Universidade de São Paulo

Faculdade de Saúde Pública

Padrões alimentares, nutrientes do metabolismo do folato e homocisteína e três desfechos em saúde

Juliana Araujo Teixeira

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

Área de concentração:

Nutrição em Saúde Pública

Orientadora:

Prof.ª Dr.ª Dirce Maria Lobo Marchioni

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ERRATA

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Conforme solicitado pela FAPESP e de acordo com a Cláusula Sétima do Termo de Outorga, cumpre-se retificar que, na página de Agradecimentos desta tese de doutorado, onde se lê: À Fundação de Amparo à Pesquisa do Estado de São Paulo pela bolsa de doutorado concedida, leia-se: À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (processo FAPESP nº 2014/12647-1) pela bolsa de doutorado concedida.

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Não basta dar os passos que nos devem levar um dia ao objetivo,
cada passo deve ser ele próprio um objetivo em si mesmo,
ao mesmo tempo que nos leva para diante.

Johann Wolfgang von Goethe

RESUMO

Teixeira JA. Padrões alimentares, nutrientes do metabolismo do folato e homocisteína e três desfechos em saúde [tese]. São Paulo: Faculdade de Saúde Pública da Universidade de São Paulo; 2018.

Introdução - Os hábitos alimentares e os nutrientes da via metabólica do folato e homocisteína possuem grande importância na manutenção da saúde. **Objetivo -** Investigar a relação entre padrões alimentares (PAs) e os nutrientes envolvidos nessa via metabólica, com medidas antropométricas do recém-nascido, duração da infecção por HPV em homens e concentrações de homocisteína (Hcy) em adultos. **Métodos** - Foram utilizados dados dos estudos de coorte ProcriAr (*Influência dos fatores nutricionais e poluentes atmosféricos urbanos na saúde pulmonar de crianças: um estudo de coorte com gestantes da zona oeste do município de São Paulo*, n=299); e HIM (*História natural da infecção por HPV em homens*, n=1.194); e do estudo transversal ISA-Capital 2008 (*Inquérito de saúde do estado de São Paulo*, n=281). Os padrões alimentares foram derivados por análise fatorial por componentes principais nos estudos ProcriAr e ISA-Capital 2008 e utilizando *reduced rank regression* (RRR) no estudo HIM. Modelos multivariados de regressão de Poisson e lineares foram utilizados nos estudos ProcriAr e HIM para identificar a relação entre PAs e medidas antropométricas do recém-nascido e duração da infecção por HPV em homens, respectivamente. Utilizando modelo de equação estrutural, investigou-se a relação entre PAs, concentrações bioquímicas de folato, vitamina B12 e ácido docosahexaenoico (DHA) e concentrações de homocisteína em adultos do estudo ISA-Capital, considerando polimorfismo da enzima metilenotetrahidrofolato redutase (MTHFR 677C>T). Os três estudos utilizaram questionário de frequência alimentar para avaliação do consumo alimentar. **Resultados** - No estudo ProcriAr, a maior adesão materna ao PA “Snacks, sanduíches, doces e refrigerantes”, rico em energia, gordura, e folato sintético, esteve diretamente associada a ter um filho pequeno ao nascer (peso e/ou comprimento ao nascer, ajustado pela idade gestacional, abaixo do percentil 10 – INTERGOWTH-21st) (RR: 2,01; IC 95%: 1.13-3.57). No estudo HIM, homens com maior adesão ao “PA3” tiveram, em média, um aumento de 1,15 (IC95% 0,09-2,21) à 1,18 (IC95% 0,11-2,24) meses na duração da infecção por HPV. O “PA3” esteve positivamente correlacionado com vitamina B6 (r = 0,59), vitamina B12 (0,27) e DFE (0,07) e negativamente correlacionado com DHA (-0,37). No estudo ISA-Capital o PA “Prudente” esteve inversamente associado à concentração de Hcy (β = -0,12). O DHA esteve diretamente associado ao PA “Prudente”; composto por verduras e legumes, peixe, frutas, frango, suco natural e batata/mandioca/polenta (cozida ou assada). **Conclusões** - Os PAs estão associados às medidas antropométricas do recém-nascido, à duração da infecção por HPV em homens e às concentrações de homocisteína em adultos. Estes resultados reforçam a importância de estudos sobre alimentação e nutrição que considerem não somente nutrientes, mas principalmente o consumo de alimentos e suas combinações, servindo como base para a elaboração de estratégias e políticas públicas de promoção à saúde.

Descritores: padrões alimentares, metabolismo do folato e homocisteína, folato, DFE, vitamina B12, ácidos graxos poliinsaturados, DHA, homocisteína, polimorfismo, ingestão habitual, comprimento ao nascer, peso ao nascer, doença cardiovascular, HPV

ABSTRACT

Teixeira JA. [Dietary patterns, nutrients involved in one-carbon metabolism and three health outcomes] [thesis]. São Paulo: Faculdade de Saúde Pública da Universidade de São Paulo; 2018. Portuguese

Introduction - The dietary habits and nutrients involved in one-carbon metabolism are of great importance in health. **Objective** - To investigate the relationship between dietary patterns (DP) and the nutrients involved in this metabolism, with newborn's anthropometric measurements, duration of HPV infection in men, and homocysteine (Hcy) levels in adults. **Methods** - Data from the cohort studies ProcriAr (*Influence of nutritional factors and urban air pollutants on the pulmonary health of children: a cohort study with pregnant women from the western region of the city of São Paulo*, n=299); and HIM (*Natural history of HPV infection in men*, n=1,194); and the cross-sectional study ISA-Capital 2008 (*São Paulo State Health Survey*, n=281) were used. The DP were estimated using factor analysis with principal component's estimation in ProcriAr and ISA-Capital 2008 studies and using reduced rank regression (RRR) in HIM study. Multivariate Poisson and linear regression models were used in the ProcriAr and HIM studies to identify the relationship between DP and newborn's anthropometric measurements and duration of HPV infection in men, respectively. Using a structural equation model, the relationship between DP, biochemical levels of folate, vitamin B12 and docosahexaenoic acid (DHA) and homocysteine levels was investigated in adults from the ISA-Capital 2008 study, considering the polymorphism of the enzyme methylenetetrahydrofolate reductase (MTHFR 677C>T). The three studies used a food frequency questionnaire to evaluate dietary intake. **Results** - In the ProcriAr study, the higher maternal adherence to the "Snacks, sandwiches, sweets and soft drinks" DP, which is a DP rich in energy, fat, and synthetic folate, was directly associated with having a child small at birth (weight and/or birth length by gestational age and sex below the 10th percentile - INTERGOWTH-21st) (RR: 2.01, 95% CI: 1.13-3.57). In the HIM study, men with higher adherence to "DP3" had, on average, an increase from 1.15 (95% CI 0.09-2.21) to 1.18 (95% CI 0.11-2.24) months in the duration of HPV infection. "DP3" was positively correlated with vitamin B6 (r = 0.59), vitamin B12

(0.27) and DFE (0.07) and negatively correlated with DHA (-0.37). In the ISA-Capital study, the "Prudent" DP was inversely associated with Hcy levels (β = -0.12). DHA was directly associated with "Prudent" DP; composed of vegetables, fish, fruits, chicken, natural juice and potato/cassava/polenta (cooked or roasted). **Conclusions** - Dietary patterns are associated with newborn’s anthropometric measurements, duration of HPV infection in men, and Hcy levels in adults. These results reinforce the importance of studies on food and nutrition that consider not only nutrients, but mainly the consumption of foods and their combinations, serving as a basis for the elaboration of public health promotion strategies and policies.

Descriptors: dietary patterns, one-carbon metabolism, folate, DFE, vitamin B12, polyunsaturated fatty acids, DHA, homocysteine, polymorphism, usual dietary intake, birth length, birth weight, cardiovascular disease, HPV

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Siglas Utilizadas

5,10-MTHF – Metilenotetrahidrofolato

5-MTHF – Metiltetrahidrofolato

95% CI – *95% confidence interval*

AGPI - Ácidos graxos poliinsaturados

AI - *Adequate intake*

ARA - Ácido araquidônico

ATP – Adenosina trifosfato

BHMT - Betaína-homocisteína S-metiltransferase

BMI - *Body mass index*

CBS - Cistationa beta-sintase

CFI - *Comparative Fit Index*

CMIA - *Chemiluminescent microparticle immunoassay*

CSE - Cistationina gama-liase / *cystathionine-gamma-lyase*

DFE - Equivalentes dietéticos de folato / *dietary folate equivalents*

DHA - Ácido docosaexaenoico / *docosahexaenoic acid*

DHFR - Dehidrofolato redutase

DM – Diabetes Mellitus

DNA - Ácido desoxirribonucleico

DP - *Dietary patterns*

dTMP - Timidina monofosfato

dUMP - Deoxiuridina monofosfato

E.g. - *Exempli gratia*

EAR – *Estimated Average Requirements*

EDTA - *Ethylenediaminetetraacetic acid*

FA - *Fatty acids*

FFQ - *Food frequency questionnaire*

FSP - Faculdade de Saúde Pública

Hcy – Homocisteína / *homocysteine*

HD - *High DHA*

HF - *High folate*

HHcy – Hiperhomocisteinemia / *hyperhomocysteinaemia*

HIM - *HPV Infection in Men*

HPV - Papilomavirus humano / *Human papillomavirus*

HT - *Hypertension*

I.e. - *Id est.*

IARC - *International Agency for Research on Cancer*

IC 95% - Intervalo de confiança 95%

INTERGROWTH-21st - *The International Fetal and Newborn Growth Consortium for the 21st Century study*

IPAC - *The International Physical Activity Questionnaire*

ISA – Inquérito de Saúde

LD - *Low DHA*

LF - *Low folate*

MAT - Metionina adenosiltransferase

MS - Metionina sintase

MTHFR - Metilenotetrahidrofolato redutase / *methylenetetrahydrofolate reductase*

MTRR - Metionina sintase redutase

n-3 PUFA – *Omega-3 polyunsaturated fatty acids*

NCC - *Nutrition Coordinating Center*

NDSR - *Nutrition Data System for Research*

P10 – *10th percentile*

PUFAs - *Polyunsaturated fatty acids*

Q1 – *1st quintile*

Q5 – *5th quintile*

RFC1 - Enzima carreadora de folato reduzido 1

RMSEA - *Root Mean Square Error of Approximation*

RNA - Ácido ribonucleico

RR - *Relative risk*

RRR - *Reduced rank regression*

r_s - *Spearman's correlation coefficients*

SAH - S-adenosil-homocisteína

SAHH - S-adenosil-homocisteína hidrolase

SAM - S-adenosilmetionina

SD - *Standard deviation*

SE - *Standard error*

SEM - *Structural equation model*

SHMT - Serina hidroximetiltransferase

STROBE - *Strengthening the Reporting of Observational Studies in Epidemiology*

T - tertile

THF – Tetrahidrofolato

TLI - *Tucker Lewis Index*

TYS - Timidilato sintase

UL - ingestão máxima tolerável

USA – *United States of America*

USDA - *United States Department for Agriculture*

WHO - *World Health Organization*

APRESENTAÇÃO

Esta tese de doutorado analisa dados de três diferentes estudos em uma tentativa de melhor compreender a relação entre combinações de alimentos, nutrientes do metabolismo do folato e homocisteína e importantes desfechos em saúde. O primeiro é o estudo ProcriAr (*Influência dos fatores nutricionais e poluentes atmosféricos urbanos na saúde pulmonar de crianças: um estudo de coorte com gestantes da zona oeste do município de São Paulo*), coordenado pelas pesquisadoras Silvia Regina D. M. Saldiva, Rossana P. V. Francisco, e Sandra Elisabete Vieira, onde foram investigados os determinantes dos padrões alimentares de mulheres no período pré-gestacional e a relação desses padrões alimentares com consumo de nutrientes e com as medidas antropométricas neonatais de seus filhos. O segundo é o estudo HIM (*História natural da infecção por HPV em homens*), coordenado pela pesquisadora Dra. Anna Giuliano e pelos co-investigadores Dr. Eduardo Lazcano e Dra. Luisa Lina Villa, onde foi estudada a associação entre padrões alimentares e consumo de nutrientes do metabolismo do folato e homocisteína e sua relação com a duração da infecção por HPV em homens. O terceiro é o estudo ISA-Capital 2008, (*Inquérito de saúde do estado de São Paulo*), coordenado pelos pesquisadores Chester Luiz G. Cesar, Marilisa Berti de Azevedo Barros, Moisés Goldbaum, Regina M. Fisberg e Dirce Maria Marchioni, onde, utilizando modelo de equação estrutural, investigou-se a relação entre padrões alimentares, concentrações bioquímicas de nutrientes e polimorfismos do metabolismo do folato e homocisteína na determinação das concentrações de homocisteína em indivíduos adultos.

Esta tese é apresentada em formato de artigos científicos, sob o respaldo das diretrizes promulgadas pela Comissão de Pós-graduação da Faculdade de Saúde Pública da Universidade de São Paulo em sua sessão 9ª/2008 de 05/06/2008, respeitando as normas de apresentação das dissertações defendidas na Faculdade de Saúde Pública (FSP), publicadas no Guia de Apresentação de Teses da FSP/USP. A tese está organizada da seguinte forma: (1) *Introdução*, que aborda alimentação no Brasil e a cidade de São Paulo como campo de estudo, o estudo de padrões alimentares, a importância dos nutrientes envolvidos no metabolismo do folato e homocisteína e, por fim, a relação entre padrões alimentares, nutrientes envolvidos no metabolismo do folato e homocisteína e os desfechos investigados; (2) *Objetivos*, onde são apresentados os propósitos do estudo; (3) *Métodos*, onde são detalhados os procedimentos empregados na coleta, tratamento e análise dos dados; (3) *Resultados e discussão* do estudo, na forma de quatro manuscritos, onde são apresentados todos os produtos das análises estatísticas e a discussão pautada nos resultados encontrados, buscando compará-los ao que se observa na literatura; e (4) *Considerações finais* que sistematizam as principais conclusões do estudo.

1 INTRODUÇÃO

Nas últimas décadas, o Brasil sofreu importantes mudanças econômicas, demográficas, ambientais e culturais. Em consequência, ocorreram alterações no padrão de saúde e consumo alimentar da população, em um processo de rápida transição epidemiológica e nutricional (Batista Filho e Rissin 2003; Conde and Monteiro 2014). As principais doenças que acometem os brasileiros deixaram de ser agudas e passaram a ser crônicas. Houve um rápido declínio na ocorrência de desnutrição energético-proteica, acompanhada por um aumento acelerado na prevalência de sobrepeso e obesidade com concomitante deficiência de micronutrientes entre crianças e adultos brasileiros (Batista Filho e Rissin 2003; Conde e Monteiro 2014). Representando este cenário, um fenômeno atual observado em países em desenvolvimento em transição nutricional é a coexistência familiar de adultos com excesso de peso e crianças com baixa estatura para idade (Garrett 2003; Barquera et al. 2007).

A identificação e isolamento de nutrientes, bem como a investigação da associação desses nutrientes com determinados desfechos em saúde marcou o surgimento da ciência da nutrição (Brasil 2014). Estes estudos foram de extrema importância para o desenvolvimento de ações de prevenção à carências nutricionais (Jacobs e Steffen 2003). No entanto, o estudo de nutrientes isoladamente foi se tornando insuficiente para identificar as relações estabelecidas entre alimentação e saúde. Estes estudos indicam que o efeito benéfico sobre a prevenção de doenças advém do alimento em si e das combinações de nutrientes e outros compostos bioativos que fazem parte da matriz do alimento, mais do que de nutrientes isolados (Willett e Skerret 2005; Maggini et al. 2007). Além disso, resultados de estudos baseados em alimentos e combinações de alimentos são mais acessíveis não somente para profissionais e pesquisadores não-nutricionistas, como também para a população em geral (Scrinis 2013) .

Os estudos de padrões alimentares têm sido cada vez mais frequentes em epidemiologia nutricional. Entretanto, não são muitos aqueles que avaliam a relação entre padrões alimentares e medidas antropométricas do recém-nascido, duração da infecção por HPV em homens ou concentrações de homocisteína, discutindo o papel dos nutrientes do metabolismo do folato e homocisteína. Assim, mais estudos neste campo se fazem necessários.

1.1 ALIMENTAÇÃO NO BRASIL E A CIDADE DE SÃO PAULO COMO CAMPO DE ESTUDO

A alimentação dos brasileiros é composta por diversas influências e atualmente é fortemente caracterizada por uma dieta tida como “tradicional” (que tem como base o arroz com feijão), combinada à alimentos ricos em energia, gorduras, sódio e açúcar e com baixo teor de micronutrientes (Brasil 2012a).

Características pessoais como sexo, escolaridade e idade influenciam os padrões alimentares. O consumo de alimentos ricos em energia, gorduras, sódio e açúcar e com baixo teor de micronutrientes é mais frequente entre os mais jovens, e este consumo tende a diminuir com o aumento da idade. O contrário é observado para o consumo de frutas e hortaliças: menor consumo entre os mais jovens, com tendência de aumento com a idade (Brasil 2017). Os adolescentes consomem menos feijão e hortaliças em geral, representando o grupo com pior perfil de dieta no Brasil (Souza et al. 2013; Veiga et al. 2013). O preço dos alimentos e os recursos disponíveis também exercem forte influência sobre a escolha dos alimentos (Contento 2008; Claro e Monteiro 2010). O consumo de alimentos de baixa qualidade nutricional como doces, refrigerantes, pizzas e salgados fritos e assados tende a crescer com o aumento da renda das famílias brasileiras (Brasil 2012a). Já os brasileiros de mais baixa renda apresentam uma dieta de melhor qualidade, com predominância do arroz e feijão, combinados à alimentos básicos como peixes e milho (Brasil 2012a).

O Brasil é geopoliticamente dividido em cinco regiões: Norte, Nordeste, Centro-Oeste, Sudeste e Sul. As regiões Sudeste e Sul são a primeira e segunda economias do Brasil, seguidas pelas regiões Centro-Oeste, Nordeste e Norte. O ingresso *per capita* das regiões Norte e Nordeste representa pouco mais de ¼ da renda individual disponível nas demais regiões brasileiras (Batista Filho e Rissin 2003). A desigual distribuição de renda pode também ser vista quando se compara regiões rurais (mais pobre) e urbanas, principalmente no Norte e Nordeste (Batista Filho e Rissin 2003). Essas desigualdades impactam os padrões de saúde e alimentação das populações e marcam o cenário epidemiológico nutricional brasileiro por fortes diferenças regionais (Batista Filho e Rissin 2003).

No Brasil houve movimentos significativos de brasileiros de uma região para outra e das regiões rurais para as regiões urbanas em busca de melhores condições de vida. As décadas de 40 a 60 foram marcadas por migrações interestaduais advindas, sobretudo, de Minas Gerais e do Nordeste em direção aos estados com maior crescimento urbano-industrial como São Paulo e Rio de Janeiro, primeiro em virtude do desenvolvimento da cultura do café e, posteriormente, com a chegada da indústria (Brito 2000). Entre as décadas de 60 e 80 houve um aumento ainda maior das desigualdades regionais, reforçando a tendência migratória rural-urbana, então facilitadas pelos avanços nos transportes e nas telecomunicações (Cavalcante et al. 2008; Albuquerque et al. 2013). Em 1872 o estado de São Paulo somava 8% da população brasileira, passando à 22% em 2010 (Baeninger 2012; Albuquerque et al. 2013). A capital do estado de São Paulo, a cidade de São Paulo, é altamente urbanizada, e considerada a cidade mais populosa e com a maior economia do hemisfério sul.

Os fluxos migratórios e as influências econômicas internas e externas determinam, ao longo da história, a oferta e a disponibilidade de alimentos. O hábito alimentar do paulistano é, então, marcado pelo ecletismo (Garcia 1997). A pizza de sábado e a macarronada de domingo simbolizam a influência italiana. Em São Paulo se encontra, facilmente, comida de todos os tipos e para todos os gostos: baiana, mineira, chinesa, japonesa, árabe, sírio-libanesa, alemã e muitas outras. Com isso, notam-se duas tendências simultâneas: a heterogeneidade e a homogeneidade, categorias peculiares às concentrações urbanas (Wirth 1987). Paralelamente a uma grande variedade de alimentos decorrente dessa alta concentração de diversidades, há uma tendência niveladora que faz parte do processo de perda da identidade cultural ou despersonalização (Garcia 1997).

O meio urbano afeta a estrutura da alimentação e provoca uma reorganização de valores e práticas que, certamente, terão implicações no padrão alimentar (Garcia 1997). A distância entre o local de trabalho e a residência, as dificuldade de deslocamento impostas pelo trânsito e pelos transportes públicos e o ritmo da cidade dificultam a realização das refeições no domicílio (Garcia 1997). Um maior número de refeições vêm sendo realizado fora do domicílio: cerca de 32% dos paulistanos fazem pelo menos uma das três refeições principais fora de casa, sendo que este comportamento alimentar esteve inversamente associado à qualidade da dieta e diretamente ao excesso de peso (Gorgulho et al. 2013).

Em função também do tempo no meio urbano, os rituais destinados à alimentação sofreram mudanças importantes. O tempo destinado ao preparo dos alimentos foi encurtado, dando espaço a um maior consumo de alimentos industrializados e prontos para o consumo. Além disso, outras atividades foram incorporadas ao momento da refeição, antes realizada à mesa junto da família, agora em companhia da televisão ou na própria mesa de trabalho, com muito menos atenção destinada àquilo que se consome (Garcia 1997; Brasil 2012a).

Há uma necessidade crescente de formulação e implantação de estratégias regionais integradas e efetivas para a redução da morbimortalidade associada à alimentação inadequada nos meios urbanos brasileiros (Brasil 2012a). Entretanto essas estratégias precisam estar alinhadas às diferentes realidades (Brasil 2012a). Estudos sobre os padrões alimentares estabelecidos pelas populações urbanas e os fatores associados a esses padrões são fundamentais para elaboração dessas estratégias.

