/ou proteção nutricionais para doenças crônicas não-transmissíveis (DCNT), bem como fatores consagrados e os pouco explorados na literatura.

Sua participação inclui:

- Responder questionário sobre saúde, ingestão alimentar, crenças e atitudes sobre obesidade via internet;
- Realizar avaliação antropométrica, de pressão arterial e da composição corporal pelo densitômetro de dupla emissão com fonte de raio-X (DXA). Este exame é um método inócuo e preciso e sua realização leva 15 minutos.
- Coleta de 30 ml de sangue com material descartável e pessoal treinado, após 12 horas de jejum;
- Obtenção de amostra de fezes.

A coleta de sangue e o recebimento da amostra de fezes serão realizados no Centro de Saúde da FSP, em dia e hora pré-agendados.

Risco: Este estudo é considerado de risco mínimo. A antropometria, bem como o DXA, não causam qualquer desconforto e não requerem preparo prévio. A coleta de sangue pode raramente gerar um pequeno hematoma (manchas roxas) no local de punção, que, em geral, desaparecem após 3 a 5 dias.

Não haverá riscos para a integridade física, mental ou moral da sua pessoa. Todas as informações coletadas serão de caráter confidencial e utilizadas somente para fins científicos descritos no protocolo desta pesquisa, sem qualquer identificação pessoal.

O consentimento está sendo pedido exclusivamente para a participação neste estudo. É garantida e respeitada a privacidade na divulgação dos resultados da pesquisa, e não haverá sua identificação.

Liberdade: É garantido o direito de desistir a qualquer momento da participação nesta pesquisa, sem qualquer prejuízo.

Informações para Contato em Caso de Intercorrências

Estaremos à disposição para informá-lo(a) sobre os procedimentos, riscos e benefícios decorrentes da pesquisa, ou qualquer outra dúvida sobre o estudo.

Caso haja quaisquer dúvidas ou perguntas relativas ao estudo, você poderá entrar em contato em qualquer momento com a Dra. Isis Tande da Silva e Dra. Sandra Roberta Gouvea Ferreira.

Telefones: 3061- 7705/7701 ou pelo e-mail: isistande@usp.br/nutrihstudy@gmail.com

Ou com o Comitê de Ética em Pesquisa - COEP/FSP
Telefones: 3061-7779 ou 3061-7742 e-mail: coep@fsp.usp.br
Endereço: Av. Dr. Arnaldo, no 715, Cerqueira César, São Paulo-SP, CEP:01246-904

☐ Declaro que li e aceito todos os itens do termo de consentimento acima.

Av. Dr. Arnaldo, 715
Cerqueira César, São Paulo - SP

Desenvolvido por: LUPHON

Na rede:

Background picture by Darren Hester

ANEXO 2. TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO DO FINHIT

25.09.2014

Fin-HIT

Fin-HIT suostumuslomake PALAUTETAAN KOULUUN

Huoltajalle

Täyttäkää seuraavat tiedot itsestänne:

Kytettäväksi pikaviestin koululle.

www.fin-hit.fi

LAPSEN tiedot

Sukunimi: _____
Etunimet: _____
Henkilötunnus: _____
Osoite (jos eräs kuin huoltajan): _____

Koulun nimi: _____
Luokka: _____

www.fin-hit.fi

HUOLTAJAN tiedot

Sukunimi: _____
Etunimet: _____
Henkilötunnus: _____
Osoite: _____

Sähköpostiosoite: _____
Puhelinnumero: _____

Katsojaan luvalla kesken päättäneen kytettömällä
osoitteella www.fin-hit.fi

Kytketty tunnus: UP28369R168B
Salasana: AGJCZUAUTUE

MILITECHNICALS OY

FOLKHÄLSAN OY

folkhälsan

[illegible]

ANEXO 3. COMPOSIÇÃO DOS GRUPOS ALIMENTARES CONSIDERADOS PARA IDENTIFICAÇÃO DOS PADRÕES ALIMENTARES

Quadro 1. Agrupamentos dos itens do questionário de frequência alimentar.