1.2 ESTUDO DOS ALIMENTOS E SUAS COMBINAÇÕES: ANÁLISE DE PADRÕES ALIMENTARES

A escolha dos alimentos e o modo como se come são constituídos por muitos significados, como identidade cultural, condição social, religião, memória familiar, o momento da história em que se vive, acesso aos alimentos, as pessoas com quem se divide o momento alimentar (Garcia 1997; Brasil 2014).

Além disso, a interação entre a atividade biológica dos alimentos consumidos e as respostas fisiologias humanas é extremamente complexa (Jacobs e Steffen 2003). Alguns estudos testaram o efeito protetor à saúde observado com o consumo de determinado alimento, porém ofertando o nutriente em isolado na forma de suplemento alimentar e não obtiveram o mesmo efeito benéfico (Maggini et al. 2007). Isso indica que a proteção à saúde provém do alimento em sua forma integral, com todos os nutrientes e compostos bioativos que compõem a sua matriz, e não de um nutriente específico em isolado (Willett e Skerret 2005). Portanto é provável que existam influências aditivas dos alimentos e seus constituintes sobre a saúde, isto é, uma sinergia alimentar (Jacobs e Steffen 2003). Dessa forma, para compreender o padrão alimentar de uma população e seus efeitos sobre a saúde, a complexidade e a interação cumulativa ou opositiva existente entre nutrientes e compostos bioativos precisam ser consideradas (Jacobs e Steffen 2003; Jacobs et al. 2009).

Os estudos de padrões alimentares podem captar a real ação sinérgica e a resposta metabólica provocada pela ingestão alimentar (Jacobs e Steffen 2003; Jacobs et al. 2009). Alguns métodos têm sido mais comumente utilizados para derivar os padrões alimentares. O primeiro deles, chamado de *a priori* ou hipótese-orientado, inclui índices numéricos que mensuram a adesão a padrões alimentares pré-estabelecidos, como recomendações nutricionais ou guias alimentares (por exemplo, dieta mediterrânea, índice de qualidade da dieta, etc.) (Hoffmann et al. 2004; USDA Evidence Analysis Library Division 2014). Neste caso se utilizam as evidências científicas disponíveis previamente para estabelecer os padrões alimentares, não se utilizando de dados do estudo para criar os padrões alimentares (Hoffmann et al. 2004). Este método foca em aspectos específicos da dieta e não leva em consideração a estrutura de correlação do consumo de alimentos e nutrientes (Hoffmann et al. 2004). Consequentemente, os escores de adesão criados não irão refletir o efeito total da dieta (Hoffmann et al. 2004).

A segunda abordagem utiliza-se de métodos estatísticos exploratórios, como análise de componentes principais e análise fatorial, para derivar os padrões alimentares (Hoffmann et al. 2004; USDA Evidence Analysis Library Division 2014). Esta abordagem ignora as evidências científicas levantadas previamente e é orientada pelos dados da população estudada, chamado de método *à posteriori* (Hoffmann et al. 2004). A aplicação destes métodos resulta em escores de padrões alimentares que não apresentam correlação entre si, e que resumem e decompõem a estrutura de correlação entre os itens alimentares originais (Hoffmann et al. 2004). Dessa forma, considera os aspectos cumulativos e interativos da dieta, gerando dados que melhor reproduzem o consumo alimentar, aumentando a capacidade de investigar os efeitos da alimentação sobre a saúde (USDA Evidence Analysis Library Division 2014). Esta abordagem recebe críticas por sua natureza subjetiva, como ocorre na consolidação dos itens alimentares em grupos de alimentos (etapa que antecede a análise de padrões), na definição do número de fatores extraídos, do método de rotação e do nome dado à cada padrão alimentar (USDA Evidence Analysis Library Division 2014).

Uma terceira abordagem que vêm sendo mais recentemente utilizada em epidemiologia nutricional para derivar padrões alimentares é a chamada regressão por redução de posto, ou *reduced rank regression* (RRR). Este método determina função linear dos alimentos pela maximização da variação explicada dos nutrientes, estes relacionados ao desfecho de interesse (Hoffmann et al. 2004). O RRR não pode ser considerado um método *à priori* e nem *à posteriori*, uma vez que utiliza as duas fontes de informação: não somente dados do próprio estudo, mas se baseia nas evidências científicas disponíveis previamente para estabelecer as variáveis resposta (nutrientes relacionados ao desfecho de interesse) (Hoffmann et al. 2004). A principal vantagem deste método é a possibilidade de escolher, baseado em conhecimentos prévios, os nutrientes relacionados ao desfecho de interesse como variáveis resposta. Assim, padrões alimentares derivados por RRR deveriam refletir com mais acurácia as associações entre alimentação e saúde.

A abordagem de pesquisa chamada '*top down*' ou 'de cima para baixo' estuda os efeitos dos padrões alimentares e combinações de nutrientes e/ou compostos alimentares sobre a saúde, considerando, então, a sinergia presente nos alimentos (**Tabela 1**) (Jacobs e Steffen 2003).

Tabela 1. Estrutura hierárquica entre padrões alimentares, alimentos e nutrientes.

Estrutura hierárquica	**Exemplos de componentes da dieta para cada nível proposto**
Nível 5: Padrões alimentares	Prudente, Tradicional, Moderno
Nível 4: Grupos de alimentos	Frutas e verduras
Nível 3: Alimento	Laranja, espinafre, couve
Nível 2: Componente/parte do alimento	Polpa, casca, talo
Nível 1: Nutrientes, compostos bioativos	Folato

(Adaptada de Jacobs e Steffen, 2003)

No exemplo acima (**Tabela 1**), os padrões alimentares foram identificados como prudente, tradicional e moderno (Nível 5). Frutas e verduras muitas vezes constituem um padrão alimentar prudente. Como as frutas possuem características em comum esses alimentos podem ser agrupados, assim como as verduras (Nível 4). Para entender detalhes desses grupos de alimentos, frutas e verduras específicas, como laranja, espinafre, couve podem ser estudados (Nível 3). Para melhor compreender os alimentos específicos, partes deles podem ser exploradas (Nível 2). Por fim, nutrientes e compostos bioativos específicos contidos nos alimentos ou padrões de alimentos são mais detalhados (Nível 1).

1.3 METABOLISMO DO FOLATO E HOMOCISTEINA: IMPORTÂNCIA DOS NUTRIENTES FOLATO, VITAMINA B6, VITAMINA B12 E ÁCIDOS GRAXOS POLIINSATURADOS

Na via metabólica do folato e homocisteína ocorre uma série de reações que envolve a transferência contínua de uma unidade de carbono para vários receptores de grupos metil (CH3) e a transformação da homocisteína em um dos principais doadores de metil, a S-adenosilmetionina (SAM) (**Figura 1**).

Primeiramente o folato é convertido à tetrahidrofolato (THF) pela ação da enzima dehidrofolato redutase (DHFR). A enzima serina hidroximetiltransferase (SHMT) catalisa a reação reversível de THF em 5,10-metilenotetrahidrofolato (5,10-MTHF), constituindo uma enzima chave na manutenção e regulação da homeostase da concentração de folato e de grupos metil intracelulares (Scheer et al. 2005). O 5,10 MTHF é, também, substrato da timidilato sintase que converte deoxiuridina monofosfato (dUMP) à timidina monofosfato (dTMP) (timina + anel de desoxirribose + ácido fosfórico). THF pode ser utilizado para a síntese de purina. Sendo o timidilato e as purinas formadores do DNA, a via metabólica do folato possui papel significante na síntese e reparo de DNA, e na síntese de proteínas (Khot et al. 2015).

A enzima carreadora de folato reduzido 1 (RFC1), localizada na membrana das células da mucosa intestinal, participa do processo de absorção do folato, realizando o transporte do 5-MTHF para o interior de uma variedade de células, constituindo um importante determinante das concentrações de folato intracelular (Finkelstein e Martin 2000).

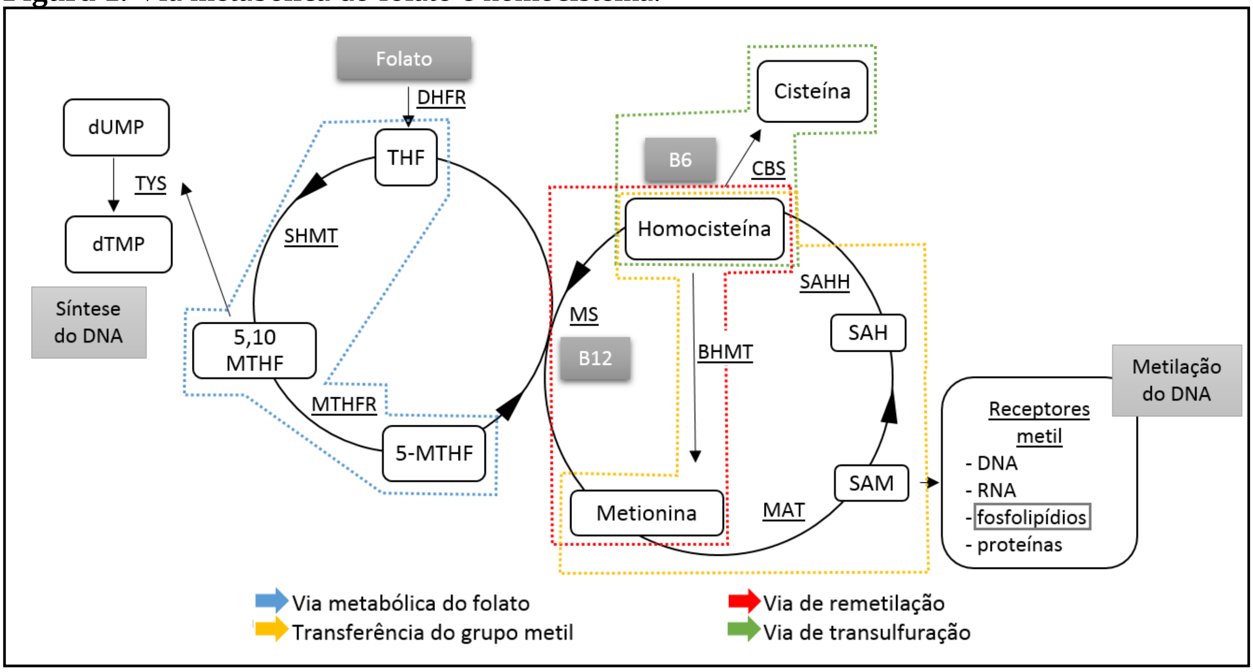
A enzima metilenotetrahidrofolato redutase (MTHFR) catalisa a conversão do 5,10-MTHF para 5-metiltetrahidrofolato (5-MTHF), a principal forma circulante de folato, que atua como doador de grupos metil para a remetilação da Hcy em metionina. Esta reação de remetilação é catalisada pela enzima metionina sintase (MS), que requer a vitamina B12 como cofator, e resulta na formação de SAM. A enzima metionina sintase redutase (MTRR) é responsável pela manutenção do estado ativo da enzima MS (Khot et al. 2015).

A metionina, por sua vez, é convertida à forma ativa da SAM pela ação da enzima metionina adenosiltransferase (MAT). MAT catalisa a formação de SAM a partir da metionina e ATP e sua reação pode ser considerada o ponto limitante da velocidade do ciclo da metionina (Markham e Pajares 2009). A SAM produzida doa o grupo metil para vários receptores metil como ácido desoxirribonucleico (DNA), ácido ribonucleico (RNA), fosfolipídios, hormônios e proteínas (Wang et al. 2014). Por esta reação de desmetilação, forma-se a S-adenosilhomocisteína (SAH), com posterior hidrólise para liberar adenosina e Hcy, completando o ciclo (Finkelstein e Martin 2000).

A enzima betaína-homocisteína S-metiltransferase (BHMT) catalisa a conversão da Hcy em metionina por uma via alternativa de remetilação, na qual o aminoácido betaína atua como doador de grupos metil (Choi e Mason 2000; Ueland et al. 2005). Quando a via de remetilação da Hcy catalisada pela enzima MS, dependente de folato, encontra-se alterada, a enzima BHMT desenvolve papel crucial na homeostase da Hcy e da SAM (Weisberg et al. 2003).

Participando também desse metabolismo, as enzimas cistationina beta-sintase (CBS) e cistationina gama-liase (CSE), dependentes de vitamina B6, convertem a Hcy em cisteína, aminoácido não essencial, na chamada via de transulfuração (Födinger et al. 2001). A cisteína é largamente utilizada na formação de glutationa que age como um importante antioxidante (Lushchak 2012).

Figura 1. Via metabólica do folato e homocisteína.



BHMT - betaína-homocisteína S-metiltransferase; CBS - cistationa beta-sintase; DHFR - dehidrofolato redutase; dTMP - timidina monofosfato; dUMP - deoxiuridina monofosfato; MAT - metionina adenosiltransferase; MS - metionina sintase; 5-MTHF - metiltetrahidrofolato; 5,10-MTHF - metilenotetrahidrofolato; MTHFR - metilenotetrahidrofolato redutase; SAH - S-adenosil-homocisteína; SAHH - S-adenosil-homocisteína hidrolase; SAM - S-adenosilmetionina; SHMT - serina hidroximetiltransferase; THF - tetrahidrofolato; TYS - timidilato sintase.
Adaptado de Khot et al., 2014.

O metabolismo do folato e homocisteína possui grande importância na manutenção da saúde. Entre outras funções, promove a formação de grupos metil (CH3) utilizados principalmente na metilação do DNA. Quando essa via metabólica se apresenta alterada, os impactos metabólicos e epigenéticos incluem desequilíbrio das concentrações de homocisteína (Hcy), metionina e vitaminas do complexo B, desequilíbrio na metilação de proteínas, histonas, DNA e RNA (Malinowska e Chmurzynska 2009). Portanto, este metabolismo exerce forte influência no desenvolvimento fetal, incluindo crescimento fetal (Blom e Smulders 2011), e também saúde cardiovascular (Williams et al. 2014). Ainda, existem evidências de que o metabolismo do folato e homocisteína influencia o sistema imunológico e, por consequência, modula a suscetibilidade à doenças infecciosas (Field et al. 2002; Piyathilake et al. 2004; Abike et al. 2011).

Apesar de não haver consenso na literatura a respeito dos processos biológicos pelos quais essas alterações ocorrem, muitos são os fatores que podem modificar o metabolismo do folato e homocisteína, incluindo doenças como anemia perniciosa, insuficiência renal, hipotireoidismo e psoríase; medicamentos (antagonistas de folato e vitamina B6), fumo, polimorfismos genéticos e consumo inadequado de nutrientes (Refsum et al. 2004; Malinowska e Chmurzynska 2009).

Em relação às inadequações nutricionais que ocorrem no desequilíbrio do metabolismo do folato e homocisteína, atenção têm sido dada ao folato, vitaminas B6, B12 e ácidos graxos poliinsaturados (AGPI). As deficiências de folato, vitaminas B6 e B12, importantes cofatores enzimáticos nesta via metabólica, estão associadas a graus diferentes de hiperhomocisteinemia (HHcy) (Lippi e Plebani 2012), fundamentando a relação entre distúrbios nutricionais e HHcy. Alguns estudos demonstraram que o aumento da concentração de AGPI em fosfolipídios plasmáticos e plaquetários, principalmente ômega-3, foi associado a um efeito protetor para doenças cardiovasculares (Li et al. 2006, 2007). Na última década os ácidos graxos começaram a ter o seu papel estabelecido como modulador do efeito dos polimorfismos da enzima metilenotetrahidrofolato redutase (MTHFR), entretanto essa interação ainda não foi claramente descrita (Li et al. 2006, 2007; Huang et al. 2011a).

Estudos que correlacionam genética ao desequilíbrio do metabolismo do folato e homocisteína apontam como fator central os polimorfismos do gene da enzima MTHFR. A MTHFR é fundamental para o metabolismo do folato e remetilação da Hcy em metionina. Em humanos, duas variantes funcionais da MTHFR C677T e A1298C são as mais estudadas (Markan et al. 2007). O genótipo homozigoto MTHFR C677T (T:T) resulta em uma enzima termolábil com atividade reduzida e consequente diminuição da concentração do folato plasmático e aumento da concentração da Hcy plasmática (Födinger et al. 2001; Zetterberg 2004). Da mesma forma, o genótipo MTHFR A1298C (C:C) também resulta em diminuição da atividade da MTHFR (van Rooij et al. 2003). Este genótipo está associado com o aumento da Hcy e do risco de hipertensão em pessoas com hipertensão essencial (Markan et al. 2007).

1.3.1 Folato

O folato, também conhecido como folacina, ácido fólico ou pteroilpoliglutamato é uma vitamina hidrossolúvel do complexo B (vitamina B9) que atua como coenzima no metabolismo dos ácidos nucléicos e aminoácidos. Dessa forma, participa da regulação, biossíntese, reparo e metilação do DNA (Duthie 2011).

São fontes naturais de folato as frutas cítricas e verduras, principalmente hortaliças de folhas verde escuras como espinafre, couve e brócolis, também leguminosas (feijões) e vísceras, em especial o fígado. A fortificação mandatória de farinhas de trigo e milho com 150 µg de ácido fólico para cada 100g de grão ocorre no Brasil desde 2004 (Ministério da Saúde 2002). Dessa forma, pães, macarrão, biscoitos, e outros produtos à base de farinha de trigo e milho são fontes de folato sintético (ou ácido fólico) no Brasil (Ministério da Saúde 2002).

A política de fortificação de alimentos com ácido fólico no Brasil contribuiu significativamente para o aumento do consumo deste nutriente (Marchioni et al. 2013; Steluti et al. 2017). Comparando os períodos pré e pós fortificação mandatória, as concentrações séricas de folato aumentaram cerca de 60% entre crianças e adolescentes, e praticamente dobraram entre adultos no Brasil (Britto et al. 2014).

A recomendação do *Institute of Medicine* (IOM 1998) é de consumo de 320 µg/d de folato na fase adulta, tanto para homens quanto para mulheres (EAR – *Estimated Average Requirements*). Essa recomendação aumenta na gestação, passando a ser de 520 µg/d. A ingestão máxima tolerável (UL) é de 1000 µg/dia, porém se aplica à sua forma sintética obtida de suplementos e/ou alimentos fortificados. Sendo assim, em locais onde há alto uso de suplementos alimentares e ainda política de fortificação mandatória há uma preocupação crescente em relação aos potenciais efeitos nocivos do consumo de altas doses de ácido fólico mantido por longos períodos (Allen 2009; Duthie 2011).

1.3.2 Vitamina B6

A vitamina B6 pertence a um grupo de compostos relacionados: piridoxal, piridoxina, piridoxamina e 5'-fosfatos e atua como coenzima no metabolismo de aminoácidos, glicogênio e bases esfingóides. Está presente naturalmente nas leguminosas (feijões), carne de aves, carne vermelha, soja e farelo de trigo.

Nenhum efeito adverso associado ao alto consumo de vitamina B6 presente naturalmente em alimentos foi relatado, entretanto, neuropatia sensorial pode ocorrer por um alto consumo de vitamina B6 na forma de suplemento.

Para adultos homens e mulheres, a recomendação de consumo (EAR) é de 1,1 mg/d. Após os 50 anos essa recomendação aumenta para 1,4 mg/d entre homens e 1,3 mg/d entre mulheres. Na gestação a recomendação aumenta para 1,6 mg/d. O UL de ingestão para essas populações é de 100 mg/d (IOM, 1998).

1.3.3 Vitamina B12

A vitamina B12, também conhecida como cobalamina, atua como coenzima no metabolismo dos ácidos nucléicos. Possui papel importante na prevenção da anemia megaloblástica. Presente naturalmente em alimentos de origem animal, como carnes, ovos, peixes, aves, vísceras, leite e derivados.

A recomendação de consumo (EAR) é igual para mulheres e homens adultos, 2,0 µg/d. Para gestantes a recomendação aumenta para 2,2 µg/d. A UL não é estabelecida (IOM, 1998).

1.3.4 Ácidos Graxos Poliinsaturados (AGPI)

São observados nos tecidos de mamíferos quatro famílias de AGPI (ômegas-3, 6, 7 e 9), mas apenas as classes ômega-3 (ácido alfa-linolênico) e ômega-6 (ácido linoleico) são ácidos graxos essenciais, ou seja, não são produzidos por humanos à partir do excesso de energia da dieta (Ross et al. 2012). Ômega-6 (e seu derivado ácido araquidônico - ARA) e ômega-3 (e seu derivado ácido docosaexaenoico - DHA) desempenham papel importante nas membranas estruturais, principalmente do tecido nervoso e da retina (Ross et al. 2012). Entretanto, uma proporção elevada de ômega-6 para ômega-3, por competirem pelas enzimas necessárias para dessaturação e alongamento, provoca depleção dos ácidos graxos ômega-3 de cadeia longa, incluindo DHA (Horrocks e Yeo 1999). Além disso, o excesso de ômega-6 e, consequentemente ARA, pode estar associado ao desenvolvimento de artrite e outras doenças crônicas inflamatórias. Por outro lado, DHA apresenta um efeito positivo não somente em doenças como artrite, aterosclerose, trombose e alguns tipos de câncer, como também no desenvolvimento cognitivo (Horrocks e Yeo 1999).

A relação entre AGPI e o metabolismo do folato e homocisteína ainda não é bem estabelecida. Acredita-se que os AGPI da dieta interajam com enzimas do metabolismo do folato e homocisteína (MTHFR e MAT), aumentando suas atividades e modificando as concentrações plasmáticas de homocisteína (Huang et al. 2010, 2011a). Pessoas com genótipos MTHFR C677T (T:T) ou A1298C (A:C/CC) que consomem maiores quantidades de AGPI possuem concentrações plasmáticas mais baixas de homocisteína comparadas àquelas com o mesmo genótipo e que consumiram menores quantidades de ômega-3 (Huang et al. 2011a). Há evidências de que o aumento das concentrações de ômega-3 nos fosfolipídios plasmáticos e plaquetários está associado a um efeito protetor cardiovascular e à diminuição das concentrações plasmáticas de homocisteína (Li et al. 2006, 2007; Huang et al. 2011a). Ainda, tem sido relatado que o consumo de folato, vitaminas B6 e B12 pode influenciar a concentração plasmática de ômega-3 pela interferência na disponibilidade de grupos metil. Sendo assim, se faz fundamental considerar os AGPI, particularmente ômega-3 e DHA, na análise do metabolismo do folato e homocisteína (Li et al. 2007).