Grupos de alimentos	ALIMENTOS DO QFA
Salgados assados	Esfiha/bauruzinho/torta
Massas	Macarrão com molho (com e/ou sem carne)/ nhoque; lasanha/canelone/panqueca
Peixe e frango (fritos)	Peixe e frango fritos
Carne processada	Embutidos (presunto, mortadela, salame); feijoada/ feijão tropeiro; nuggets/almôndega; carne seca/carne de sol; linguiça; salsicha; bacon
Carne suína	Carne de porco (lombo, bisteca)
Fast food	Batata/mandioca/ polenta (fritas); salgados fritos (pastel, coxinha, rissoles, bolinho); pizza; hot-dog/hambúrguer
Snacks	Salgadinhos; pipoca
Temperos industrializados	Shoyo; catchup/mostarda
Bebidas industrializadas	Suco industrializado; refrigerante; achocolatado; bebida a base de soja
Bebidas alcoólicas	Cerveja; vinho; caipirinha/destilados
Doces/ sobremesas	Biscoito sem recheio (doce, salgado); biscoito recheado/ waffer/ amanteigado; bolo simples; bolo recheado; cereal matinal; bala/ pirulito; chantilly/ leite de coco/ leite condensado; chocolate/bombom; doce de frutas; gelatina; doce de confeitaria (torta, pudim, mousse); sorvete
Tubérculos e sopas	Batata/mandioca/inhame (cozida, assada ou purê); farofa/farinha de mandioca ou milho; salada de maionese com legumes; sopa de legumes/canja; sopa creme
Verduras e legumes	Alface; espinafre/ escarola; agrião/ rúcula; couve; repolho; couve flor/brócolis; tomate; cenoura; berinjela; beterraba; chuchu; abóbora; pepino; abobrinha; cebola; molho vinagrete
Frutas	Laranja/mexerica; banana; maçã/ pêra; mamão; melão/ melancia; abacaxi, manga; abacate; goiaba; caqui; uva
Suco natural	Suco natural
Aveia	Aveia
Chá	Chá
Pães integrais	Pães integrais
Iogurte e vitamina de frutas	Iogurte integral (natural/ com frutas); vitamina de frutas
Óleo/azeite	Óleo/azeite para tempero de salada
Peixe e frango (assado)	Peixe e frango assados/ cozidos/ ensopados
Ovos	Ovo cozido; ovo frito/omelete
Carne bovina	Carne assada/cozida/ensopada; carne frita; carne com legumes
Arroz integral	Arroz integral
Lentilha	Lentilha/ervilha seca/grão de bico
Sal	Sal para tempero de salada
Oleaginosas	Amendoim/ castanha do Pará/ nozes
Pão francês/forma	Pão francês/forma
Café	Café
Leite integral	Leite integral/ semidesnatado
Feijão	Feijão
Arroz branco	Arroz branco
Gorduras	Manteiga/margarina; maionese/molho para salada/patê
Queijos	Amarelos (muçarela, prato, parmesão, provolone); brancos (minas, ricota)
Leite desnatado	Leite desnatado
Miúdos (vísceras)	Vísceras de boi ou frango (coração, fígado)

ANEXO 4. PUBLICAÇÃO MANUSCRITO 2

*Journal of Developmental
Origins of Health and Disease*

www.cambridge.org/doh

Original Article

Cite this article: Eshriqui I, Folchetti LD, Valente AMM, de Almeida-Pititto B, and Ferreira SRG. Breastfeeding duration is associated with offspring's adherence to prudent dietary pattern in adulthood: results from the Nutritionist's Health Study. *Journal of Developmental Origins of Health and Disease* <https://doi.org/10.1017/S204017441900031X>

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Address for correspondence:

Sandra Roberta G. Ferreira, Department of Epidemiology, School of Public Health, University of São Paulo, São Paulo, Brazil. Email: sandrafv@usp.br

Breastfeeding duration is associated with offspring's adherence to prudent dietary pattern in adulthood: results from the Nutritionist's Health Study

Ilana Eshriqui¹, Luciana Dias Folchetti², Angélica Marques Martins Valente³, Bianca de Almeida-Pititto⁴ and Sandra Roberta G. Ferreira⁵

¹Graduation Program in Public Health Nutrition, School of Public Health, University of São Paulo, São Paulo, Brazil;

²School of Public Health, University of São Paulo, São Paulo, Brazil; ³Graduation Program in Epidemiology, School of Public Health, University of São Paulo, São Paulo, Brazil; ⁴Department of Preventive Medicine, Federal University of São Paulo, São Paulo, Brazil and ⁵Department of Epidemiology, School of Public Health, University of São Paulo, São Paulo, Brazil

Abstract

Little is known about the long-term effect of breastfeeding on dietary habits. We examined the association between breastfeeding duration and adherence to current dietary patterns of young women. This was a cross-sectional analysis of 587 healthy women aged ≤ 45 years, undergraduates or nutrition graduates. Maternal characteristics and breastfeeding duration [<6 ; $6-12$; ≥ 12 months (reference)] were recalled. Diet was assessed using a food frequency questionnaire and patterns were identified using factor analysis by principal component. Adherence to patterns was categorized in tertiles; the first (T1 = reference) was compared to T2 + T3 (moderate-to-high adherence). Logistic regression was performed considering the minimal sufficient adjustment recommended by the directed acyclic graph. Median age was 22 (interquartile range (IQR) 20; 27) years and body mass index (BMI) 22.2 (IQR 20.4; 25.0) kg/m². The four dietary patterns identified (*Processed*, *Prudent*, *Brazilian* and *Lacto-vegetarian*) explained 27% of diet variance. Women breastfed for <6 months showed lower chance of moderate-to-high adherence to the *Prudent* pattern (odds ratio (OR) = 0.53, $p = 0.04$). Breastfeeding was not associated with the other patterns. Maternal pre-pregnancy BMI was directly associated with moderate-to-high adherence to the *Processed* pattern (OR = 2.01, $p = 0.03$) and inversely to the *Prudent* pattern (OR = 0.52, $p = 0.02$). Higher adherence to the *Brazilian* pattern was associated with proxies of low socioeconomic status and the *Lacto-vegetarian* pattern with the opposite. Confirmation in prospective studies of the association found in this study between breastfeeding with the *Prudent* pattern in adult offspring could suggest that early feeding practices influence long-term dietary habits, which could then affect the risk of nutrition-related diseases.

Introduction

The first 1,000 days of life have been shown to be important for adult health. According to the Developmental Origins of Health and Disease theory, exposure to certain conditions in this period may trigger metabolic programming that could increase susceptibility to chronic diseases, such as obesity, diabetes and cardiovascular disease later in life.^{1–3} In this context, type of early feeding (breast milk or formula) is potentially associated with long-term health outcomes.^{4,5} The World Health Organization's breastfeeding recommendation until 24 months of life⁶ coincides with the end of the 1,000-day period. In Brazil, approximately 40% of children aged 9–12 months are no longer breastfed,⁷ and this scenario seems to be even worse in high-income countries, where more than 70% of 12-month-old children are not breastfed.⁸

The development of food preferences and dietary habits in the short and long run could be influenced by breastfeeding.^{9–13} Proposed underlying mechanisms are related to the presence of leptin in human milk but not in infant formulas, which plays a role in hypothalamic programming of satiety,^{14,15} and to the central reward systems.¹⁶ Additionally, being exposed to the flavors from the maternal diet during breastfeeding or even earlier during gestation via amniotic liquid may be important for the development of food preferences and propensity of accepting new foods in early life.^{16,17}

Longer breastfeeding has been associated with lower consumption of processed foods,¹⁰ preference for natural sources of animal protein¹¹ and higher adherence to healthy dietary patterns¹⁸ in 2–8-year old children. Although these findings probably reflect the mother's lifestyle, a Finnish cohort suggested reasonable stability of dietary patterns identified throughout infancy, adolescence and adulthood.¹⁹ In adult life, this association is scarcely reported and controversial.^{12,13,20} As far as

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ANEXO 5. PUBLICAÇÃO MANUSCRITO 3

European Journal of Clinical Nutrition
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ARTICLE

Epidemiology

Early life feeding and current dietary patterns are associated with biomarkers of glucose and lipid metabolism in young women from the Nutritionist's Health Study

Ilana Eshriqui¹ · Luciana Dias Folchetti² · Angélica Marques Martins Valente³ · Bianca de Almeida-Pititto⁴ · Sandra Roberta G. Ferreira⁵

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Abstract

Background/objectives We investigated if breastfeeding duration and current dietary patterns (DP) were associated with glucose and lipid metabolism biomarkers in women from the Nutritionist's Health Study.

Subjects/methods This is a cross-sectional analysis of 200 healthy undergraduates and nutrition graduates aged ≤45 years. Total [<6; ≥6 months] and predominant [<3; ≥3 months] breastfeeding were recalled using questionnaires. Diet were assessed using a food frequency questionnaire. DP obtained by factor analysis by principal component were categorized into tertiles of adherence (T1 = reference). Glucose and lipid biomarkers were categorized into tertiles (T1 + T2 = reference). Logistic regression was applied considering minimal sufficient adjustment recommended by directed acyclic graphs.

Results Median (interquartile range) age and BMI were 23.0 (20.0; 28.5) years and 22.6 (20.7; 25.4) kg/m², respectively. Mean ± SD values of glucose, LDL-c and HDL-c were 82.0 ± 9.0, 101.1 ± 29.6 and 54.4 ± 12.4 mg/dL, respectively. Women breastfed for <6 months had higher chance of being classified into T3 of insulin (OR = 2.87; 95%CI = 1.28–6.40). Predominant breastfeeding <3 months was associated with insulin levels (OR = 2.27; 95%CI = 1.02–5.02) and HOMA-IR (OR = 2.36; 95%CI = 1.06–5.26). Breastfeeding was not associated with lipids. The *Processed* pattern was directly associated with LDL-c (T3: OR 6.08; 95%CI 1.80–20.58; *P*-trend = 0.004), while the *Prudent* pattern was inversely associated with LDL-c (T3: OR 0.26; 95%CI 0.08–0.87; *P*-trend = 0.029) and LDL-c/HDL-c ratio (T3: OR 0.28; 95%CI 0.08–0.97; *P*-trend = 0.046).