O ômega-3 está naturalmente presente em óleos vegetais, peixes (carpa, salmão, sardinha, atum, arenque, truta), semente de abóbora e gema de ovo. O IOM recomenda, como *Adequate Intake* (AI) para adultos, um consumo de ômega-3 de 1,6 g/d para homens, 1,1 para mulheres e 1,4 g/d para gestantes (IOM 2005).

1.4 RELAÇÃO ENTRE PADRÕES ALIMENTARES, NUTRIENTES DO METABOLISMO DO FOLATO E HOMOCISTEÍNA E DESFECHOS EM SAÚDE

1.4.1 Medidas Antropométricas do Recém-Nascido

Peso, comprimento e perímetro cefálico ao nascer são medidas antropométricas do recém-nascido que permitem avaliar o crescimento ocorrido no período intrauterino. Uma em cada quatro mortes neonatais no mundo são atribuídas ao baixo peso ao nascer (Lee et al. 2017). Aproximadamente 1/7 das mortes globais de crianças menores de 5 anos é atribuída à baixa estatura para idade (Black et al. 2008). A restrição no crescimento intrauterino aumenta a morbidade, mortalidade e se associa a consequências funcionais adversas, além de promover um risco aumentado para o desenvolvimento de doenças crônicas na idade adulta (Anderson e Hay 2008; Black et al. 2008; Victora et al. 2008; Lee et al. 2013; Mosites et al. 2016).

A alimentação materna é um dos mais importantes fatores associados ao crescimento intrauterino (Black et al. 2013b). Padrões alimentares maternos nomeados como prudente ou saudável estiveram associados a um menor risco, enquanto os padrões alimentares nomeados como Ocidental ou 'à base de trigo' estiveram associados a um maior risco de ter um filho pequeno para a idade gestacional (Chen et al. 2016). Há poucos estudos sobre a influência da alimentação materna no baixo comprimento ao nascer, ajustado pela idade gestacional e sexo, um indicador mais refinado do crescimento intrauterino do que peso ou comprimento ao nascer. Um estudo identificou que o padrão alimentar materno rico em gordura/açúcar/alimentos prontos para o consumo no período pré-gestacional esteve associado ao baixo comprimento ao nascer, mas essa medida não foi ajustada pela idade gestacional (Grieger et al. 2014).

Padrões alimentares maternos pobres em micronutrientes cruciais à gestação poderiam limitar o desenvolvimento e crescimento fetal (Black et al. 2013a). A orientação nutricional preconizada pelo Ministério da Saúde brasileiro direcionada ao período gestacional é qualitativa e baseada em alimentos e comportamento alimentar, mas ressalta a importância de nutrientes como ferro, cálcio e folato (Brasil 2012b).

A partir da década de 70, estudos verificaram uma forte relação entre concentrações sanguíneas diminuídas de folato e defeitos no tubo neural do feto. Com base nestes achados, foi estabelecido como protocolo em vários países a suplementação diária de 400 a 800µg/d de ácido fólico no período pré-gestacional e primeiro trimestre da gestação (Smithells et al. 1976; Blom e Smulders 2011). Além da suplementação, alguns países adotaram a fortificação mandatória de alimentos com ácido fólico com o objetivo de reduzir a incidência de má-formação do tubo neural (Atta et al. 2016). No Brasil, a recomendação do Ministério da Saúde é para suplementar todas as mulheres com 5.000µg/d de ácido fólico no período pré-gestacional e primeiro trimestre da gestação (Brasil 2012b). No entanto, esta recomendação é apenas parcialmente efetiva uma vez que a maioria das gestações não são planejadas e o uso do suplemento de ácido fólico varia muito de acordo com as características maternas.

Desde a introdução da fortificação mandatória das farinhas de trigo e milho com ácido fólico no Brasil, o consumo alimentar deste nutriente entre mulheres aumentou, mas ainda segue abaixo da recomendação (Ministério da Saúde 2002). Em 2003, 95% das mulheres adultas da cidade de São Paulo apresentavam inadequação do consumo de folato total segundo estudo de base populacional. Após a fortificação mandatória, em 2008, essa prevalência diminuiu para 38%, valor ainda alto de inadequação de consumo (Marchioni et al. 2013).

Além da diminuição da incidência dos defeitos de tubo neural, o estado nutricional adequado de folato durante a gestação se associa a um risco reduzido para outros defeitos congênitos como anomalias cardíacas e fenda palatina (Ionescu-Ittu et al. 2009; Poletta et al. 2018). Ainda, o estado nutricional materno dos micronutrientes do metabolismo do folato e homocisteína possui um importante papel na determinação do crescimento intrauterino pelas alterações epigenéticas relacionadas à esse metabolismo (Khot et al. 2015). Um estudo de revisão sistemática com metanálise (n=21.326) identificou uma chance aumentada em 25% de o filho nascer com baixo peso quando a mãe tinha concentrações elevadas de Hcy (Hogeveen et al. 2012). O *Horland Homocysteine Study* relatou uma tendência de restrição do crescimento intrauterino conforme o aumento do número de polimorfismos do gene da enzima MTHFR do metabolismo do folato e homocisteína (Nurk et al. 2004). Ainda, estudos experimentais observaram efeito benéfico da suplementação materna combinada de vitamina B12 e ômega-3 sobre desfechos gestacionais e marcadores cardiometabólicos do filho (Khaire et al. 2016), podendo estar associado também à redução do estresse oxidativo e inflamação na pré-eclâmpsia (Kemse et al. 2014). Cabe ressaltar que esses efeitos não foram observados quando os suplementos de vitamina B12 e ômega-3 foram administrados separadamente.

Até agora, há poucas pesquisas sobre padrões alimentares na gestação e sua influência no risco de dar à luz a bebês com baixo peso e / ou comprimento ao nascer (Chen et al. 2016). Assim, é importante investigar de que modo várias combinações e padrões de alimentos dentro de uma população podem afetar a prevalência de déficit no crescimento. Sabendo que o crescimento intrauterino é influenciado pela alimentação e por alterações no metabolismo do folato e homocisteína materno, acredita-se que os padrões alimentares e o consumo alimentar de folato, vitaminas B6, B12 e AGPI da gestante possam influenciar as medidas antropométricas neonatais de seu filho.

1.4.2 Infecção por HPV

Infecção pelo papilomavirus humano (HPV) é a mais comum infecção viral do trato reprodutivo (WHO 2016). A maioria das pessoas será infectada pelo HPV em algum aumento de sua vida. Entre homens, a prevalência de infecções por HPV é de cerca de 65.2% (Giuliano et al. 2008). Portanto, entender a história da infecção por HPV entre os homens é um componente essencial para a prevenção de doenças relacionadas ao vírus tanto em homens, quanto em mulheres (Giuliano et al. 2008, 2011).

A maior parte dessas infecções é assintomática e remite antes que qualquer diagnóstico seja feito. Mas a infecção persistente por tipos oncogênicos de HPV é preponderante para o desenvolvimento do câncer do colo do útero (Repp et al. 2012; Crosbie et al. 2013). O câncer do colo do útero é o terceiro tumor mais comum e a quarta causa de morte por câncer entre as mulheres no Brasil (5.727 óbitos/ano) (INCA 2017). Cânceres de ânus, vulva, vagina, pênis e orofaringe também estão relacionados à infecção por HPV (Crosbie et al. 2013). Os tipos não oncogênicos de HPV são responsáveis pelo desenvolvimento de verrugas.

Diversos fatores podem influenciar a persistência, a remissão e a progressão da infecção por HPV (IARC 2012). Embora já tenha sido reportado que alimentação pode influenciar o risco de infecção e persistência da infecção por HPV, essas evidências permanecem inconsistentes (Burchell et al. 2006; Moscicki et al. 2006; IARC 2012). Alguns estudos demonstraram efeito protetor de frutas, verduras e legumes (Rajkumar et al. 2003), vitamina C (Giuliano et al. 2003), carotenóides (Giuliano et al. 1997), tocoferóis (Giuliano et al. 1997), vitamina B12 (Sedjo et al. 2002) e folato (Weinstein et al. 2001), mas a *International Agency for Research on Cancer* (IARC) enfatiza a necessidade de mais estudos sobre a interação entre alimentação e infecção por HPV, principalmente estudos envolvendo nutrientes do metabolismo do folato e homocisteína (IARC 2012).

Há evidências de que o estado nutricional de nutrientes envolvidos na via metabólica do folato e homocisteína influencia a história natural da infecção por HPV, dada a sua relação direta com a biossíntese de ácidos nucléicos e proteínas, essenciais à produção de anticorpos e citocinas e, portanto, às funções imunológicas (Dhur et al. 1991; Hernandez et al. 2003). Um estudo prospectivo com mulheres (n=345) mostrou que aquelas com baixo status de folato tinham maior risco de adquirir e se reinfectar com HPV oncogênico do que mulheres com status adequado de folato (Piyathilake et al. 2004). A investigação dos padrões alimentares tornou-se um importante campo de pesquisa, uma vez que reproduz de forma mais acurada a interação entre os alimentos e nutrientes consumidos (USDA Evidence Analysis Library Division 2014). Entretanto, não há evidências consistentes sobre a influência dos padrões alimentares e do status de folato e outros nutrientes do metabolismo do folato e homocisteína na história da infecção por HPV em homens (IARC 2012; Flatley et al. 2014).

Acredita-se que os padrões alimentares e o consumo de nutrientes envolvidos no metabolismo do folato e homocisteína como folato, vitaminas B6, B12 e AGPI pode influenciar a história da infecção por HPV e o desenvolvimento de doenças relacionadas ao vírus (Bailey e Gregory 1999; Choi e Mason 2000; Sedjo et al. 2002).

1.4.3 Concentrações de Homocisteína

Concentrações elevadas de homocisteína tem sido associadas à doenças cardiovasculares e cerebrovasculares (Kim et al. 2016), como aterosclerose, doença de Alzheimer e demência (Selhub 2008). O mecanismo biologicamente plausível para o efeito da Hcy no desenvolvimento dessas doenças consistiria na sua auto oxidação e geração de peróxido de hidrogênio, tendo como consequência a lesão celular, levando ao efeito trombogênico e proliferação de células musculares lisas nos vasos, com efeito aterogênico (Hankey e Eikelboom 1999; Sharma et al. 2014). Entretanto, ainda não há consenso em que medida a Hcy é um fator de risco ou um biomarcador nas relações apresentadas acima (Selhub 2008).

Entre as causas da hiperhomocisteinemia (HHcy) estão fatores genéticos e a alimentação. Além das vitaminas do complexo B (folato, vitaminas B6 e B12), recentemente tem sido investigado o papel dos AGPI, em especial o ômega-3, na diminuição da Hcy plasmática. O consumo aumentado de AGPI está relacionado ao aumento da concentração de AGPI em fosfolipídios plasmáticos. Esse fator tem um efeito protetor contra as DCV e HHcy (Piolot et al. 2003; Li et al. 2006; Zeman et al. 2006; Pooya et al. 2010). Estudo experimental sugere que os AGPI podem exercer uma regulação sobre a expressão genética e sobre a atividade de enzimas do metabolismo do folato e homocisteína (Huang et al. 2010). O uso de suplementos de vitaminas do complexo B e ômega-3 tem se mostrado eficaz na redução das concentrações de Hcy (Huang et al. 2011b; Martí-Carvajal et al. 2015), entretanto não há evidência de que essa redução de fato diminua a mortalidade (Li et al. 2015; Martí-Carvajal et al. 2015).

Evidências maiores são encontradas sobre o efeito protetor do consumo regular de frutas, verduras e legumes, cereais integrais, oleaginosas, leite e derivados com teor reduzido de gordura e peixes e do efeito danoso do consumo regular de carnes vermelhas e processadas, alimentos e bebidas adoçadas sobre a saúde cardiovascular (USDA Evidence Analysis Library Division 2014). Esses achados suportam a hipótese de que um padrão alimentar variado e saudável, onde há uma combinação de micronutrientes e compostos bioativos não encontrada em suplementos alimentares, é que, de fato, pode estar associado a desfechos mais favoráveis em saúde, como, por exemplo, concentrações adequadas de Hcy (Jacobs e Steffen 2003).

A interação entre Hcy, nutrientes e variantes polimórficas envolvidos no metabolismo do folato e homocisteína têm sido mais extensamente avaliada em relação às vitaminas B6, B12 e folato. O estudo dos padrões alimentares e da interação entre AGPI e este metabolismo permanece inadequadamente descrita (Huang et al. 2011a). Dessa forma, a relação entre padrões alimentares, folato, vitaminas B6 e B12 e concentrações de homocisteína pode ser melhor elucidada se for considerada nessa interação polimorfismos envolvidos no metabolismo do folato e homocisteína e os AGPI.

1.5 JUSTIFICATIVA

Os padrões alimentares e os nutrientes do metabolismo do folato e homocisteína podem estar associados à desfechos importantes em saúde, como medidas antropométricas do recém-nascido, infecção por HPV e concentrações de homocisteína. Entretanto, em virtude das lacunas na literatura, mais estudos são necessários para que essas relações sejam claramente estabelecidas e auxiliem na formulação de políticas públicas em alimentação e nutrição.

2 OBJETIVOS

1 – Investigar a relação entre padrões alimentares e consumo de folato, vitamina B6, vitamina B12 e AGPI de gestantes e medidas antropométricas neonatais de seus filhos, como marcador do crescimento intrauterino, em uma coorte de gestantes da cidade de São Paulo, SP (Estudo ProcriAr).

2 – Investigar a relação entre padrões alimentares e consumo de folato, vitamina B6, vitamina B12 e AGPI e a duração da infecção por HPV em um estudo de coorte com homens da cidade de São Paulo, SP (Estudo HIM).

3 – Investigar a relação entre padrões alimentares, estado nutricional de folato, vitamina B12, e AGPI e concentrações de homocisteína, intermediados por uma variante polimórfica do metabolismo do folato e homocisteína em um estudo transversal com adultos da cidade de São Paulo, SP (Estudo ISA-Capital 2008).

3 MÉTODOS

Serão utilizados dados dos estudos ProcriAr, HIM e ISA-Capital 2008 para identificar os padrões alimentares e os nutrientes do metabolismo do folato e homocisteína associados à medidas antropométricas do recém-nascido, infecção por HPV e concentrações de homocisteína, respectivamente. Para a apresentação dos métodos utilizados em cada um dos três estudos, o *Strengthening the Reporting of Observational Studies in Epidemiology* (STROBE) foi utilizado como guia (von Elm et al. 2007).

3.1 MEDIDAS ANTROPOMÉTRICAS DO RECÉM-NASCIDO - ESTUDO PROCRIAR

Tabela 2. Características do estudo ProcriAr

	ProcriAr
	Influência dos fatores nutricionais e poluentes atmosféricos urbanos na saúde pulmonar de crianças: um estudo de coorte em gestantes da zona oeste do município de São Paulo
Objetivo do estudo	Avaliar a influência de fatores nutricionais e poluentes atmosféricos urbanos na saúde respiratória das crianças
Localização	São Paulo, Brasil
Desenho do estudo	Coorte
Tipo de amostra	Conveniência
Definição do tamanho amostral	O tamanho amostral foi calculado para detectar uma alteração de ≥ 5% nos parâmetros funcionais pulmonares com um poder de estudo ≥ 80%, resultando em um tamanho de amostra requerido de 400 indivíduos
Tamanho amostral	454
Período de recrutamento	Março/2011 à Dezembro/2013
Locais ou fontes de recrutamento	Quatro unidades básicas de saúde pertencentes ao Distrito de Saúde Escola do Butantã (localizadas na região oeste da cidade de São Paulo)
Critérios de elegibilidade	Mulheres com teste positivo para gravidez: feto único, idade gestacional < 14 semanas (estimada pela data da última menstruação e confirmada por ultrassonografia) e ausência de doenças crônicas pré-existentes
Período de seguimento	Até 1 ano de idade da criança
Momento da primeira entrevista	Primeiro trimestre da gestação (idade gestacional media: 10.7 semanas, min. 6, máx. 16 semanas)
Método utilizado na primeira entrevista	Entrevista domiciliar
Dado utilizado	Primeira entrevista (*baseline*) e dados do parto
Tipo de análise	Transversal
Variáveis (dados disponíveis)	Consumo alimentar: n=454. Comprimento ao nascer: n=299

Tabela 3. Variáveis do estudo ProcriAr consideradas no presente trabalho

	ProcriAr
	Influência dos fatores nutricionais e poluentes atmosféricos urbanos na saúde pulmonar de crianças: um estudo de coorte em gestantes da zona oeste do município de São Paulo
<u>Variáveis dietéticas (exposição)</u>	
Consumo alimentar	QFA com 110 itens foi utilizado para avaliar o consumo alimentar pré-gestacional (12 meses anteriores a data da entrevista). "*Com que frequência você costuma comer esse item*?". Os participantes poderiam responder de 0 a 10 vezes por dia/semana/mês/ano. O número de porções consumidas por pessoa por dia (g/dia) foi calculado dividindo a frequência de ingestão (1-10) pelo número de dias (1, 7, 30 ou 365) para todos os itens. A base de dados do software *Nutrition Data System for Research* foi utilizada para calcular o consumo de energia e nutrientes.
Padrões alimentares	Os 110 itens do QFA foram classificados em 51 grupos de alimentos. Os padrões alimentares foram derivados por análise fatorial por componentes principais. Foram derivados quatro padrões alimentares, representando 25,5% da variância na ingestão alimentar: "Lentilhas, grãos integrais e sopas", "*Snacks*, sanduíches, doces e refrigerantes", "Legumes temperados e carnes magras", e "Sucos adoçados, pão e manteiga, arroz e feijão".
Nutrientes avaliados (consumo alimentar)	Energia, carboidrato, proteína, gordura, álcool, cafeína, sódio, ferro, cálcio, vitamina D, ácido docosaexaenoico (DHA), equivalentes dietéticos de folato (DFE), folato natural, folato sintético (ácido fólico), metionina, colina, betaína e vitaminas B6 e B12.
<u>Desfechos (definição)</u>	Informações sobre sexo, peso e comprimento ao nascer da criança foram compiladas dos prontuários hospitalares. Recém-nascidos com peso e/ou comprimento ao nascer, ajustado por sexo e idade gestacional, abaixo do percentil 10 (INTERGROWTH-21st) foram considerados pequenos ao nascer, sendo este um indicador de restrição de crescimento intrauterino neste estudo.
<u>Covariáveis (número de *missings*)</u>	Idade, escolaridade (2), emprego, raça (3), região de nascimento, status de relacionamento (1), estado nutricional (índice de massa corporal), uso de suplementos dietéticos, história familiar de hipertensão (4) e/ou diabetes (3) (mãe e/ou pai), tabagismo (1), comportamento sedentário (12), e paridade (2)

3.1.1 Análise dos Dados

Variáveis sociodemográficas (covariáveis) foram descritas de acordo com a adesão das mulheres aos padrões alimentares (quintis). Testes qui-quadrado foram utilizados para a identificação de diferenças entre os grupos. Modelos de regressão linear foram utilizados para o estudo dos fatores sociodemográficos (variáveis independentes) associados aos padrões alimentares (variável dependente).

Coeficientes de correlação de Spearman foram calculados para avaliar correlações entre os padrões alimentares e o consumo de energia e nutrientes e apresentados em gráfico de radar. Modelos multivariados de regressão linear foram utilizados para investigar as associações entre a ingestão de energia e nutrientes (variáveis contínuas) e os escores de padrões alimentares (variáveis contínuas). Neste caso, os modelos foram ajustados pelas características maternas (covariáveis).

Modelos multivariados de regressão de Poisson foram utilizados para avaliar a associação entre os padrões alimentares das mulheres (escores em quintis, variável independente) e ter um filho com peso e/ou comprimento ao nascer abaixo do percentil 10, ajustado por sexo e idade gestacional (INTERGROWTH-21st), independentemente das características maternas (covariáveis).

O pacote estatístico Stata® (versão 12.0, 2011, StataCorp LP, College Station, TX) foi utilizado para todas as análises (StataCorp 2011). $P<0.05$ foi considerado estatisticamente significante.

3.2 INFECÇÃO POR HPV - ESTUDO HIM

Tabela 4. Características do estudo HIM

	HIM *HPV Infection in Men*
Objetivo do estudo	Compreender a história natural das infecções por HPV em homens
Localização	São Paulo, Brasil
Desenho do estudo	Coorte
Tipo de amostra	Conveniência
Definição do tamanho amostral	Estipulado para resultar em estimativas precisas de incidência e persistência de infecções por HPV e poder estatístico apropriado para calcular os fatores de risco independentes para persistência ou remissão de tais infecções. Foi antecipado que 80% dos homens que comparecessem à consulta de inserção iriam retornar para a consulta inicial e que 80% dos homens que comparecessem à consulta inicial iriam completar os quatro anos de estudo, resultando em um tamanho de amostra de 3.000 homens (1.000 homens por sítio)
Tamanho amostral	1.412
Período de recrutamento	Julho/2005 à Setembro/2009
Locais ou fontes de recrutamento	Clientes de um Centro de Referência e Treinamento DST/AIDS em São Paulo e população em geral recrutada por publicidade veiculada em várias instituições e meios de comunicação
Critérios de elegibilidade	Homens com idades entre 18 e 70 anos, residentes em São Paulo, sem diagnóstico anterior de câncer de pênis ou de ânus, que não apresentaram diagnóstico de verrugas genitais ou anais no momento da inclusão no estudo, não portadores de HIV ou diagnóstico de AIDS e dispostos a comparecer às consultas do estudo. Foram excluídos os indivíduos com diagnóstico de DST no momento da inclusão no estudo, exceto infecção por HPV
Período de seguimento	4 anos
Momento da primeira entrevista	Julho/2005 à Setembro/2009
Método utilizado na primeira entrevista	Questionário eletrônico auto preenchido (presencial)
Dado utilizado	Visita 2 (*baseline*) à visita 6 (5 medidas)
Tipo de análise	Longitudinal
Variáveis (dados disponíveis)	n=1.194

Tabela 5. Variáveis do estudo HIM consideradas no presente trabalho

	HIM *HPV Infection in Men*
Variáveis dietéticas (exposição)	
Consumo alimentar	QFA validado com 54 itens foi utilizado para avaliar o consumo alimentar no ano anterior às visitas 2 e 6. "*Com que frequência você costuma comer esse item*?". Os participantes poderiam responder de 0 a 10 vezes por dia/semana/mês/ano. O número de porções consumidas por pessoa por dia (g/dia) foi calculado dividindo a frequência de ingestão (1-10) pelo número de dias (1, 7, 30 ou 365) para todos os itens. A média dos dois QFA representou o consumo usual dos participantes. A base de dados do software *Nutrition Data System for Research* foi utilizada para calcular o consumo de energia e nutrientes.
Padrões alimentares	Os 54 itens do QFA foram classificados em 41 grupos de alimentos. Os padrões alimentares foram derivados por *reduced rank regression* (RRR). Os nutrientes DHA, vitamina B6, vitamina B12 e DFE foram as variáveis resposta escolhidas. Foram derivados quatro padrões alimentares, representando 13,0% da variância na ingestão alimentar e 87,5% da variância na ingestão dos nutrientes selecionados.
Nutrientes avaliados (consumo alimentar)	DHA, vitamina B6, vitamina B12 e DFE
Desfechos (definição)	Para cada resultado positivo para infecção por HPV foram considerados 6 meses de duração da infecção (intervalo entre as visitas do estudo). Como foram feitas 5 medidas de infecção por HPV, a duração de qualquer tipo de infecção por HPV variou entre 0 e 30 meses.
Covariáveis (número de *missings*)	Idade, raça (13), escolaridade (11), renda familiar (72), status de relacionamento (8), tabagismo (11), estado nutricional (índice de massa corporal), circuncisão (2), número de parcerias sexuais ao longo da vida (9), número de parceiras sexuais nos últimos 3 meses (665), número de parceiros sexuais nos últimos 3 meses (6).