Conclusion Early feeding could be a protective factor against insulin resistance development, while current DP were associated with lipid profile. This evidence indicates that from early life until early adulthood, dietary habits might influence women's cardiometabolic risk profile.

Supplementary information The online version of this article (<https://doi.org/10.1038/s41430-019-0516-8>) contains supplementary material, which is available to authorized users.

✉ Sandra Roberta G. Ferreira
sandrafv@usp.br

¹ Graduation Program in Public Health Nutrition, School of Public Health, University of São Paulo, São Paulo, Brazil

² School of Public Health, University of São Paulo, Paulo, Brazil

³ Graduation Program in Epidemiology, School of Public Health, University of São Paulo, São Paulo, Brazil

⁴ Department of Preventive Medicine, Federal University of São Paulo, São Paulo, Brazil

⁵ Department of Epidemiology, School of Public Health, University of São Paulo, São Paulo, Brazil

Introduction

Glucose and lipid metabolism disturbances are important risk factors for cardiovascular disease, a major public health problem worldwide [1]. Insulin resistance and increased LDL-c should be monitored to prevent cardiovascular events [2, 3]. In studies of risk factors for cardiovascular diseases, the female sex has been underrepresented, despite being recognized biological and psychosocial gender differences [4].

The impact of diet on glucose and lipid metabolism and cardiometabolic outcomes may also differ between sexes [5–9]. Once these abnormalities are more common after menopause [4], most studies have assessed its impact in middle-aged women [8, 10–12]. Considering that menopausal hormonal changes could modify the effect of diet on

ANEXO 6. PUBLICAÇÃO DO MANUSCRITO 4

Eshriqui et al. *International Breastfeeding Journal* (2020) 15:42
<https://doi.org/10.1186/s13006-020-00285-w>

International Breastfeeding
Journal

RESEARCH

Open Access

Breastfeeding may have a long-term effect on oral microbiota: results from the Fin-HIT cohort

Ilana Eshriqui^{1,2}, Heli T. Viljakainen^{3,4*} , Sandra R. G. Ferreira⁵, Sajan C. Raju^{3,6}, Elisabete Weiderpass⁷ and Rejane A. O. Figueiredo^{3,6}

Abstract

Background: Breastfeeding contributes to gastrointestinal microbiota colonization in early life, but its long-term impact is inconclusive. We aimed to evaluate whether the type of feeding during the first six months of life was associated with oral microbiota in adolescence.

Methods: This is a cross-sectional sub-study using baseline information of 423 adolescents from the Finnish Health in Teens (Fin-HIT) cohort. Type of feeding was recalled by parents and dichotomized as (i) No infant formula; (ii) Infant formula (breastmilk + formula or only formula). Saliva microbiota was analysed using 16S rRNA (V3–V4) sequencing. Alpha diversity and beta diversity were compared between feeding type groups using ANCOVA and PERMANOVA, respectively. Differential bacteria abundance was tested using appropriate general linear models.

Results: Mean age and body mass index were 11.7 years and 18.0 kg/m², respectively. The No formula group contained 41% of the participants. *Firmicutes* (51.0%), *Bacteroidetes* (19.1%), and *Proteobacteria* (16.3%) were the most abundant phyla among all participants. Alpha and beta diversity indices did not differ between the two feeding groups. Three Operational Taxonomic Units (OTUs) belonging to *Eubacteria* and *Veillonella* genera (phylum *Firmicutes*) were more abundant in the No formula than in the Infant formula group (log₂fold changes/ *p* - values – 0.920/ < 0.001, – 0.328/ 0.001, – 0.577/ 0.004).

Conclusion: Differences exist in abundances of some OTUs in adolescence according to feeding type during the first six months of life, but our findings do not support diversity and overall oral microbiota composition in adolescents being affected by early feeding type.

Keywords: Microbiota, Saliva, Breastfeeding, Adolescent

* Correspondence: heli.viljakainen@helsinki.fi

¹Folkhälsan Research Center, Topeliuksenkatu 20, FI-00250 Helsinki, Finland

²Department of Food and Nutrition, University of Helsinki, Helsinki, Finland

Full list of author information is available at the end of the article

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