3.2.1 Análise dos Dados

Coeficientes de correlação de Spearman foram usados para investigar as correlações entre padrões alimentares e os nutrientes DFE, vitamina B6, vitamina B12 e DHA. Medianas (intervalo de confiança de 95%) desses nutrientes e dos cinco principais itens alimentares (gramas) com associações direta e inversa com os padrões alimentares foram apresentadas de acordo com o tercil dos escores dos padrões alimentares. Regressões lineares univariadas foram utilizadas para avaliar tendência entre os tercis dos padrões alimentares.

Regressões logísticas univariadas foram usadas para testar associações significativas entre fatores sociodemográficos e estilo de vida (variáveis independentes) e adesão aos padrões alimentares (variável dependente, comparando terceiro com o primeiro tercil, sendo o terceiro tercil o grupo de referência).

Regressões lineares multivariadas foram utilizadas para identificar a associação entre a duração da infecção pelo HPV (variável dependente) e os escores dos padrões alimentares (variáveis independentes, em tercis). As variáveis: raça, escolaridade, renda familiar, estado civil, tabagismo, estado nutricional, circuncisão, número de parceiras sexuais nos últimos 3 meses e número de parceiros sexuais nos últimos 3 meses foram selecionadas para serem testadas nos modelos multivariados. Realizou-se análise de regressão linear univariada e, das variáveis acima, as que apresentaram $p < 0,20$ foram selecionadas para os modelos multivariados. O modelo final para cada padrão alimentar foi estabelecido com uma seleção *forward-stepwise*. Idade, número total de parceiras sexuais e status de HPV no início do estudo foram mantidos nos modelos finais por essas covariáveis serem fatores importantes na epidemiologia da infecção pelo HPV.(6) Além disso, todos os modelos foram ajustados pelo consumo total de energia.

O SAS Studio (SAS Institute) foi utilizado para a análise RRR. As outras análises foram realizadas utilizando o Stata Statistical Software (versão 12 de 2011, StataCorp LP, College Station, TX) (StataCorp 2011). $P <0,05$ foi considerado estatisticamente significante.

3.3 CONCENTRAÇÕES DE HOMOCISTEÍNA - ESTUDO ISA-CAPITAL 2008

Tabela 6. Características do estudo ISA-Capital 2008

	ISA-Capital 2008
	Inquérito de Saúde de base populacional no Município de São Paulo
Objetivo do estudo	Conhecer os aspectos e monitorar as condições de saúde da população paulistana ao longo do tempo e contribuir na avaliação do impacto das políticas de saúde
Localização	São Paulo, Brasil
Desenho do estudo	Transversal
Tipo de amostra	Amostragem aleatória probabilística em dois estágios, setores censitários e domicílios.
Definição do tamanho amostral	Para estimar uma prevalência de 0,5 com erro de 0,07, nível de confiança de 95% e efeito de delineamento de 1,5, foi considerado um tamanho amostral de 600 homens e mulheres adultos (20-59 anos).
Tamanho amostral	1,162
Período de recrutamento	Setembro/2008 à Agosto/2009
Locais ou fontes de recrutamento	Indivíduos residentes em domicílios particulares na área urbana da cidade de São Paulo.
Critérios de elegibilidade	Indivíduos que participaram da segunda fase da coleta de dados (entre 2010 e 2011), com amostra sanguínea e medidas antropométricas (entrevista domiciliar), além de QFA (entrevista telefônica).
Período de seguimento	-
Momento da primeira entrevista	2008 a 2009
Método utilizado na primeira entrevista	Entrevista domiciliar
Dado utilizado	Primeira entrevista (no domicílio; dados sociodemográficos) e segunda fase (por telefone; dieta e dados bioquímicos)
Tipo de análise	Transversal
Variáveis (dados disponíveis)	n=281

Tabela 7. Variáveis do estudo ISA-Capital 2008 consideradas no presente trabalho

	ISA-Capital 2008 Inquérito de Saúde de base populacional no Município de São Paulo
Variáveis dietéticas (exposição)	
Consumo alimentar	QFA com 38 itens foi utilizado para avaliar a frequência do consumo alimentar no ano anterior à entrevista. "*Com que frequência você costuma comer esse item*?". Os participantes poderiam responder de 0 a 10 vezes por dia/semana/mês/ano. Frequência de consumo por pessoa por dia foi calculada dividindo a frequência de ingestão (1-10) pelo número de dias (1, 7, 30 ou 365) para todos os itens
Padrões alimentares	Após a exclusão de 6 itens do QFA por não apresentarem correlação com nenhum outro item (matriz de correlação) ou por não contribuírem com nenhum padrão alimentar, 32 itens do QFA foram utilizados para derivar os padrões alimentares por análise fatorial por componentes principais. Foram derivados três padrões alimentares: "Moderno", “Tradicional” e “Prudente”, representando 26,0% da variância na ingestão alimentar
Nutrientes avaliados (concentrações bioquímicas)	Folato total, vitamina B12 e DHA
Desfechos (definição)	Concentrações bioquímicas de homocisteína
Covariáveis (número de *missings*)	Sexo, idade, escolaridade (3), raça, tabagismo, consumo de álcool, estado nutricional (índice de massa corporal) (2), atividade física (1) e polimorfismo MTHFR C677T

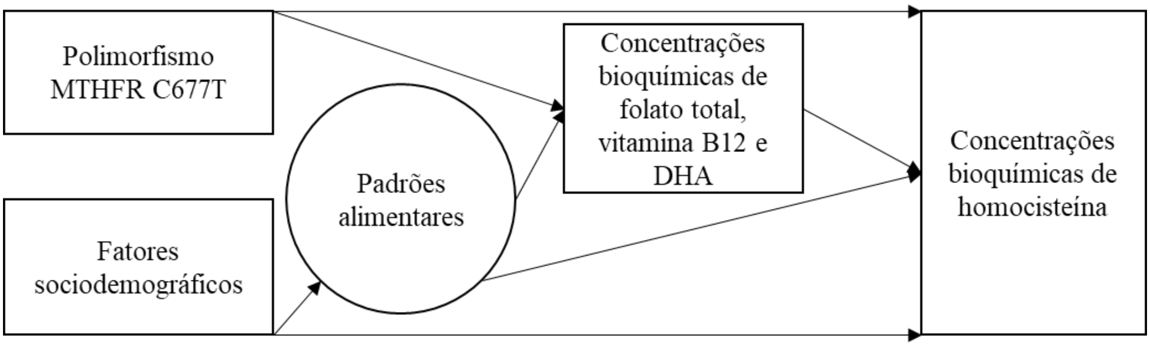
3.3.1 Análise dos Dados

Concentração de homocisteína (µmol / mL) foi categorizada em tercil de acordo com o sexo. O terceiro tercil foi considerado indicador de risco cardiovascular aumentado em relação ao primeiro e segundo tercis. Para identificar diferenças significativas entre grupos com diferentes concentrações de Hcy (tercis 1/2 *vs* tercil 3) de acordo com fatores sociodemográficos e de estilo de vida, estado nutricional, MTHFR C677T polimorfismo, vitaminas e concentrações de DHA foram utilizados os testes de Kruskal-Wallis (variáveis contínuas) e qui-quadrado (variáveis categóricas).

A população foi categorizada estando acima ou abaixo das concentrações bioquímicas médias de folato total e DHA. Teste Kruskal-Wallis foi utilizado para identificar diferenças nas concentrações de homocisteína (contínuo) de acordo com as concentrações de folato e DHA.

Foi utilizado modelo de equações estruturais para testar quais nutrientes do metabolismo do folato e homocisteína (folato total, vitamina B12 e DHA) estariam associados à concentração de homocisteína; a associação de DHA com concentração de homocisteína; e quão distante é a relação entre os padrões alimentares e as concentrações de homocisteína e em qual medida essa relação é mediada por folato total, vitamina B12 e DHA. O modelo de equações estruturais considerou um modelo teórico onde os padrões alimentares estavam diretamente associados às concentrações de homocisteína, e indiretamente através de concentrações bioquímicas de folato, vitamina B12 e DHA (**Figura 2**). As variáveis sexo, idade, etnia e polimorfismo MTHFR C677T foram incluídas no modelo.

Figura 2. Modelo teórico sobre as relações entre as variáveis de exposição, covariáveis e variável desfecho no estudo ISA-Capital 2008



MTHFR: metilenotetrahidrofolato redutase; DHA: ácido docosaexaenoico

Todas as variáveis contínuas foram padronizadas (escores dos padrões alimentares, folato total, vitamina B12, DHA, homocisteína e idade). O coeficiente padronizado expressa o efeito de um preditor sobre a variável desfecho em unidades de desvio padrão. Isso permite comparações entre os efeitos de preditores que usam diferentes escalas.

O pacote estatístico Stata® (versão 12.0, 2011, StataCorp LP, College Station, TX) foi utilizado para todas as análises (StataCorp 2011), com exceção do modelo de equações estruturais, derivado no software R (R Core Team 2016) usando o pacote Lavaan (Yves Rosseel 2012). $P<0.05$ foi considerado estatisticamente significante.

3.4 ASPECTOS ÉTICOS

Os três estudos principais foram aprovados por seus respectivos Comitês de Ética (ProcriAr - Comitê de Ética em Pesquisa da Prefeitura do Município de São Paulo e do Hospital das Clínicas da Faculdade de Medicina da USP; HIM – Comitê de Ética do Centro de Referência e Treinamento DST/AIDS; ISA-Capital 2008 – Comitê de Ética da Secretaria Municipal de Saúde de São Paulo e Faculdade de Saúde Pública da USP). Todos os participantes assinaram Termo de Consentimento Livre e Esclarecido. O projeto desta tese foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Saúde Pública da Universidade de São Paulo (Parecer nº 1.501.677) (**ANEXO 1**).

4 RESULTADOS E DISCUSSÃO

Os resultados e discussão deste estudo são apresentados em forma de quatro manuscritos. O primeiro manuscrito buscou identificar os padrões alimentares pré-gestacionais de mulheres do estudo ProcriAr e investigar os fatores sociodemográficos e nutrientes associados a esses padrões. O segundo manuscrito avaliou a associação dos padrões alimentares com ter um filho pequeno ao nascer, também no estudo ProcriAr. O terceiro teve como objetivo investigar as relações entre os padrões alimentares e consumo alimentar de folato total, vitaminas B6, B12 e DHA de homens do estudo HIM e duração da infecção por HPV. O quarto manuscrito buscou testar relações múltiplas e simultâneas entre fatores sociodemográficos, padrões alimentares, concentrações bioquímicas de folato, vitamina B12 e DHA e seus efeitos na concentração de homocisteína no estudo ISA-Capital 2008.

<u>Primeiro Manuscrito:</u>

Teixeira JA, Castro TG, Grant CC, Wall CR, Castro ALS, Francisco RPV, Vieira SE, Saldiva SRDM, Marchioni DM. *Dietary patterns are influenced by socio-demographic conditions of women in childbearing age: a cohort study of pregnant women.* BMC Public Health. 2018 Mar 1;18(1):301. doi: 10.1186/s12889-018-5184-4.

Segundo Manuscrito:

Teixeira JA, Hoffman DJ, Castro TG, Francisco RPV, Vieira SE, Saldiva SRDM, Marchioni DM. *Maternal dietary pattern is associated with newborn size: results from ProcriAr study*. (Submetido em periódico internacional)

Terceiro Manuscrito:

Teixeira JA, Baggio ML, Villa LL, Giuliano AR, Fisberg RM, Marchioni DM. *Dietary pattern derived using reduced rank regression is associated with duration of HPV infection in men: evidence from HIM Study.*

Quarto Manuscrito:

Teixeira JA, Alencar GP, Steluti J, Gorgulho BM, Carioca AA, Fisberg RM, Marchioni DM. *Dietary pattern influences homocysteine level more than specific nutrients: a structural equation model approach.* (Sob revisão em periódico internacional)

4.1 PRIMEIRO MANUSCRITO - *DIETARY PATTERNS ARE INFLUENCED BY SOCIO-DEMOGRAPHIC CONDITIONS OF WOMEN IN CHILDBEARING AGE: A COHORT STUDY OF PREGNANT WOMEN*

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Abstract

Background: Background: Women's health during their reproductive years and whilst pregnant has implications for their children's health, both in utero and during childhood. Associations of women's pre-pregnancy dietary patterns (DP) with maternal socio-demographic characteristics and nutrient intake were investigated in ProcriAr cohort study in São Paulo/Brazil, 2012. **Methods**: The DPs of 454 women were investigated by principal component factor analysis, using dietary information from a validated 110-item food frequency questionnaire. Multiple linear regression models identified independent associations between DPs and maternal socio-demographic characteristics and Spearman's correlation determined associations between DPs and nutrients intake. **Results**: Participants' mean age was 26.1 years (standard deviation = 6.3), 10.3% had more than 8 years of formal education, 30% were migrants from outside of the Southeast of Brazil, 48% were employed, 13% were smokers, and 51% were overweight/obese. Four DPs were derived: ‘Lentils, whole grains and soups,’ ‘Snacks, sandwiches, sweets and soft drinks,’ ‘Seasoned vegetables and lean meats,’ and ‘Sweetened juices, bread and butter, rice and beans’. The ‘Lentils, whole grains and soups’ score was positively related to maternal age, being non-smoker and born in the South, North or Midwest of Brazil. The ‘Snacks, sandwiches, sweets and soft drinks’ score was positively related to higher maternal education, and negatively related to age, lack of formal work and being born in the Northeast region. The ‘Seasoned vegetables and lean meats’ score was positively related to higher maternal education. The ‘Sweetened juices, bread and butter, rice and beans’ score was positively related to unemployment and to no family history of hypertension, and negatively related to maternal overweight and obesity. Dietary intake of fruits and vegetables, foods that require preparation, nutrients from one-carbon metabolism, protein, iron, calcium and vitamin D were correlated with the ‘Seasoned vegetables and lean meats’. Dietary intake of sugar-sweetened and alcoholic beverages, processed and takeaway foods, and foods rich in sugar, energy, fat, and synthetic folate were correlated with the ‘Snacks, sandwiches, sweets and soft drinks’. **Conclusions**: Findings from this study add perspectives to be considered in the implementation of health interventions, which could improve women’s nutritional status and provide an adequate environment for the developing fetus.

Keywords: Dietary pattern, Principal component analysis, Socioeconomic factors, Childbearing age, Epidemiology, Public health nutrition

Background

Women’s health during the reproductive years and particularly during pregnancy is an important determinant of their children’s health, both in utero and during childhood.[1] Inadequate maternal nutrition during pregnancy is associated with reduced fetal growth and increased risk of respiratory disease in early childhood and then, later in life, cardiovascular diseases, type 2 diabetes, obesity and osteoporosis.[2–6] A focus on nutrition as a component of preconception care is recognized as essential if this care is to promote the health of the mother and to optimize fetal development. Thus, it is necessary to understand how social, demographic and behavioral factors of women of childbearing age can influence their broader dietary intake patterns as well as their intake of specific foods and nutrients.[7] Dietary patterns can be derived by using different methods, including numerical indexes aimed to measure adherence to specific patterns (e.g., Mediterranean Diet, Healthy Eating Index, or a nutritional guideline) or data-driven methods that use mathematics to empirically derive dietary patterns within the study population (e.g., cluster or factor analysis).[8] Principal component factor analysis takes into account the cumulative and interactive diets' aspects and, thus, generates data that better reproduce the actual dietary consumption rather than the description of specific foods and nutrients intake.[8] This method reflects food components interactions and improves the capacity to investigate the effects of diet on health.[8]

To date, most of the nutritional recommendations concerning healthy pregnancy outcomes relate to specific foods, food groups or nutrients, making it difficult to translate appropriate dietary advice for non-dietitian health care professionals and for the women to whom advice is being given.[9] There is a need for nutritional interventions based on patterns of dietary intake but currently there is a lack of studies in developing countries that used this holistic approach. The aim of this study was to identify the dietary patterns of pregnant women from ProcriAr study during the pre-pregnancy period using principal component factor analysis, and to investigate the socio-demographic factors and nutrients associated with these patterns.

Methods

The ProcriAr Cohort Study

The present study used data from ProcriAr study (The Influence of Nutritional Factors and Urban Air Pollutants on Children's Respiratory Health: A Cohort Study in Pregnant Women), which was conducted in the west region of São Paulo – Southeast, Brazil.[10,11] As lung function in infants was the principal outcome, the sample size was calculated to detect a change of ≥ 5% in pulmonary functional parameters with a study power of ≥ 80%,[12] resulting in a required sample size of 400 individuals. Recruitment occurred between March 2011 and December 2013 in four primary health care units. During home visits, all women with positive pregnancy tests who met the eligibility criteria (single fetus, gestational age <14 weeks and absence of pre-existing chronic diseases) were invited by Community Health Agents to take part of the study. Gestational age was estimated based on the last menstrual period and confirmed by ultrasonography performed in the first trimester of pregnancy. Of the

619 women with positive pregnancy tests, a sample of 454 met the eligibility criteria, provided Informed Consent Form and completed all the questionnaires (Figure 1).

Food intake assessment

A validated 110-item quantitative food frequency questionnaire (FFQ) was used to assess the pre-pregnancy food intake of the population.[13,14] The interviewer-administered FFQ assessed the dietary intake of women for the previous 12 months that is the usual dietary intake prior to becoming pregnant. Frequency of intake of each food item over the pre-pregnancy period and the portion size typically eaten (small, medium or large) were asked during the first home visit, when most of the women were in the first trimester of pregnancy (mean gestational age: 10.7 weeks, range: 6-16 weeks). Foods and recipes listed in the FFQ were converted into grams using Brazilian specific tables and manuals.[15] Daily intakes were calculated by multiplying the portion size by the frequency of intake (1-10) and dividing by the days (daily-1, weekly-7, monthly-30 or yearly-365).

The Nutrition Data System for Research software version 2.0 (2007) (NDSR), developed by the Nutrition Coordinating Centre, University of Minnesota, Minneapolis, MN was used to calculate the dietary intake of energy and nutrients. The nutritional information of the NDSR software is based on data from food composition databases published by the United States Department for Agriculture (USDA). The food contents of NDSR were compared with the Brazilian food composition tables, ensuring that the foods used had an 80-120% match between tables for energy and macronutrients.[16]

Daily intake of energy, carbohydrate, protein, fat, alcohol, caffeine, sodium, iron, calcium, vitamin D, docosahexaenoic acid (DHA), dietary folate equivalents (DFE), natural folate, synthetic folate (from fortified foods), methionine, choline, betaine, and vitamins B6 and B12 were analyzed, since those nutrients are part of the nutritional recommendation for a healthy pregnancy.[9,17–20]

Assessment of socio-demographic and lifestyle factors

The choice of the socio-demographic and lifestyle factors that could influence the dietary pattern of pregnant women was based on previous studies that addressed the determinants of dietary intake among adult population,[21,22] and, also, based on the data collected in the first home visit of the ProcriAr study, through face-to-face interviews. Therefore, age, education, work status, ethnicity, region of birth, relationship status, nutritional status, dietary supplements use, family history of disease (mother or father), smoking habits and sedentary behavior represented the socio-demographic and lifestyle factors considered in this study.[21]

The two primary indicators of socioeconomic status (education - as accumulation of schooling - and income) are reportedly associated with one another and also associated with health and disease [23,24]. Because in ProcriAr study 20% of the participants answered that they did not know or did not want to inform their family income, in this study only education was analyzed as a proxy for socioeconomic status.

Weight (kilograms) and height (meters) were measured according to World Health Organization (WHO) protocol.[25] Body mass index (BMI) was calculated and categorized according to WHO criteria, generating the variable nutritional status variable.[26]

Statistical analyses

Dietary patterns were estimated using principal component factor analysis and were based on the average daily amount of intake derived from the FFQ food items. Low-fat milk, skim milk, butter/margarine light, unsweetened coffee, unsweetened tea and diet/light soda were consumed by less than 5% of the population and were not included in the analysis. The grouping scheme was based on the correlations between food items and composition similarities, resulting in 51 food items, which were included in the dietary pattern analysis. Food items, grouping description, frequency of intake, and the daily amount of intake for each item were presented in Supplement Table 1.

To identify the number of dietary patterns to be retained, the eigenvalue > 1.0 criterion was used, retaining seventeen factors with low interpretability.[27] The Scree test analysis and the interpretation of each factor were considered, resulting in four dietary patterns for further analyses. Varimax orthogonal rotation was performed to simplify the factor matrix and to facilitate data interpretation by generating nonrelated factors. Rotated factor loadings > 0.25 or < -0.25 were considered to significantly contribute to a pattern.[8,28,29] We excluded the items fruit smoothies, sweetened coffee, farofa, cassava or corn (flour), offal, pasta with meatless sauce and vinaigrette from the final analysis because they did not load on any of the retained factors. The highest factor loadings were considered when identifying a name for each of the dietary patterns.

The dietary pattern scores were divided into quintiles. Socio-demographic and lifestyle factors were described according to the maternal adherence to a dietary pattern (lower adherence: 1st quintile; higher adherence: 5th quintile). Chi-square tests were used to determine if there were any significant differences between the groups of women classified in the 1st versus 5th quintile for a specific dietary pattern.

Associations between the component scores of each dietary pattern and the covariates were tested in multivariate linear regression models. Age was used in years; underweight, overweight and obese were defined in relation to normal weight using a dummy variable for nutritional status. Northeast and other regions (South, North and Midwest) were defined in relation to Southeast using a dummy variable for region of birth. The following variables were treated as dichotomous (yes or no): eight or more years of education, white skin, dietary supplements use, lacking of formal work, having a partner, no family history of hypertension, no family history of diabetes, not currently smoking and watching 2 or more hours of TV/day. Each model was adjusted for the other dietary patterns and also for the primary health care unit. The assumed linear relationship between the variables was evaluated using residual plots.

Spearman's correlation coefficients (r_s) were calculated for testing associations between the dietary patterns (factors) and the nutrients intake. As the orthogonal rotation of all patterns ensured that they were uncorrelated, the sum of the squared correlations between absolute nutrient availability and the factor scores could be interpreted as the proportion of variance of the nutrient intake explained by the patterns.[30] A radar chart was generated with energy and nutrients represented on the axes to visualize the correlations established with the dietary patterns.

All analyses were performed using Stata Statistical Software (release 12, 2011, StataCorp LP, College Station, TX).[31] Two-sided significance was determined at $P<0.05$.

Results

Four dietary patterns were retained in the principal component factor analysis and accounted for 25.5% of the variance in food intake. The patterns were named ‘Lentils, whole grains and soups,’ ‘Snacks, sandwiches, sweets and soft drinks,’ ‘Seasoned vegetables and lean meats,’ and ‘Sweetened juices, bread and butter, rice and beans’ (Table 1).

The mean age of the women was 26.1 years (Standard deviation (SD) 6.3; range 14-49), and 51% were overweight/obese. Only 10.3% (n=45) had more than 8 years of formal education, the majority was non-white (n=271, 60%), and 30% (n=135) were migrants from outside of the Southeast region. Almost half of them (n=220, 48%) were formally working, and the majority had elementary occupations, such as store attendant, saleswoman, cashier and housekeeper. Forty-one percent (n=186) and 15% (n=68) had a family history of hypertension and diabetes, respectively. Forty-two percent (n=184) spent 2 or more hours/day watching TV and 13% (n=61) were current smokers (Table 2).

The women who adhered more to the ‘Lentils, whole grains and soups’ dietary pattern were older, had higher level of education and were born outside of the Southeast region, when compared with the women with low adherence to this pattern. Those who adhered more to the ‘Snacks, sandwiches, sweets and soft drinks’ dietary pattern were younger, had higher level of education and were born outside of the Northeast region. Higher adherence to the dietary pattern ‘Seasoned vegetables and lean meats’ was more frequent among those who belonged to a specific primary health care unit, and lower adherence to this pattern was verified among single women and with a formal work. Higher adherence to the ‘Sweetened juices, bread and butter, rice and beans’ dietary pattern was more frequent among underweight or normal weight women and with no family history of hypertension (Table 2).

Age, being born in the South, North or Midwest of Brazil and not being a current smoker were positively associated to the ‘Lentils, whole grains and soups’ score. Higher level of education was positively associated to the ‘Snacks, sandwiches, sweets and soft drinks’ score, while age, lack of formal work and being born in the Northeast region of Brazil were negatively associated to this score. Higher level of education was also positively associated to the ‘Seasoned vegetables and lean meats’ score. Lack of formal work was positively associated to the ‘Sweetened juices, bread and butter, rice and beans’ score, as well as no family history of hypertension. Overweight and obesity were negatively associated to the ‘Sweetened juices, bread and butter, rice and beans’ score (Table 3). The residual plots indicated that the assumed linear relationships between the variables were acceptable for all the four multivariate models (data not presented).

Most of the nutrients investigated were more strongly correlated to ‘Seasoned vegetables and lean meats’ than to the other patterns. In descending order of correlation, these nutrients were natural folate (rs=0.51), vitamin B6 (0.41), iron (0.41), choline (0.38), DFE (0.38), protein (0.37), sodium (0.36), methionine (0.35), calcium (0.34), vitamin B12 (0.31), carbohydrate (0.30), vitamin D (0.28) and caffeine (0.20). ‘Lentils, whole grains and soups’ had higher correlation coefficients with DHA (0.32) and betaine (0.30). Total fat (0.45) was more strongly associated with ‘Snacks, sandwiches, sweets and soft drinks’, as were alcohol (0.41), energy (0.39) and synthetic folate (0.32). Nevertheless, the four retained patterns accounted for a relatively low proportion of the variance of the studied nutrients, ranging from 27.6% (sodium) to 3.5% (caffeine) (Figure 2).

Discussion

This study of women’s pre-pregnancy dietary intake revealed four dietary patterns namely ‘Lentils, whole grains and soups,’ ‘Snacks, sandwiches, sweets and soft drinks,’ ‘Seasoned vegetables and lean meats,’ and ‘Sweetened juices, bread and butter, rice and beans.’ Maternal age, education, work status, region of birth, nutritional status, family history of hypertension and smoking were the factors associated with the dietary patterns. Fruits and vegetables, foods that require preparation, protein, carbohydrate, nutrients related to one-carbon metabolism pathway, DHA, vitamin D, iron, sodium, calcium and caffeine were more strongly correlated to the ‘Seasoned vegetables and lean meats,’ which was the dietary pattern that could contribute most to a healthy pre-pregnancy nutrition,[9,17–20] followed by ‘Lentils, whole grains and soups’ pattern. ‘Snacks, sandwiches, sweets and soft drinks’ pattern was composed of sugar-sweetened and alcoholic beverages, processed and takeaway foods, and foods rich in sugar, energy, fat, and synthetic folate, and could be considered an unhealthy dietary pattern.[8,32–35]

Dietary patterns analyses consider the totality of a diet and enables the data collected from observational studies to be translated into descriptions of eating behaviors that can inform public health guidelines and recommendations.[36] In this study, the variance in food intake explained by the dietary patterns was similar to the variance explained by the dietary patterns of a group of 327 pregnant women from the Rio de Janeiro, Brazil (25.3%).[37] The Brazilian study used an 81-item FFQ to evaluate the dietary intake during pregnancy and identified the following three dietary patterns: ‘healthy’ (legumes, vegetables and fruits), ‘mixed’ (candy, butter and margarine, and snacks) and ‘traditional’ (beans and rice).[37] The explained variances found in the dietary pattern analyses in both studies have been well accepted in the field of nutritional epidemiology.[37–39] A study review on 54 papers describing maternal dietary patterns and pregnancy outcomes verified that the patterns were frequently classified as prudent or healthy (healthful, health conscious, fruit and low-fat dairy, cooked vegetables, high-protein/fruit, Mediterranean), traditional (common-Brazilian, Nordic, Southern), or as Western or processed (meats/snacks/sweets, high-fat/sugar/takeaway, junk, snack). The majority of the studies identified by the mentioned review study used FFQs to measure the dietary intake and applied principal component analysis to derive the dietary patterns.[20]

Higher adherence to dietary patterns consisting of discretionary food items in the pre-pregnancy period has been linked with negative outcomes for both mother and child,[20] including maternal uncontrolled asthma,[38] gestational diabetes mellitus,[39] preterm delivery,[40] earlier gestation and shorter birth length.[40] However, these same kind of pre-pregnancy dietary patterns have not been shown to be associated with hypertension[41] nor depressive symptoms[32] in pregnancy, nor with early fetal growth[33] emphasizing the necessity for more studies in this field.[34]

Older women appeared to adhere more to a healthy dietary pattern peri-conceptionally and during pregnancy.[37,42] With increasing age, women from ProcriAr study adhered more to the ‘Lentils, whole grains and soups’ dietary pattern. In contrast, a higher score on the ‘Snacks, sandwiches, sweets and soft drinks’ pattern was associated with being younger. Despite the low levels of education reported by this population, higher education was associated to the dietary patterns ‘Snacks, sandwiches, sweets and soft drinks’ and ‘Seasoned vegetables and lean meats’, which are patterns that included more expensive foods. Studies in Brazil have shown that the proportion of consumption of food groups such as milk and dairy, fruits and vegetables, animal fats, processed meats, alcoholic beverages, soft drinks and ready meals tends to increase consistently with the level of household income, demonstrating the mixed effects of education and income in determining food intake.[43,44]

Migrants typically move to achieve better living conditions, but in Brazil important socioeconomic differences persist when migrants are compared with the native population[45] and are likely to reflect in dietary behavior.[46] Brazil is geopolitically divided into five regions: North, Northeast, Central-West, Southeast and South. The Southeast and South regions are the first and second economies of Brazil, followed by Central-West, Northeast, and North. São Paulo is part of the Southeast region of Brazil, and is considered the most populous city with the largest economy by gross domestic products in the Southern Hemisphere. Almost 30% of the population in this study was not born in Southeast Brazil. Being born in the South, North or Midwest of Brazil was positively related to ‘Lentils, whole grains and soups' pattern. In contrast, being born in the Northeast region of Brazil was negatively related to the 'Snacks, sandwiches, sweets and soft drinks' pattern, demonstrating the poorer socioeconomic conditions and/or the persistence of eating habits acquired in one's region of birth. In fact, previous study has identified that, for all nine states of the Northeast region of Brazil, the prevalence of fruits and vegetables intake was below the observed national prevalence (average of 26% in Northeast versus 37% in Brazil – including Northeast).[47]

Unemployment was associated with a dietary pattern composed mostly by foods that require preparation ('Sweetened juices, bread and butter, rice and beans'). In contrast, employment was related to the pattern rich in fast foods and takeaway foods ('Snacks, sandwiches, sweets and soft drinks'). Being employed is an important determinant of food-related decisions.[48] Van der Horst & Siegrist[48] found correlations between cooking and working status, with workers spending less time cooking and reporting fewer cooking skills. In ProcriAr study, despite of the fact that the unemployed women cooked more than those who were employed, 61% of them watched TV for 2 or more hours/day. In contrast, only 22% of the women formally working had the same sedentary behavior (Chi-square test, $p<0.001$, unpublished results).

Socio-demographic and lifestyle factors were determinants of the women's pre-pregnancy dietary patterns in this study, suggesting that extra resources may be necessary for disadvantaged mothers to ensure good nutrition during pregnancy.[49,50] These results highlight that attention should be prioritized to young employed women of low socioeconomic status, and who are born in urban and highly industrialized regions. Women with these characteristics were more likely to have an unhealthy dietary habit [44,51–53], and this knowledge should be considered in an individualized antenatal care.

The Brazilian health system consists of a range of public and private organizations, and people can use both depending on ease of access or their ability to pay.[54] Therefore, socioeconomic inequalities exist between individuals that use the public or the private sectors.[54] Those women who have their antenatal care in the public health service are more likely to be poor, when compared with those who have their antenatal care in the private sector.[55] The women in this study were recruited from public primary health care units, and represent a population from a lower socioeconomic area, with a low level of education and the majority of whom had elementary occupations. This population characteristic can be observed in several pre-conceptional women's settings in Brazil, and the evidence of this study may be applicable to those settings.[55] The coverage of antenatal care in Brazil is high, and the majority of visits are made to public primary health care units (89.6%).[56] This represents a window of opportunity to interventions in food and nutrition, since lifestyle characteristics are prone to change in association with pregnancy.[20,56] A special focus on diet within the antenatal care framework could have a greater impact on maternal and child health, and at a lower cost, than strategies based on postnatal therapy to those with health issues that occurred as a consequence of the pregnancy.[57]

Individuals and environmental interventions could be implemented during the antenatal care,[7,58] such as encouraging the involvement of the whole family in meals planning and preparation,[59] understanding which are the barriers for cooking and eating more fruit and vegetables (working with participative troubleshooting models),[60] encouraging the intake of seasonal fruit and vegetables, implementing garden-based fruit and vegetables intervention, negotiating the establishment of food markets with local producers,[61,62] and offering culinary workshops.[63] However, to be more effective, nutritional interventions should approach the complex set of dietary behaviors determinants, such as women's social and material resources, social and cultural environment, psychosocial factors, and accessibility of food.[64–66].

This study highlights the relevance and application of the investigation of food patterns and their association with socio-demographic factors during the peri-conceptional period. Additionally, the topic of dietary patterns is a growing area of research that is relevant to nutrition policies and programs.[8] However, the use of factor analysis has been criticized for its subjective nature, including the consolidation of food items into food groups, the number of factors to be extracted and the methods of rotation and labelling.[8] In order to improve the assessment, interpretability and comparability of our results, we have addressed in this manuscript all the items suggested by the STROBE guidelines.[67] A potential limitation of this study is the use of a FFQ as a tool to evaluate the dietary intake, which is a method that rely on memory and recall bias are more likely to occur. The use of foreign food composition tables to estimate the population dietary intake can be also considered a limitation of this study. Unfortunately, the Brazilian food composition tables have few nutrients analyzed, which makes it difficult to use them in dietary intake studies. Yet, the lack of detailed information about the dietary supplements taken (such as brands and quantities) could be underestimating the dietary intake of micronutrients. However, the use of dietary supplements was very low in this population and was not associated with any of the dietary patterns. Still, dietary intake is the product of different factors and interactions, many of which could not be accomplished in this study. Thus, future research are needed in order to fully investigate the relationship between the social determinants of health and women's dietary patterns.[68] Although ProcriAr study was a cohort study, our study used a cross-sectional analysis. A subsequent research is planned to investigate whether the maternal dietary patterns identified here are related to better or worse outcomes in pregnancy and in the children's health, both in utero and later in life.

Conclusions

The dietary pattern analysis led to a better understanding of the pre-pregnancy eating behaviors and their determinant factors among women of childbearing age in ProcriAr study. The analysis of pre-pregnancy food intake produced four distinctive dietary patterns. The 'Snacks, sandwiches, sweets and soft drinks' dietary pattern (composed of sugar-sweetened and alcoholic beverages, processed and takeaway foods, and foods rich in sugar, energy, fat, and synthetic folate) was associated with being younger, more educated, formally employed and born in the Southeast region of Brazil. Based on its food and drink contents, this dietary pattern could be considered the unhealthiest eating behavior for pregnancy. As women's health is a public health priority, the findings of this study add perspectives to be considered in the implementation of health promotion practices and interventions that will enable the improvement of women's nutritional status and provide an adequate environment for a healthy fetal development.

Figure 1. Description of the sample selection, ProcriAr study – São Paulo/Brazil, 2012

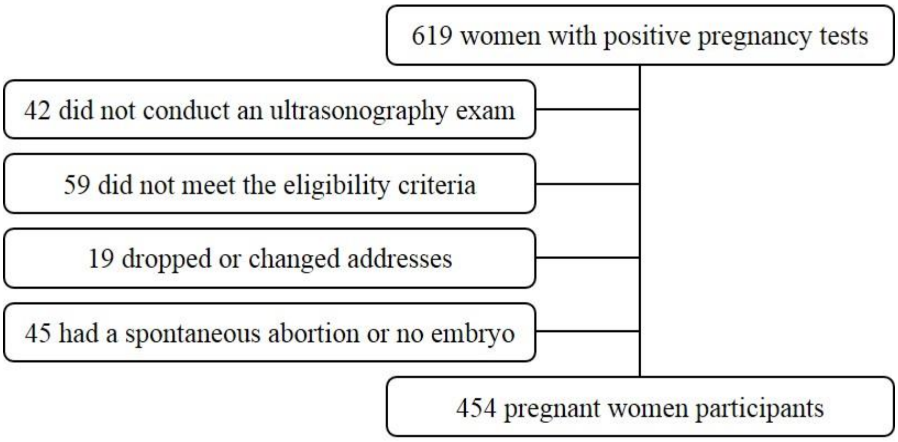


Table 1. Pre-pregnancy dietary patterns for women, ProcriAr study (n = 454) - São Paulo/Brazil, 2012.

	Lentils, whole grains and soups	**Snacks, sandwiches, sweets and soft drinks**	**Seasoned vegetables and lean meats**	**Sweetened juices, bread and butter, rice and beans**
Food items[a]	**Rotated factor loadings**			
Lentils	**0.54**	0.01	0.15	-0.10
Wheat bread and brown rice	**0.51**	-0.10	-0.03	-0.04
Soups	**0.47**	0.01	0.21	0.07
Popcorn	**0.47**	0.17	0.04	0.09
Cereal ready to eat and Oats	**0.46**	0.02	0.16	0.04
White cheese	**0.44**	0.07	0.08	-0.01
Desserts with fruits and jelly	**0.44**	0.00	0.23	-0.09
Simple cakes	**0.41**	0.06	0.01	0.13
Soy beverages	**0.41**	-0.02	0.11	0.00
Beef jerky	**0.40**	0.13	-0.06	0.08
Nuts	**0.40**	0.23	0.00	-0.05
Crackers	**0.35**	0.09	-0.01	-0.03
Soy sauce	**0.35**	0.08	0.10	-0.02
Tea (sweetened)	**0.30**	0.00	0.19	-0.02
Beef (roasted, cooked or soaked)	**0.29**	0.22	0.00	-0.07
Processed meats, sandwiches and snacks	-0.05	**0.59**	0.04	-0.03
Sandwich sauces	0.07	**0.54**	-0.04	-0.09
Desserts and sweets	0.19	**0.50**	-0.05	0.06
Soft drinks	-0.14	**0.48**	-0.16	0.10
Pasta with meat sauce and gnocchi	0.13	**0.44**	0.16	-0.02
Stuffed pasta (cannelloni, lasagne)	**0.32**	**0.39**	0.02	0.05
Yogurt with flavour (whole milk)	0.06	**0.39**	0.03	-0.19
Pork and Frankfurters	-0.01	**0.39**	0.06	0.09
Bakery with filling (cake and cookies)	0.12	**0.38**	-0.09	0.19
Fried beef and fried chicken	-0.09	**0.38**	0.10	0.13
Fried egg or omelette	0.14	**0.36**	0.12	**0.33**
Potato salad, with vegetables and mayonnaise	0.03	**0.36**	**0.27**	0.14
Alcoholic beverages (beer, wine and *caipirinha*[b])	0.03	**0.35**	-0.02	**-0.29**
Chocolate milk (powder)	0.01	**0.33**	0.03	0.25
Feijoada[c]	**0.31**	**0.32**	0.05	0.12
Potato or cassava (fried)	-0.06	**0.32**	-0.08	**0.30**
Mozzarella cheese	0.22	**0.30**	0.20	-0.04
Vegetables	0.20	-0.05	**0.69**	-0.11
Oil (for salad dressing)	-0.03	0.04	**0.67**	-0.05
Salt	-0.07	0.00	**0.66**	-0.03
Lean meats and fish	0.25	0.00	**0.53**	0.02
Potato or cassava (boiled or roasted)	0.21	0.13	**0.39**	0.06
Fruits	**0.29**	-0.09	**0.31**	-0.07
Sweetened juices (natural or artificial)[d]	0.08	-0.02	-0.13	**0.70**
Butter or margarine (regular and salted)	-0.19	0.17	0.19	**0.46**
French bread and white rice	**-0.27**	0.11	**0.32**	**0.39**
Beans	-0.01	0.09	0.23	**0.36**
Whole milk (3.5-4% fat)	-0.07	0.04	0.16	**0.36**
Yogurt (whole milk)	**0.26**	**-0.32**	0.05	**0.33**
Unsweetened juices (natural or artificial)	0.04	0.04	**0.29**	**-0.59**
Percentage of variance explained (%)	**9.9**	**6.6**	**4.7**	**4.3**

In bold are the rotated factor loadings > 0.25 or < -0.25. [a]The food items fruit smoothies, sweetened coffee, *farofa*, cassava or corn (flour), offal, pasta with meatless sauce, and vinaigrette were excluded from this analysis because they did not load on any of the retained factors. Traditional recipes: [b]*Caipirinha* - a drink made with *cachaça* (a hard liquor from sugar cane), fresh limes, sugar and ice; [c]*Feijoada*: black bean stew. [d]Natural juices are made with fresh fruits or frozen fruit pulps, with the addition of water or not. [e]Artificial juices are artificial powdered drink mixes, fruit nectars, or sweetened processed juice.

Table 2. Socio-demographic and lifestyle characteristics of pregnant women according to their dietary patterns, ProcriAr study (n = 454) - São Paulo/Brazil, 2012

Characteristics	Total	Lentils, whole grains and soups		Snacks, sandwiches, sweets and soft drinks		Seasoned vegetables and lean meats		Sweetened juices, bread and butter, rice and beans	
		Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Years of age, mean (SD)	26.1 (6.3)	23.9 (5.7)	29.1 (5.7)***	27.3 (6.8)	25.1 (5.7)*	25.3 (6.7)	26.5 (6.3)	26.8 (5.4)	25.9 (6.6)
Years of formal education									
≤ 5	111 (24.6)	33 (36.3)	11 (12.2)***	31 (34.1)	18 (20.0)*	26 (28.6)	19 (21.1)	23 (25.3)	29 (32.6)
6 - 7	96 (21.2)	20 (22.0)	18 (20.0)	24 (26.4)	17 (18.9)	22 (24.2)	14 (15.6)	18 (19.8)	19 (21.4)
≥ 8	245 (54.2)	38 (41.8)	61 (67.8)	36 (39.6)	55 (61.1)	43 (47.3)	57 (63.3)	50 (55.0)	41 (46.1)
Ethnicity[a]									
Parda[b]	216 (47.9)	45 (50.0)	41 (45.6)	49 (53.9)	44 (48.9)	41 (45.1)	45 (50.0)	45 (50.0)	44 (48.9)
White	180 (39.9)	37 (41.1)	39 (43.3)	31 (34.1)	32 (35.6)	39 (42.9)	36 (40.0)	35 (38.9)	40 (44.4)
Black	53 (11.8)	8 (8.9)	8 (8.9)	10 (11.0)	14 (15.6)	10 (11.0)	9 (10.0)	10 (11.1)	6 (6.7)
Other[c]	2 (0.4)	0 (0.0)	2 (2.2)	1 (1.1)	0 (0.0)	1 (1.1)	0 (0)	0 (0.0)	1 (0.0)
Relationship status									
Married or in common law marriage	271 (59.8)	46 (50.6)	60 (67.4)	60 (65.9)	46 (51.1)	45 (49.5)	58 (64.4)*	59 (65.6)	52 (57.8)
Single	178 (39.3)	45 (49.5)	28 (31.5)	30 (33.0)	43 (47.8)	46 (50.6)	30 (33.3)	30 (33.3)	38 (42.2)
Divorced or widower	4 (0.9)	0 (0.0)	1 (1.1)	1 (1.1)	1 (1.1)	0 (0.0)	2 (2.2)	1 (1.1)	0 (0.0)
Region of birth									
Southeast	319 (70.3)	73 (80.2)	57 (63.3)*	47 (51.7)	67 (74.4)**	58 (63.7)	66 (73.3)	59 (64.8)	61 (67.8)
Northeast	122 (26.9)	18 (19.8)	28 (31.1)	42 (46.2)	18 (20.0)	31 (34.1)	19 (21.1)	29 (31.9)	25 (27.8)
Other[d]	13 (2.8)	0 (0.0)	5 (5.6)	2 (2.2)	5 (5.6)	2 (2.2)	5 (5.6)	3 (3.3)	4 (4.4)

Table 2. Socio-demographic and lifestyle characteristics of pregnant women according to their dietary patterns, ProcriAr study (n = 454) - São Paulo/Brazil, 2012 (continued)

Characteristics	Total	Lentils, whole grains and soups		Snacks, sandwiches, sweets and soft drinks		Seasoned vegetables and lean meats		Sweetened juices, bread and butter, rice and beans	
		Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Nutritional status									
Underweight	15 (3.3)	3 (3.3)	3 (3.3)	2 (2.2)	4 (4.4)	4 (4.4)	2 (2.2)	1 (1.1)	5 (5.6)*
Normal weight	210 (46.3)	45 (49.5)	34 (37.8)	36 (39.6)	37 (41.1)	45 (49.5)	45 (50.0)	32 (35.2)	46 (51.1)
Overweight	143 (31.5)	30 (33.0)	32 (35.6)	41 (45.1)	30 (33.3)	25 (27.5)	31 (34.4)	34 (37.4)	24 (26.7)
Obese	86 (18.9)	13 (14.3)	21 (23.3)	12 (13.2)	19 (21.1)	17 (18.7)	12 (13.3)	24 (26.4)	15 (16.7)
Dietary supplements use	19 (4.2)	6 (6.6)	3 (3.3)	2 (2.2)	3 (3.3)	3 (3.3)	3 (3.3)	3 (3.3)	3 (3.3)
No formal work	234 (51.5)	57 (62.6)	39 (43.3)	51 (56.0)	35 (38.9)*	40 (44.0)	53 (58.9)*	39 (42.9)	50 (55.6)
Family history of disease (mother or father)									
No family history of hypertension	264 (58.7)	57 (64.0)	47 (52.2)	52 (57.1)	51 (58.0)	55 (61.1)	50 (56.2)	40 (44.0)	58 (64.4)**
No family history of diabetes	383 (84.9)	79 (87.8)	74 (82.2)	75 (82.4)	79 (89.8)	76 (84.4)	75 (83.3)	73 (80.2)	75 (83.3)
Not a current smoker	392 (86.5)	65 (72.2)	85 (94.4)	84 (92.3)	74 (83.2)	81 (90.0)	80 (88.9)	75 (82.4)	79 (87.8)
≥ 2 hours/day watching TV	184 (41.6)	46 (52.9)	22 (25.3)	35 (38.9)	38 (43.7)	33 (38.4)	37 (43.0)	40 (44.9)	31 (34.8)
Primary health care unit[e]									
1	185 (40.7)	36 (39.6)	42 (46.7)	42 (46.2)	33 (36.7)	48 (52.7)	32 (35.6)**	33 (36.3)	38 (42.2)
2	213 (46.9)	45 (49.4)	41 (45.5)	39 (42.9)	43 (47.8)	30 (33.0)	50 (55.5)	48 (52.7)	38 (42.2)
3	43 (9.5)	8 (8.8)	5 (5.6)	9 (9.9)	9 (10.0)	9 (9.9)	7 (7.8)	8 (8.8)	13 (14.5)
4	13 (2.9)	2 (2.2)	2 (2.2)	1 (1.1)	5 (5.5)	4 (4.4)	1 (1.1)	2 (2.2)	1 (1.1)

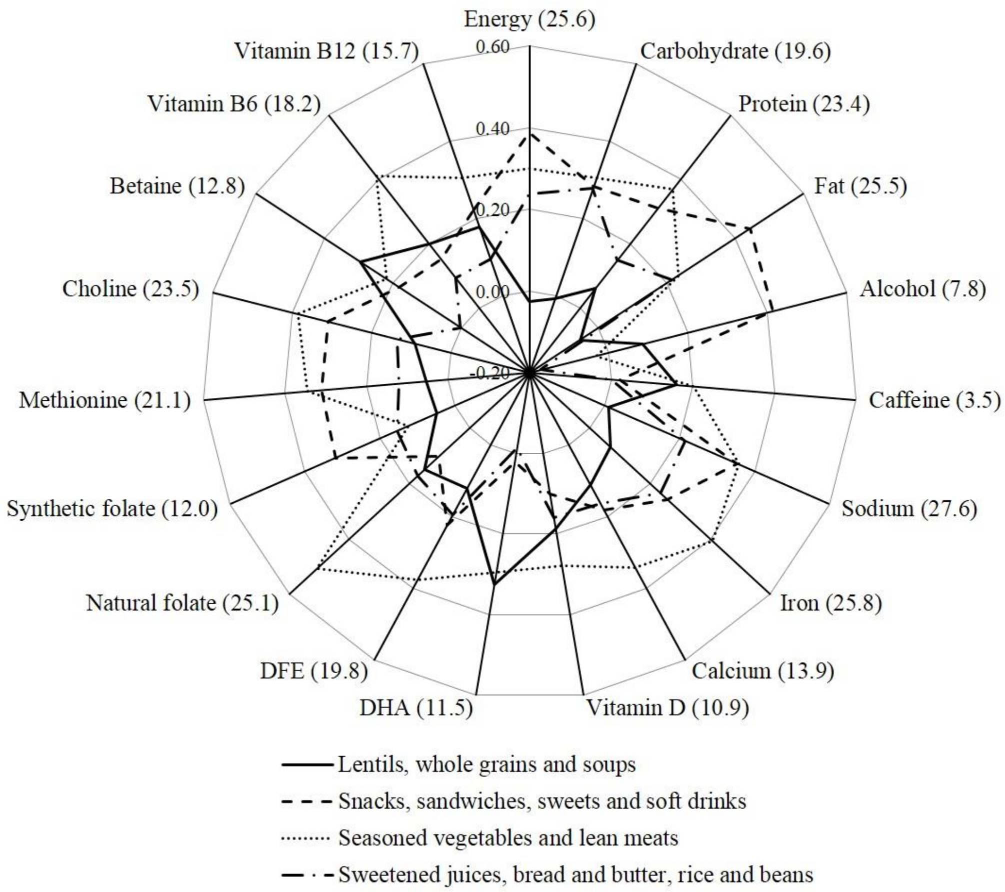
Q1: 1st quintile; Q5: 5th quintile. Chi-square tests were used to determine if there were any significant differences between the 1st and the 5th quintile of each dietary pattern score regards to the women's characteristics *p<0.05, **p<0.01, ***p<0.001. Missing number of cases for: Years of formal education (2), Ethnicity (3), Relationship status (1), Family history of hypertension (4), Family history of diabetes (3), Smoking (1), and Hours/day watching TV (12). [a]Based on self-reported skin colour. [b]*Parda* ethnicity means a mixed-ethnicity, brown skin. [c]Other in Ethnicity includes Asian and Indigenous population. [d]Other in Region of Birth includes South, North and Midwest of Brazil. [e]Located in the District of Butantã, West region of Sao Paulo city.

Table 3 Association between socio-demographic factors and pre-pregnancy dietary patterns of women, ProcriAr study (n = 454) - São Paulo/Brazil, 2012

Socio-demographic and lifestyle characteristics	Lentils, whole grains and soups[a]				Snacks, sandwiches, sweets and soft drinks				Seasoned vegetables and lean meats				Sweetened juices, bread and butter, rice and beans			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	βi	95%CI	βi	95%CI	βi	95%CI	βi	95%CI	βi	95%CI	βi	95%CI	βi	95%CI	βi	95%CI
Age (years)	0.05	0.03;0.06	**0.04**	**0.03;0.06**	-0.02	-0.04;-0.01	**-0.02**	**-0.04;-0.01**	0.02	0.00;0.03	0.01	0.00;0.03	-0.01	-0.03;0.00	-0.01	-0.03;0.01
≥8 years of education	0.29	0.11;0.47	0.08	-0.10;0.27	0.26	0.07;0.44	**0.27**	**0.07;0.46**	0.22	0.03;0.40	**0.21**	**0.01;0.41**	-0.07	-0.25;0.12	-0.03	-0.23;0.17
White skin (ethnicity)[b]	0.08	-0.11;0.26	0.06	-0.13;0.24	0.02	-0.17;0.21	0.03	-0.16;0.22	0.00	-0.19;0.19	-0.08	-0.28;0.11	0.07	-0.12;0.26	0.06	-0.13;0.25
Having a partner	0.22	0.04;0.41	-0.04	-0.23;0.16	-0.17	-0.35;0.02	-0.07	-0.28;0.14	0.24	0.06;0.43	0.19	-0.02;0.40	-0.04	-0.22;0.15	0.10	-0.11;0.31
Region of birth																
Southeast	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
Northeast	0.29	0.08;0.49	0.14	-0.07;0.36	-0.44	-0.65;-0.24	**-0.31**	**-0.53;-0.09**	-0.12	-0.33;0.09	-0.17	-0.40;0.06	-0.10	-0.31;0.11	-0.10	-0.33;0.12
Other[c]	0.73	0.18;1.28	**0.55**	**0.03;1.07**	0.24	-0.30;0.79	0.31	-0.24;0.85	0.29	-0.26;0.85	0.22	-0.34;0.79	0.06	-0.50;0.61	-0.04	-0.59;0.52
Nutritional status																
Underweight	0.09	-0.43;0.62	-0.04	-0.55;0.47	0.09	-0.43;0.61	0.12	-0.41;0.66	-0.22	-0.74;0.31	-0.14	-0.68;0.41	0.25	-0.27;0.77	0.13	-0.41;0.67
Normal weight	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-
Overweight	0.08	-0.14;0.29	-0.01	-0.22;0.19	-0.24	-0.45;-0.03	-0.13	-0.35;0.08	0.08	-0.13;0.30	0.07	-0.16;0.29	-0.26	-0.47;-0.05	**-0.24**	**-0.46;-0.03**
Obese	0.28	0.03;0.53	0.22	-0.02;0.47	0.02	-0.23;0.27	0.02	-0.24;0.28	-0.08	-0.33;0.17	-0.17	-0.44;0.10	-0.43	-0.67;-0.18	**-0.39**	**-0.65;-0.13**
Dietary supplements use	-0.20	-0.66;0.26	-0.19	-0.62;0.24	0.06	-0.40;0.52	0.18	-0.27;0.63	0.03	-0.43;0.49	-0.05	-0.52;0.41	0.13	-0.33;0.59	0.14	-0.31;0.60
No formal work	-0.21	-0.39;-0.03	-0.03	-0.22;0.17	-0.24	-0.43;-0.06	**-0.40**	**-0.60;-0.20**	0.15	-0.04;0.33	0.19	-0.03;0.40	0.22	0.04;0.40	**0.25**	**0.04;0.46**
No family history HT	-0.16	-0.35;0.03	-0.02	-0.22;0.18	0.04	-0.15;0.23	-0.06	-0.26;0.15	-0.09	-0.28;0.09	-0.08	-0.29;0.14	0.34	0.15;0.52	**0.33**	**0.12;0.53**
No family history DM	-0.23	-0.49;0.03	0.00	-0.26;0.26	0.13	-0.12;0.39	0.17	-0.11;0.44	-0.04	-0.30;0.22	-0.01	-0.29;0.27	0.09	-0.17;0.35	-0.11	-0.39;0.16
Not a current smoker	0.49	0.23;0.76	**0.46**	**0.19;0.72**	-0.12	-0.39;0.15	-0.14	-0.41;0.14	-0.06	-0.34;0.21	-0.07	-0.35;0.22	0.17	-0.10;0.44	0.16	-0.12;0.44
≥ 2 hours/day of TV	-0.27	-0.46;-0.09	-0.13	-0.32;0.07	0.05	-0.14;0.24	0.16	-0.04;0.37	0.02	-0.17;0.21	-0.03	-0.24;0.18	-0.05	-0.24;0.14	-0.11	-0.32;0.10

95%CI - 95% confidence interval; DM – Diabetes Mellitus; HT - Hypertension. Statistically significant β is presented in bold. Missing number of cases for: Years of formal education (2), Ethnicity (based on self-reported skin colour) (3), Relationship status (1), Family history of hypertension (4), Family history of diabetes (3), Smoking (1), and Hours/day watching TV (12). [a]Each dietary pattern regression model was adjusted by the others and also by the primary health care unit. Interactions not included in this model. [b]White skin compared to non-white skin (*parda*, black and other ethnicities). [c]Other in Region of birth includes South, North and Midwest of Brazil.

Figure 2. Radar graph of the correlations[a] (total % of explained variance[b]) between energy and nutrients and the dietary patterns, ProcriAr Study (n = 454) - São Paulo/Brazil, 2012



DHA - docosahexaenoic acid; DFE - dietary folate equivalents. [a]Correlation coefficients ≥0.09 or ≤-0.09 were significantly different from 0 (P ≤ 0.05). [b]The variance proportion of energy and nutrient intake explained by the patterns is presented in parentheses.

Supplement Table 1. Food items, grouping description, frequency of intake and daily amount of intake of women in the pre-pregnancy period, ProcriAr Study (n=454) - São Paulo/Brazil, 2012.

Food items	**Grouping description**	**Intake (%)**	**Daily amount of intake (g/day)**	
			Median	**95% CI**
Whole milk (3.5-4% fat)	FFQ	80.0	128.9	113.2;128.9
Fruit smoothie (whole milk)	FFQ	62.1	8.3	8.3;16.6
Yogurt (whole milk)	FFQ	25.5	0.0	0.0;0.0
Yogurt with flavour (whole milk)	FFQ	62.8	14.2	11.0;23.7
Cereal ready to eat and Oats	'Cereal ready to eat', 'oats'	44.5	0.0	0.0;0.0
Mozzarella cheese	FFQ	74.2	5.7	5.7;5.7
White cheese	FFQ	38.1	0.0	0.0;0.0
Crackers	FFQ	80.6	10.8	10.8;11.1
Simple cakes	FFQ	72.5	6.7	4.0;8.6
Bakery with filling (cake and cookies)	'Cake with filling', 'cookies with filling'	85.2	8.6	7.5;10.2
Butter or margarine (regular and salted)	FFQ	89.9	11.6	7.7;13.5
Coffee (sweetened)	FFQ	66.7	81.1	60.1;81.1
Tea (sweetened)	FFQ	44.9	0.0	0.0;0.0
Chocolate milk (powder)	FFQ	66.3	3.6	3.3;3.6
Wheat bread and brown rice	'Wheat bread', 'brown rice'	26.9	0.0	0.0;0.0
French bread and white rice	'French bread', 'white rice'	100.0	269.4	249.2;286.0
Potato or cassava (boiled or roasted)	FFQ	91.0	20.0	18.4;20.0
Potato or cassava (fried)	FFQ	81.5	34.0	34.0;34.0
Farofa[a], cassava or corn (flour)	FFQ	61.2	2.5	1.1;3.6
Potato salad, with vegetables and mayonnaise	FFQ	73.6	3.0	3.0;4.9
Beans	FFQ	94.9	104.8	86.0;104.8
Lentils	FFQ	28.8	0.0	0.0;0.0
Feijoada[b]	FFQ	66.3	7.0	3.1;7.0
Lean meats and fish	'Beef with vegetables', 'roasted, cooked or soaked chicken', 'boiled egg', 'roasted, cooked or soaked fish', 'fried fish'	98.9	61.3	56.3;66.8
Beef (roasted, cooked or soaked)	FFQ	85.7	24.5	17.1;28.6
Pork and Frankfurters	'Bacon', 'pork sausage', 'frankfurters', 'pork (loin and chops)'	94.9	23.6	22.2;28.1
Fried beef and fried chicken	'Fried beef', 'fried chicken'	92.1	49.3	41.1;56.0
Fried egg or omelette	FFQ	76.4	7.1	7.1;7.1
Beef jerky	FFQ	33.9	0.0	0.0;0.0

Supplement Table 1. (continue) Food items, grouping description, frequency of intake and daily amount of intake of women in the pre-pregnancy period, ProcriAr Study (n=454) - São Paulo/Brazil, 2012.

Food items	**Grouping description**	**Intake (%)**	**Daily amount of intake (g/day)**	
			Median	**95% CI**
Offal (heart and liver)	FFQ	33.5	0.0	0.0;0.0
Pasta with meat sauce and gnocchi	FFQ	75.1	11.0	7.3;12.1
Stuffed pasta (cannelloni, lasagne)	FFQ	78.8	4.8	4.8;6.3
Pasta with meatless sauce	FFQ	78.4	28.6	20.0;28.6
Soups	‘Cream soup’, ‘vegetable soup’	70.9	12.6	7.0;14.1
Fruits	‘Orange’, ‘banana’, ‘apple’, ‘papaya’, ‘melon’, ‘pineapple’, ‘mango’, ‘avocado’, ‘guava’, ‘persimmon’, ‘grape’	99.8	304.8	279.0;325.7
Vegetables	‘Lettuce’, ‘spinach’, ‘watercress’, ‘kale’, ‘cabbage’, ‘cauliflower’, ‘tomato’, ‘carrot’, ‘eggplant’, ‘beets’, ‘chayote’, ‘pumpkin’, ‘cucumber’, ‘zucchini’, ‘onion’	98.9	106.7	99.0;115.8
Oil (for salad dressing)	FFQ	89.6	3.3	3.3;4.6
Salt	FFQ	94.5	0.4	0.3;0.4
Soy sauce	FFQ	17.8	0.0	0.0;0.0
Vinaigrette	FFQ	43.6	0.0	0.0;0.0
Processed meat, sandwiches and snacks	‘Hamburger/chicken nuggets/meatball’, ‘ham/mortadella/salami’, ‘sandwich: hot dog/hamburger’, ‘fried snacks’, ‘baked savoury’, ‘pizza’, ‘snacks’	99.1	69.3	62.0;75.8
Sandwich sauces	‘Mayonnaise’, ‘ketchup/mustard’	62.1	0.6	0.4;0.9
Popcorn	FFQ	59.5	0.7	0.7;1.1
Nuts	FFQ	36.3	0.0	0.0;0.0
Desserts with fruits and jelly	‘Desserts with fruits’, ‘jelly’	70.5	5.0	3.7;7.0
Desserts and sweets	‘Candy/lollipop’, ‘whipped cream/coconut milk/condensed milk’, ‘chocolate’, ‘ice cream’, ‘sweet pies/pudding/mousse’	96.5	34.6	30.1;38.6
Soy beverages	FFQ	33.9	0.0	0.0;0.0
Soft drinks	FFQ	81.9	59.3	52.4;71.9
Sweetened juices (natural[d] or artificial[e])	‘Artificial juice (sweetened)’, ‘natural juice (sweetened)’	88.1	51.1	42.5;60.1
Unsweetened juices (natural or artificial)	‘Artificial juice (unsweetened)’, ‘natural juice (unsweetened)’	32.6	0.0	0.0;0.0
Alcoholic beverages (beer, wine and *caipirinha*[c])	‘Beer’, ‘wine’, *‘caipirinha’*	41.4	0.0	0.0;0.0

95%CI - 95% confidence interval. Traditional recipes: [a]*Farofa*: manioc flour toasted in butter or olive oil/cooking oil, sometimes mixed with meat or eggs; [b]*Feijoada*: black bean stew; [c]*Caipirinha* - a drink made with “cachaça” (a hard liquor from sugar cane), fresh limes, sugar and ice. [d]Natural juices are made with fresh fruits or frozen fruit pulps, with the addition of water or not. [e]Artificial juices are artificial powdered drink mixes, fruit nectars, or sweetened processed juice.

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4.2 SEGUNDO MANUSCRITO - *MATERNAL DIETARY PATTERN IS ASSOCIATED WITH NEWBORN SIZE: RESULTS FROM PROCRIAR STUDY*

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Abstract

Background: Intrauterine growth restriction is an important public health nutrition problem given that it is associated with an increased risk of morbidity, mortality and adverse functional consequences. **Objective**: To determine whether maternal pre-pregnancy dietary patterns are associated with delivering newborns with small size in the ProcriAr Cohort Study, Sao Paulo-Brazil, 2012. **Study design**: Pre-pregnancy dietary patterns of women (n=299) were investigated using factor analysis with principal component's estimation, based on intake reported on a validated 110-item food frequency questionnaire. Newborns whose weight or length, adjusted by gestational age and sex, were below the 10th percentile according to INTERGROWTH-21st standards were considered small at birth. Multivariate Poisson regression models with robust error variance estimated the relationship between small size at birth and women's dietary patterns, adjusted by maternal age, education, body mass index, work status, relationship status, smoking status, and number of previous births. Linear regression models were used to determine associations between maternal energy and nutrient intakes and dietary patterns. **Results**: The maternal adherence to the highest quintile of the dietary pattern composed of processed meat, sandwich, snack, sweet, and sugar-sweetened beverage ('Snacks, sandwiches, sweets and soft drinks' dietary pattern) during the pre-pregnancy period was significantly and independently associated with small size at birth (Relative Risk, 95% Confidence Interval: 2.01; 1.13-3.57). This dietary pattern was associated with higher intakes of total fat and lower intakes of carbohydrate, calcium, vitamin D, natural folate and dietary folate equivalents. **Conclusion**: This study verified that women's dietary behavior characterized by an energy-dense, nutrient-poor food intake was a risk factor for delivering newborns with low weight and/or length at birth. Investments in education and improved access to healthful food and nutritional information during childbearing age should be prioritized due to its potential impact on maternal and child health. However, further studies are warranted to identify specific nutrients and metabolic pathways that may be underlying these associations.

Keywords: Maternal and child health; newborn stunting; length by gestational age; food, nutrition; dietary pattern; health-related behaviors; life stages

Introduction

Approximately one in five children in low and middle income countries is born small for gestational age (below the 10th percentile of birth weight),[1] and one in four neonatal deaths is attributed to low birth weight.[2] In 2015 it was estimated that nearly 25% of children in the world were stunted (below the 10th percentile of birth length or height),[3,4] and approximately one-seventh of global deaths in children less than 5 years of age was attributed to childhood stunting.[5] It is well documented that an unhealthy living environment, poor parental education, lack of access to quality health care, and household food insecurity are the main determinants of intrauterine growth restriction, i.e., low weight and/or length at birth (below the 10th percentile).[3,6] However, it is still unclear how these factors interact with dietary patterns, rather than caloric or nutrient intake, in ways that may moderate the risk for intrauterine growth restriction. Thus, the objective of this study was to determine how specific pre-pregnancy dietary patterns are associated with the risk of delivering a small at birth.

Intrauterine growth restriction is an important public health nutrition problem given that it is associated with an increased risk of morbidity, mortality and adverse functional consequences, including poor cognition and educational performance, and a long-term risk of nutrition-related chronic diseases in adulthood.[5,6] Among the many factors that are associated with fetal growth, it is clear that a poor diet during pregnancy is an important determinant of poor fetal growth.[7] To address this, potential evidence-based interventions have focused on improving the diet of women of reproductive age and during pregnancy with supplements of folic acid, iron, calcium, and iodine.[8] Yet, it is also important that nutrient recommendations be met through dietary intake as a more sustainable solution compared to supplements.[9] So far, there is limited research about pre-pregnancy dietary patterns and its influence on the risk of delivering babies with low weight and/or length at birth.[10] Thus, it is important to improve our understanding of how various combinations and food patterns within a population may impact the overall prevalence of reduced fetal growth as a means to develop new strategies using food patterns to improve maternal diet.

Dietary patterns are frequently studied using the factor analysis with principal component's estimation, a powerful statistical method that determines food intake patterns once the cumulative and interactive effects of a diet are taken into account, and it is more similar to actual dietary consumption.[11] The main outcome associated with maternal dietary pattern in previous studies were generally birth weight.[10] It has been reported that a prudent or healthful maternal dietary pattern is associated with a decreased risk and Western or 'wheat products' pattern with an increased risk of having a small for gestational age newborn.[10] Yet, few studies describe the association between pre-pregnancy dietary patterns and newborn stunting.[10,12] Therefore, to design more effective nutritional interventions to improve maternal and child health, it is important to understand the relative contributions of women dietary patterns on the risk of low weight and/or length in the newborn. The main hypothesis of this study was that an unhealthful dietary pattern in the pre-pregnancy period increases the risk of delivering a newborn who is small at birth, defined by low weight and/or length by gestational age and sex.

Materials and Methods

Study Design

We analyzed data from the birth cohort ProcriAr (The Influence of Nutritional Factors and Urban Air Pollutants on Children's Respiratory Health: A Cohort Study in Pregnant Women), which was conducted with women who attended to three primary health care units from the west region of São Paulo – Southeast/Brazil. Between March/2011 and December/2013, 619 women with positive pregnancy tests were invited to participate in the study. Fifty-nine of those women did not met the study eligibility criteria (single fetus, gestational age <14 weeks and absence of pre-existing chronic diseases). Among the remaining 560 women for this study, we excluded: 42 women who did not have the ultrasonography exam, 45 women who had a miscarriage or no embryo; 19 women dropped out of the study or moved out to another place in the beginning of the study, and 155 women whose follow-up was lost. A final sample of 299 pregnant women who completed the face-to-face home interview were followed until delivery. This study was approved by the Ethical Committee of Municipal Department of Health of São Paulo (nº 430/10), the Ethical Committee of School of Medicine, University of São Paulo (nº 0068/10), and the Ethical Committee of School of Public Health, University of São Paulo (nº 1.501.677/16). Participants provided written informed consent to be involved in the study.

Exposure assessment

A validated 110-item quantitative food frequency questionnaire (FFQ) was used to assess the pre-pregnancy food intake of the population.[13,14] In the beginning of their pregnancy (mean gestational age: 11.1 weeks, range: 6-17 weeks), women were asked about the frequency of their consumption of each food item over the past year in addition to the portion size consumed (small, medium or large). Brazilian manuals were used to convert foods and recipes from the FFQ into grams.[15] Daily intakes were calculated by multiplying the portion size by the frequency of intake (1-10) and dividing by the days.

Dietary patterns were estimated using factor analysis with principal component's estimation and were based on the daily amount of intake derived from the FFQ.[11] After grouping, remained 51 food items for the pattern analysis. To identify the number of dietary patterns to be retained, the eigenvalue >1.0 criterion, the Scree test analysis, and the interpretation of each factor were considered, resulting in four dietary patterns. Varimax orthogonal rotation was performed, generating nonrelated factors. The retained dietary patterns accounted for 25.5% of the variance in food intake and were named 'Lentils, whole grains and soups,' 'Snacks, sweets and soft drinks,' 'Seasoned vegetables and lean meats,' and 'Sweetened juices, bread and butter, rice and beans'.

Daily intake of energy, carbohydrate, protein, fat, caffeine, iron, calcium, vitamin D, dietary folate equivalents, natural folate, synthetic folate, methionine, choline, betaine, vitamin B6, vitamin B12, omega-3, and docosahexaenoic acid (DHA) were analyzed using

Nutrition Data System for Research software version 2.0 (2007), which was developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN, US. The foods used had an 80-120% match for energy and macronutrients between the NDSR and the Brazilian composition tables.[16] The amount of folic acid added to fortified foods was corrected due to differences among US and Brazilian fortification policies. The choice of nutrients investigated was based in recommendations for healthy pregnancy outcomes.[10,17–19] The adjustment of the nutrients for energy was performed by the residuals method.[20]

Outcome assessment

Information on newborn's gender and anthropometric measurements were retrieved from hospital medical records by researchers engaged in this study. The newborn measures of weight (in grams), height (in centimeters) and head circumference (in centimeters) were assessed by the health care teams of the different hospitals where the women gave birth using, respectively, pediatric scales, stadiometers, and measurement tape. The estimate of the conception date was based on the last menstrual period and confirmed by ultrasonography performed in the first trimester of pregnancy. Gestational age was determined by the difference between conception and childbirth dates.

We have standardised birth weight and length by gestational age and sex using as reference The International Fetal and Newborn Growth Consortium for the 21st Century study (INTERGROWTH-21st).[21,22] Newborns with birth weight or birth length by gestational age and sex below the 10th percentile were considered small at birth, as indicator of newborn's intrauterine growth restriction.[23]

Covariates assessment

Information on maternal socio-demographic and lifestyle factors were collected during the first home face-to-face interview. Also, antenatal weight and height were self-reported by the pregnant women. Maternal body mass index (BMI) was calculated and classified in accordance with WHO criteria.[24] Due to the low prevalence of underweight in this population (n=6, 2.0%), it was grouped in one category as follow: under and normal weight ≤ 24.9 kg/m^2; overweight: 25.0-29.9 kg/m^2; and obese: ≥ 30.0 kg/m^2.

Socio-demographic and lifestyle factors previously associated with fetal growth were chosen as covariates, according to data availability.[25,26] Therefore, maternal characteristics, such as age (years), gestational age (weeks), education (< or ≥ 8 years), work status (yes or no), relationship status (married or not), smoking status (yes or no), sedentary behavior (< or ≥2 hours watching TV/day), number of previous births (0, 1, or ≥ 2), supplement use (yes or no), and BMI were used to describe the population. Some newborn characteristics such as gender (male/female), weight (g), length (cm), and head circumference (cm) were also described.

Statistical Analyses

Variables were described as percentages and medians (95% confidence intervals - 95% CI). Kruskal Wallis test for continuous variable and chi-square test for categorical variable were implemented to compare the population of this study (n=299) with the original study sample (n=454). Also these tests were used to determine if any significant differences existed between the groups of newborns classified as < 10th or ≥ 10th percentile for birth weight and/or birth length by gestational age and sex according to maternal and newborn characteristics.

Multivariate Poisson regression models with robust error variance were used to identify the relationship between small size at birth and women's dietary patterns, adjusted by maternal characteristics. Newborn size at birth was considered the outcome variable. The component scores of each maternal dietary pattern were categorized into quintiles, considered the exposure variable in this analysis. Maternal age was used in years. Eight or more years of education, lacking formal work, being married and smoking (pre-pregnancy period) were treated as dichotomous variables (yes or no options). Overweight and obese were defined in relation to under and normal weight, using a dummy variable for BMI. None or two or more previous births were defined in relation to one previous birth using a dummy variable for number of previous births.

Multivariate linear regression models were used to examine associations between energy and nutrient intakes and the scores of dietary patterns, both variables as continuous. All models were adjusted by maternal age, education, BMI, work status, relationship status, smoking status, and number of previous births.

All analyses were performed using Stata Statistical Software (release 12, 2011, StataCorp LP, College Station, TX).[27] Two-sided statistical significance was determined at

P<0.05.

Results

The studied women were assumed to be a random subsample of the original study population as there were no statistically significant differences in terms of socio-demographic and lifestyle factors, except for the number of previous births (Supplement Table S1). This population had, in median, 25.9 years old, more frequently had under and normal weight (51.8%), had formal work (50.2%), were married or in common law marriage (59.9%), non-smoker (86.9%), watched TV for less than 2 hours a day (58.6%), had given previous birth (50.2%), used folic acid (80.9%) and iron (80.3%) supplements during pregnancy, and had female babies (54.5%) (**Table 1**).

The majority of the newborns had birth weight classified between the 10th and 90th percentiles (79%), and the prevalence of low birth weight was 9%. The prevalence of low birth length (25%) was almost 2.8 times higher than low birth weight (**Figure 1**). Babies with low birth weight had younger mothers whom more frequently were smokers in the pre-pregnancy period and were not married or in common law marriage, when compared with mothers of babies >10th percentile for the index (**Table 1**). Babies with low birth length had mothers with less years of formal education and whom more frequently were smokers in the pre-pregnancy period, when compared with mothers of babies >10th percentile for the index (**Table 1**).

Overall, the prevalence of newborns small at birth was 26.1% (78/299), where 29.5% (23/78) of them presented both low birth weight and length, 64.1% (50/78) presented only low birth length, and 6.4% (5/78) presented only low birth weight. When compared the newborns with small size at birth with the ones who were not, no differences in the proportion of gender were verified (**Table 2**). However, smaller median values of birth weight, birth length and head circumference were verified for the first group of newborns (**Table 2**). When compared with mothers of newborns non-small at birth, the mothers of newborns small at birth had less years of formal education and more frequently were smokers in the pre-pregnancy period (**Table 2**).

In multivariate Poisson regression models, offspring of mothers in the fifth vs. first quintile of the 'Snacks, sandwiches, sweets and soft drinks' dietary pattern had a significant increased relative risk (RR (95% CI): 2.01 (1.13-3.57)) of being small, independently of maternal characteristics. It was also verified a trend effect of adherence to dietary pattern 'Snacks, sandwiches, sweets and soft drinks' among the newborns small at birth (p=0.036). No relationships between small size at birth and quintiles of score in the other three dietary patterns were found (**Table 3**).

The only dietary pattern negatively correlated to the intake of carbohydrate, calcium, vitamin D, DFE, and natural folate was the 'Snacks, sandwiches, sweets and soft drinks'. Likewise, the one and only positive correlation was found between 'Snacks, sandwiches, sweets and soft drinks' and fat (**Table 4**).

Comment

Many middle and lower income countries are now experiencing the nutrition transition that is accompanied by an increased prevalence of obesity and a decreased prevalence of underweight.[28] However, the problem of chronic growth retardation (stunting) continues to be very prevalent with more than 150 million children worldwide being too short for their age.[29] At the same time, in South America, the prevalence of stunting among pre-school children decreased from 21% in 1990 to 12% in 2010.[30] It is well established that stunting is associated with poor environmental and socioeconomic, illness and/or inappropriate feeding practices,[30–33] but specifics on maternal pre-conception diet are not well known. Results from our analysis of the ProcriAr cohort study showed that maternal adherence to an unhealthful dietary pattern before pregnancy was significantly associated with being small at birth, independent of maternal characteristics. These findings provide an important foundation upon which dietary recommendations can be made or modified to ensure that rapid changes in the food environment do not promote excess weight gain or poor fetal outcomes in countries undergoing rapid development.

With regards to maternal diet and newborn nutritional status, there is abundant research on maternal diet during gestation and the impact it has on fetal outcomes,[5] but there is relatively little research on pre-pregnancy dietary patterns and offspring length by gestational age and sex, a more refined indicator of nutritional status than birth weight or length.[10] Of recently published research on maternal dietary patterns and health, the main outcomes reported included infertility, hypertensive disorders, depressive symptoms, preterm birth, asthma in children, gestational diabetes mellitus, and birth weight.[10] Yet, only two studies focused on length by gestational age and, of these, one focused on preconceptional diet,[34] and the other focused on diet during the last two trimesters of gestation.[35] However, one study of pre-pregnancy dietary patterns did find that a high-fat/sugar/takeaway dietary pattern was associated with shorter birth length, but not adjusted for gestational age.[12] Our results extend this research given that we were able to assess fetal growth using weight and length for gestational age rather than only birth weight or length. It is important to note that relative weight and length is a proxy for growth retardation and this specific indicator of nutritional status is very complex and influenced by a number of dietary and environmental factors.

Considering the relationship between maternal periconceptional dietary patterns and growth of their offspring requires an interdisciplinary approach given the interactions that occur between diet and other factors that promote or limit growth. For example, we found that women with lower maternal education (<8 years of education) or who smoked in the pre-pregnancy period had a higher risk of having a small child compared to women with higher education or nonsmokers. This finding is consistent with other research in which women with lower educational levels and/or unhealthy behavior (smoking and high intake of food rich in energy and poor in micronutrients) were more likely to have children with growth deficits.[10,36] This pattern constitutes what is termed as the vicious cycle of poverty, under-nutrition and poor growth. Poverty contributes to under-nutrition and poor growth as low income leads to poor dietary intake of the pregnant women, increasing the risk of poor intra-uterine growth of her fetus.[37,38] Further nutritional insults contribute to prolonged growth delays, resulting in stunting in childhood and even adulthood, both of which are associated with physical and cognitive delays, reduced human capital and potential income, that is, social disadvantages throughout life.[38,39] Improvement of women dietary intake presents an important opportunity to contribute to the disruption, even in part, of this unfair process. If policymakers want to effectively improve maternal and child health in developing countries, they should not disregard the potential of policies that will promote more equitable access to education and natural healthy food to women of childbearing age.

Potential explanations for the results reported in this study are most likely related to the fact that a lack of crucial nutrients during pregnancy interrupts or limits important developmental processes in utero with a long-term impact on health and disease of the offspring.[8] Poor maternal diet has been reported to be associated with diabetes,[40,41] preeclampsia,[42] hypertension,[43] leading to increased perinatal mortality as well long-term effects on development and health status of the fetus.[44] Again, we found that offspring of mothers in the fifth vs. first quintile of the 'Snacks, sandwiches, sweets and soft drinks' dietary pattern had a higher risk of having a small child. In addition, the dietary pattern 'Snacks, sandwiches, sweets and soft drinks' was the only pattern negatively correlated with calcium, vitamin D, natural folate and dietary folate equivalents. This is an important point as recommendations of the American Academy of Nutrition and Dietetics for healthy pregnancy outcomes includes an appropriate intake of calcium and folate.[18] The importance of folate in pregnancy has been highlighted since the 1970s when it found to be associated with neural tube defects[45] given that perturbations in the folate-mediated synthesis of precursors used in DNA synthesis, repair, and methylation.[46] Maternal inadequate nutrition can disrupt these processes and results epigenetic changes central to the generation of pathologic phenotypes throughout the life course.[44] Thus, what may be reported as simply a “dietary pattern” may actually have profound biochemical triggers that prevent chronic diseases throughout life.

As with any research study, there are a number of strengths and limitations that need to be discussed to have a full appreciation of the results presented. For example, loss to follow-up is a problem in most cohort studies and may lead to bias and loss of statistical power.[47] In the ProcriAr study, we were able to follow-up 66% of the study sample and the subsample described in this paper (n=299) had previous births less frequently when compared to the total sample, indicating that the sample studied was possibly more concerned about health. Regardless, the fact that we found a statistically significant relationship between an unhealthful pre-pregnancy dietary pattern and offspring growth restriction, even in this population, suggests that this relationship is not random. At the same time, it is worth noting that our results were statistically significant even with a loss to follow-up that could contribute to a decrease in the statistical power of study and the magnitude of the association found may be even higher than the results presented in this paper. In addition, a FFQ was used to assess the dietary intake of women and this instrument has been reported to provide misclassifications in dietary intake[10] that could bias the magnitude of the observed effects towards the null hypothesis. Furthermore, the use of Brazilian food composition tables was not possible, as they have a limited number of nutrients available. Still, a major strength of this study was the use of the INTERGROWTH-21st standards to evaluate the newborn length by gestational age and sex. INTERGROWTH-21st is a multicentre, multiethnic, population-based project conducted in eight sites in eight countries, including Pelotas, Brazil.[21] The same conceptual framework as the WHO Multicentre Growth Reference Study[48] was used to produce these international prescriptive standards for newborn size.[21] Therefore, we are confident that the results are sound given that a reputable standard for offspring growth was our main outcome of interest. Despite the weaknesses discussed above, the use of validated measurement tools protect against serious bias that could undermine the interpretation of the results presented.

There are a number of important public health implications that merit discussion. Some studies have reported that an energy-dense, nutrient-poor food environment is harmful to the health not only of the generation that consumes it, but also to the next generation,[10] as suggested by the present study. Therefore, strategies to improve the quality of food intake should be implemented, especially for women of childbearing age. One approach is to improve the dietary diversity of a woman's diet to increase micronutrient intake without the added expense generally associated with the use of micronutrient supplements.[49] A policy-based approach to supporting dietary diversity is modeled by the Dietary Guidelines for the Brazilian Population 2014 in which a food-based guidelines encourage a whole-foods diet with minimal intake of ultra-processed foods.[50] Diets with a higher intake of fruits, vegetables, legumes and fish, instead of higher intake of processed and takeaway food, have positive pregnancy outcomes in general and this evidence should be communicated not only to women specifically, but also to her social support.[10]

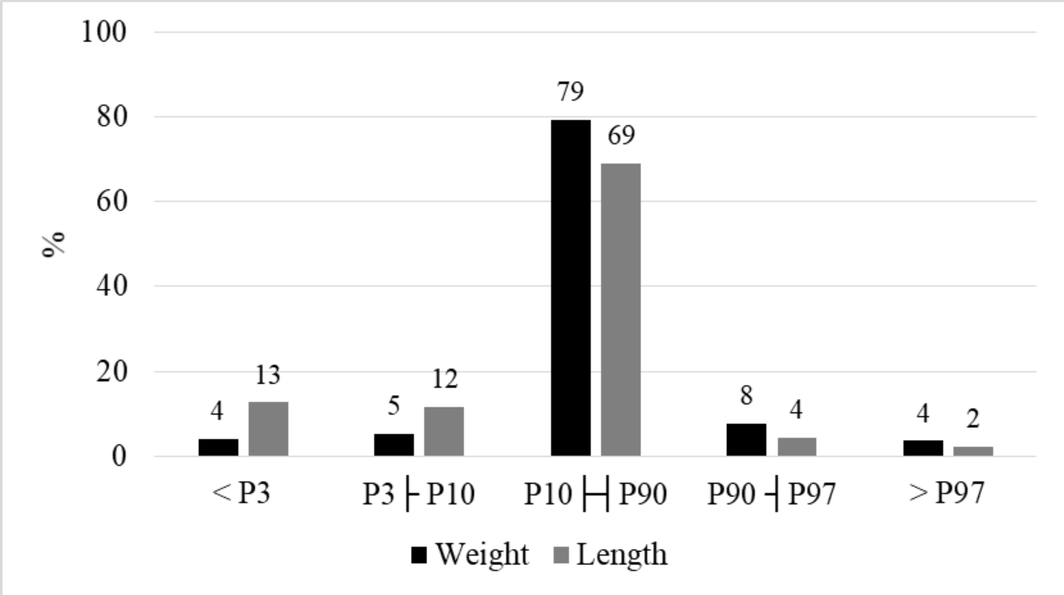
In conclusion, we found that a pre-pregnancy dietary pattern characterized by energy-dense, nutrient-poor foods increases the risk of having a newborn with low weight and/or length by gestational age and sex. Intrauterine growth restriction is the product of many interacting factors apart from diet, such as paternal size, maternal morbidity, pregnancy complications, poor hygiene, low access to clean water, violence, or psychological aspects, which could not be accomplished in this study. Thus, with what limited knowledge there is investigating pre-pregnancy diet and birth outcomes, it is necessary that future research complement their data with these measures, including measurements of stress, violence, and autonomy to more fully understand how these interact with diet to limit fetal and childhood growth.[51] Investments in education and improved access to healthful food and nutritional information should be prioritized due to its potential impact on maternal and child health. However, further studies are warranted to identify specific nutrients and metabolic pathways that may be underlying these associations.

Table 1. Newborn weight and length by gestational age according to maternal and newborn characteristics in the ProcriAr study - Sao Paulo/Brazil, 2012‡

	Total	Weight by gestational age			Length by gestational age		
		<P10	≥P10	*p**	<P10	≥P10	*p**
	299 (65.9%)	28 (9%)	271 (91%)		73 (24%)	226 (76%)	
Maternal characteristics							
Age (years), median (95% CI)	25.9 (24.8,26.6)	23.4 (21.0,25.5)	26.2 (25.4,26.8)	**0.043**	25.1 (23.6,26.9)	26.1 (24.9,26.8)	0.630
≥8 years of education, n (%)	163 (54.9)	12 (42.9)	151 (56.1)	0.179	32 (43.8)	131 (58.5)	**0.029**
Nutritional status[a], n (%)				0.108			0.518
Under and normal weight	155 (51.8)	19 (67.9)	136 (50.2)		41 (56.2)	114 (50.4)	
Overweight	94 (31.4)	4 (14.3)	90 (33.2)		19 (26.0)	75 (33.2)	
Obese	50 (16.7)	5 (17.9)	45 (16.6)		13 (17.8)	37 (16.4)	
No formal work, n (%)	149 (49.8)	12 (42.9)	137 (50.6)	0.438	39 (53.4)	110 (48.7)	0.480
Married or in common law marriage, n (%)	179 (59.9)	11 (39.3)	168 (62.0)	**0.020**	49 (67.1)	130 (57.5)	0.146
Current smoker (pre-pregnancy period), n (%)	39 (13.1)	9 (32.1)	30 (11.1)	**0.002**	18 (24.7)	21 (9.3)	**0.001**
≥2 hours watching TV/day, n (%)	121 (41.4)	14 (50.0)	107 (40.5)	0.333	35 (49.3)	86 (38.9)	0.122
Dietary patterns, 5th quintile, n (%)							
Lentils, whole grains and soups	60 (20.1)	2 (7.1)	58 (21.4)	0.085	15 (20.6)	45 (19.9)	0.521
Snacks, sweets and soft drinks	58 (19.4)	10 (35.7)	48 (17.7)	0.178	20 (27.4)	38 (16.8)	0.201
Seasoned vegetables and lean meats	60 (20.1)	3 (10.7)	57 (21.0)	0.110	11 (15.1)	49 (21.7)	0.264
Sweetened juices, bread and butter, rice and beans	66 (22.1)	5 (17.9)	61 (22.5)	0.579	14 (19.2)	52 (23.0)	0.901
Number of previous births, n (%)				0.078			0.588
0	147 (49.8)	19 (67.9)	128 (47.9)		37 (51.4)	110 (49.3)	
1	95 (32.2)	4 (14.3)	91 (34.1)		20 (27.8)	75 (33.6)	
2 or more	53 (18.0)	5 (17.9)	48 (18.0)		15 (20.8)	38 (17.0)	
Folic acid supplement use, n (%)	242 (80.9)	21 (75.0)	221 (81.6)	0.401	60 (82.2)	182 (80.5)	0.753
Iron supplement use, n (%)	237 (80.3)	23 (82.1)	214 (80.2)	0.801	60 (82.2)	177 (79.7)	0.646
Gestational age (weeks), median (95% CI)	39.6 (39.4,39.7)	40.0 (39.6,40.1)	39.6 (39.3,39.7)	0.1507	39.4 (39.1,39.9)	39.6 (39.4,39.9)	0.6615
Newborn characteristics							
Gender, female, n (%)	163 (54.5)	18 (64.3)	145 (53.5)	0.275	43 (58.9)	120 (53.1)	0.386
Weight (g), median (95% CI)	3230 (3200,3290)	2600 (2493,2667)	3290 (3220,3321)	**<0.001**	2810 (2726,2988)	3310 (3280,3370)	**<0.001**
Length (cm), median (95% CI)	48 (48,49)	46 (45,46)	49 (48,49)	**<0.001**	46 (45,46)	49 (49,49)	**<0.001**
Head circumference (cm), median (95% CI)	34 (34,34)	33 (32,34)	34 (34,35)	**<0.001**	34 (33,34)	34 (34,35)	**<0.001**

P10: 10th percentile; 95% CI: 95% confidence interval. ‡Kruskal Wallis test (continuous variable) or chi-square test (categorical variable) were used to determine if any significant differences existed amongst the groups according to maternal and newborn characteristics (p<0.05; there is difference) (n = 299). [a]BMI; underweight and normal weight: ≤ 24.9 kg/m2 ; overweight: 25.0-29.9 kg/m2; and obese: ≥ 30.0 kg/m2. Prevalence of underweight was 2.0% (n=6).

Figure 1. Prevalence of percentiles of weight and length by gestational age in the ProcriAr study - Sao Paulo/Brazil, 2012‡



‡According to The International Fetal and Newborn Growth Consortium for the 21st Century study (INTERGROWTH-21st).

Table 2. Small newborns by gestational age according to maternal and newborn characteristics in the ProcriAr study - Sao Paulo/Brazil, 2012‡

	Small newborns[a]		
	Yes	No	*p**
	78 (26%)	221 (74%)	
Maternal characteristics			
Age (years), median (95% CI)	24.9 (23.5,26.7)	26.1 (25.2,26.9)	0.500
≥8 years of education, n (%)	35 (44.9)	128 (58.5)	**0.039**
Nutritional status[b], n (%)			0.290
Under and normal weight	45 (57.7)	110 (49.8)	
Overweight	19 (24.4)	75 (33.9)	
Obese	14 (17.9)	36 (16.3)	
No formal work, n (%)	40 (51.3)	109 (49.3)	0.766
Married or in common law marriage, n (%)	50 (64.1)	129 (58.4)	0.375
Current smoker (pre-pregnancy period), n (%)	19 (24.4)	20 (9.1)	**0.001**
≥2 hours watching TV/day, n (%)	38 (50.0)	83 (38.4)	0.078
Dietary patterns, 5th quintile, n (%)			
Lentils, whole grains and soups	15 (19.2)	45 (20.4)	0.413
Snacks, sweets and soft drinks	23 (29.5)	35 (15.8)	0.077
Seasoned vegetables and lean meats	13 (16.7)	47 (21.3)	0.195
Sweetened juices, bread and butter, rice and beans	14 (18.0)	52 (23.5)	0.638
Number of previous births, n (%)			0.396
0	42 (54.5)	105 (48.2)	
1	20 (26.0)	75 (34.4)	
2 or more	15 (19.5)	38 (17.4)	
Folic acid supplement use, n (%)	64 (82.1)	178 (80.5)	0.771
Iron supplement use, n (%)	64 (82.1)	173 (79.7)	0.657
Gestational age (weeks), median (95% CI)	39.6 (39.1,39.9)	39.6 (39.3,39.9)	0.8382
Newborn characteristics			
Gender, female, n (%)	46 (59.0)	117 (52.9)	0.358
Weight (g), median (95% CI)	2800 (2715,2972)	3310 (3284,3370)	**<0.001**
Length (cm), median (95% CI)	46 (45,46)	49 (49,49)	**<0.001**
Head circumference (cm), median (95% CI)	34 (33,34)	34 (34,35)	**<0.001**

P10: percentile 10th; 95% CI: 95% confidence interval. ‡Kruskal Wallis test (continuous variable) or chi-square test (categorical variable) were used to determine if any significant differences existed amongst the groups according to maternal and newborn characteristics (p<0.05; there is difference) (n = 299). [a]Weight and/or length by gestational age below the 10th percentile, total agreement: 80.6% (length <P10, n=50; weight <P10, n=5; length and weight <P10, n=23). [b]BMI; underweight and normal weight: ≤ 24.9 kg/m2 ; overweight: 25.0-29.9 kg/m2; and obese: ≥ 30.0 kg/m2. Prevalence of underweight was 2.0% (n=6).

Table 3. Adjusted associations between maternal dietary patterns and small newborns in the ProcriAr study - Sao Paulo/Brazil, 2012

Maternal characteristics	Small newborns[a]							
	RR (95%CI)	*p*	RR (95%CI)	*p*	RR (95%CI)	*p*	RR (95%CI)	p
Dietary pattern	Lentils, whole grains and soups		Snacks, sweets and soft drinks		Seasoned vegetables and lean meats		Sweetened juices, bread and butter, rice and beans	
1st quintile	1.00	-	1.00	-	1.00	-	1.00	-
2nd quintile	1.11 (0.63,1.94)	0.725	1.12 (0.58,2.16)	0.733	0.84 (0.45,1.57)	0.582	0.93 (0.54,1.60)	0.787
3rd quintile	0.75 (0.39,1.44)	0.388	1.34 (0.71,2.55)	0.365	0.83 (0.47,1.47)	0.524	0.72 (0.43,1.22)	0.220
4th quintile	1.29 (0.78,2.13)	0.314	0.97 (0.51,1.87)	0.930	1.43 (0.87,2.34)	0.155	0.76 (0.44,1.31)	0.320
5th quintile	1.18 (0.62,2.25)	0.604	2.01 (1.13,3.57)[b]	**0.017**	0.85 (0.46,1.57)	0.601	0.69 (0.38,1.26)	0.226
Age (years)	1.01 (0.97,1.05)	0.745	1.00 (0.96,1.04)	0.953	1.00 (0.96,1.04)	0.954	1.00 (0.96,1.04)	0.933
≥8 years of education	0.61 (0.40,0.92)	**0.017**	0.60 (0.40,0.89)	**0.012**	0.65 (0.43,0.98)	**0.040**	0.59 (0.40,0.88)	**0.010**
Nutritional status[c]								
Under and normal weight	1.00	-	1.00	-	1.00	-	1.00	-
Overweight	0.81 (0.52,1.25)	0.333	0.80 (0.51,1.25)	0.323	0.79 (0.51,1.21)	0.280	0.81 (0.52,1.25)	0.334
Obese	0.94 (0.54,1.61)	0.809	0.99 (0.58,1.69)	0.964	0.91 (0.55,1.51)	0.724	0.95 (0.57,1.60)	0.859
No formal work	1.15 (0.79,1.67)	0.477	1.14 (0.78,1.68)	0.498	1.14 (0.78,1.67)	0.492	1.16 (0.80,1.70)	0.432
Married or in common law marriage	1.49 (1.00,2.22)	0.050	1.59 (1.05,2.40)	**0.028**	1.57 (1.06,2.33)	**0.026**	1.49 (0.99,2.25)	0.056
Current smoker (pre-pregnancy period)	2.13 (1.42,3.21)	**<0.001**	2.28 (1.50,3.46)	**<0.001**	2.11 (1.43,3.12)	**<0.001**	2.28 (1.55,3.37)	**<0.001**
Number of previous births								
0	1.46 (0.92,2.32)	0.113	1.47 (0.92,2.34)	0.104	1.50 (0.95,2.38)	0.083	1.42 (0.89,2.29)	0.144
1	1.00	-	1.00	-	1.00	-	1.00	-
2 or more	1.10 (0.66,1.85)	0.714	1.04 (0.61,1.75)	0.895	1.16 (0.69,1.96)	0.576	1.07 (0.63,1.81)	0.814

RR: relative risk; 95%CI: 95% confidence interval. Models adjusted by the other dietary patterns. Loss of 5 individuals in the models because of missing data in the adjustment variables education (2), smoking status (1), and parity (2). [a]Weight and/or length by gestational age below the percentile 10th, total agreement: 80.6% (length <P10, n=50; weight <P10, n=5; length and weight <P10, n=23). [b]Trend effect: p=0.036. [c]BMI; underweight and normal weight: ≤ 24.9 kg/m2 ; overweight: 25.0-29.9 kg/m2; and obese: ≥ 30.0 kg/m2. Prevalence of maternal underweight was 2.0% (n=6).

Table 4. Pregnant women's dietary intake of energy and nutrients according to their dietary patterns in the ProcriAr study - Sao Paulo/Brazil, 2012‡

Dietary Intake	**Lentils, whole grains and soups**[a]	**Snacks, sweets and soft drinks**[b]	**Seasoned vegetables and lean meats**[c]	**Sweetened juices, bread and butter, rice and beans**[d]
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Energy (kcal)	71.5 (-33.6,176.6)	390.4 (290.8,490.0)*	294.9 (189.5,400.2)*	274.4 (172.9,375.8)*
Carbohydrate (g)	5.6 (0.6,10.6)*	-11.7 (-16.5,-7.0)*	-1.0 (-6.0,4.0)	2.6 (-2.3,7.4)
Protein (g)	2.2 (0,4.4.0)	-0.2 (-2.3,1.9)	5.1 (2.8,7.3)*	-2.4 (-4.5,-0.2)*
Fat (g)	-2.2 (-3.8,-0.7)*	3.0 (1.5,4.5)*	-1.3 (-2.9,0.3)	0.3 (-1.2,1.9)
Caffeine (mg)	72.3 (-8.3,152.9)	-57.1 (-133.6,19.5)	40.0 (-41.1,121.0)	-83.3 (-161.3,-5.3)*
Iron (mg)	1.1 (0.7,1.5)*	-0.4 (-0.8,0.0)	1.0 (0.6,1.5)*	0.1 (-0.3,0.6)
Calcium (mg)	57.9 (24.2,91.7)*	-50.9 (-83.4,-18.4)*	41.5 (7.1,76.0)*	-27.3 (-60.5,5.8)
Vitamin D (calciferol) (mcg)	0.7 (0.4,1.0)*	-0.5 (-0.8,-0.1)*	0.3 (-0.1,0.6)	-0.2 (-0.5,0.1)
Dietary Folate Equivalents (mcg)	43.3 (30.6,56.1)*	-26.9 (-39.9,-13.9)*	19.5 (5.7,33.2)*	-10.0 (-23.2,3.2)
Natural Folate (mcg)	27.0 (17.6,36.5)*	-32 (-41.5,-22.6)*	31.7 (21.7,41.7)*	-10.9 (-20.5,-1.3)*
Synthetic Folate (mcg) - Food	9.9 (3.8,15.9)*	2.9 (-2.9,8.7)	-7.8 (-14,-1.6)*	0.4 (-5.5,6.3)
Methionine (g)	0.0 (0.0,0.1)	0.0 (0.0,0.1)	0.1 (0.1,0.2)*	-0.1 (-0.1,0.0)*
Choline (mg)	14.3 (5.9,22.7)*	-6.0 (-14.1,2.1)	21.5 (12.9,30.0)*	-11.0 (-19.3,-2.8)*
Betaine (mg)	96.6 (68.3,124.8)*	-24.5 (-53.3,4.3)	-2.1 (-32.6,28.3)	-49.2 (-78.5,-19.9)*
Vitamin B6 (mg)	0.2 (0.2,0.3)*	-0.2 (-0.3,-0.1)*	0.2 (0.1,0.3)*	-0.2 (-0.3,-0.1)*
Vitamin B12 (mcg)	0.5 (0.3,0.7)*	-0.1 (-0.3,0.1)	0.2 (0.0,0.5)*	-0.3 (-0.5,-0.1)*
Omega-3	-0.01 (-0.08,0.06)	-0.05 (-0.12,0.01)	0.07 (0.00,0.14)*	0.01 (-0.05,0.08)
DHA (g)	0.04 (0.02,0.05)*	-0.02 (-0.04,-0.01)*	0.02 (0.00,0.04)*	-0.03 (-0.05,-0.01)*

β: linear regression coefficients; 95% CI: 95% confidence interval. ‡Linear regression models were used to determine the association between energy and nutrients intake and the dietary patterns. All models were adjusted by maternal age, education, BMI, work status, relationship status, smoking status, and parity. Loss of 5 individuals in the models because of missing data in the adjustment variables education (2), smoking status (1), and parity (2). *p<0.05. [a]Dietary pattern includes high factor loadings for lentils, whole grains, soups, popcorn, cereal ready to eat, oats, and white cheese. [b]Dietary pattern includes high factor loadings for processed meats, sandwiches and snacks, sandwich sauces, desserts and sweets, soft drinks, pasta with meat sause and gnocchi, and pork and frankfurters. [c]Dietary pattern includes high factor loadings for vegetables, oil (for salad dressing), salt, lean meats, and fish. [d]Dietary pattern includes high factor loadings for sweetened juices (natural or artificial), butter or margarine (regular and salted), refined rice, and bread.

Supplement Table 1. Maternal characteristics in terms of loss of follow up in the ProcriAr study - São Paulo, 2012‡

Maternal characteristics	Original sample	Final sample	*p**
	454 (100.0%)	299 (65.9%)	
Age (years), median (95% CI)	25.8 (24.8,26.4)	25.9 (24.8,26.6)	0.587
≥8 years of education, n (%)	245 (54.2)	163 (54.9)	0.480
Married or in common law marriage, n (%)	271 (59.8)	179 (59.9)	0.519
No formal work, n (%)	234 (51.5)	149 (49.8)	0.446
Current smoker (pre-pregnancy period), n (%)	61 (13.5)	39 (13.1)	0.892
Nutritional status[b], n (%)			0.120
Under and normal weight	225 (49.6)	155 (51.8)	
Overweight	143 (31.5)	94 (31.4)	
Obese	86 (18.9)	50 (16.7)	
Dietary patterns (5th quintile), n (%)			
Lentils, whole grains and soups	91 (20.0)	60 (20.1)	0.588
Snacks, sweets and soft drinks	91 (20.0)	58 (19.4)	0.883
Seasoned vegetables and lean meats	91 (20.0)	60 (20.1)	0.309
Sweetened juices, bread and butter, rice and beans	91 (20.0)	66 (22.1)	0.215
≥2 hours watching TV, n (%)	184 (41.6)	121 (41.4)	0.592
Number of previous births, n (%)			<0.001
0	149 (39.3)	147 (49.8)	
1	143 (37.7)	95 (32.2)	
2 or more	87 (23.0)	53 (18.0)	

95% CI: 95% confidence interval; BMI: body mass index. [a]BMI; underweight and normal weight: ≤ 24.9 kg/m2; overweight: 25.0-29.9 kg/m2; and obese: ≥ 30.0 kg/m2. Prevalence of underweight was 2.0% (n=6). ‡Kruskal Wallis test (continuous variable) or chi-square test (categorical variable) were used to determine if any significant differences existed amongst the groups in terms of maternal characteristics (*p<0.05; there is difference).

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4.3 TERCEIRO MANUSCRITO - *DIETARY PATTERN DERIVED USING REDUCED RANK REGRESSION IS ASSOCIATED WITH DURATION OF HPV INFECTION IN MEN: EVIDENCE FROM HIM STUDY*

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Abstract

Background. HPV infection is a major public health issue due to its high carcinogenic potential. This study aimed to investigate the association between men's dietary patterns and duration of HPV infection. **Methods**. Two measures (first and last visits) of a validated 54-item food frequency questionnaire (FFQ) was used to evaluate the dietary intake of 1,412 men from the Brazilian cohort of the HPV Infection in Men (HIM) study. Dietary intakes (grams) were an average across FFQ. The RRR method was used to derive the dietary patterns, considering as response variables the nutrients dietary folate equivalents (DFE), vitamins B6 and B12, and docosahexaenoic acid (DHA). Samples of penile and scrotal cells were obtained at five visits every 6 months. Clinical and laboratory measurements were detection of HPV DNA by use of PCR and genotyping. For each positive test result, for at least one of 37 HPV genotypes, consecutive or non-consecutive, was considered 6 months of infection duration (variation from 0 to 30 months). Multivariate linear regressions were used to identify the association between duration of HPV infection (dependent variable) and the dietary pattern scores (independent variables in tertiles), adjusted by age, total number of female partners, and HPV status at enrolment. **Results**. This study found three dietary patterns associated with the response variables. A dietary pattern positively correlated to vitamin B6 (r_s=0.59), vitamin B12 (0.27) and DFE (0.07), but negatively correlated to DHA (-0.37) was associated with longer duration of HPV infection. This dietary pattern was directly associated with the intake of beef and offal, natural juice, *feijoada* (a traditional Brazilian recipe, black bean stew), fruits and beer. **Conclusions**. Men's dietary pattern is associated with duration of HPV infection.

However, the biological role that diet plays in HPV infection remains understudied and limited epidemiological data exist, especially among men, emphasizing the necessity for more studies in this field.

Keywords: human papillomavirus, diet, dietary pattern, one-carbon metabolism, DFE, vitamin B6, vitamin B12, DHA

Background

Human papillomavirus (HPV) infection is the most common viral infection of the reproductive tract.(1) From the initiation of sexual activity, the majority of women and men will be infected by HPV at some point of their lives.(1) Worldwide, approximately 291 million women (10.4%) are estimated to have HPV, though this prevalence is higher among young women and those who live in developing countries, i.e, in sub-Saharan Africa, Latin America, India, Mongolia, China.(2,3) Among men, an international multicentre study found an overall HPV infection prevalence of 65.2%, but highest in Brazil (72.3%) and lowest in the United States (61.3%) and Mexico (61.9%; p = 0.03).(4) Most HPV infection is harmless and clears naturally but persistent infection with high-risk HPV is the cause for all cervical cancer,(2) which is the fourth most common cancer in women, and the seventh overall, accounting for 7.5% of all female cancer deaths in the world.(5) Cancers of the anus, vulva, vagina, penis, and oropharynx are also associated with HPV infection.(2)

Several factors can influence HPV infection, persistence and remission. The number of sexual partners has been shown to be the main determinant of ano-genital HPV infection in both men and women.(6,7). Increased age, circumcision, and condom use are associated with reduced risk of infection.(7) Although smoking, oral contraceptives use, parity, other sexually transmitted agents, age at first intercourse, host susceptibility, and diet may influence the risk of acquisition and persistence of HPV infection, epidemiological evidence is still inconsistent.(7–9)

The study of the nutrients role as HPV cofactors in cervical cancer, precursor lesions, and duration of infection remains with inconsistent results. Studies suggest a protective effect of fruits and vegetables(10), vitamin C(11) carotenoids(12), tocopherols(12), vitamin B12(13) and folate(14). The nutritional status of nutrients involved in the one-carbon metabolism, such as folate, vitamin B6, vitamin B12 and DHA may influence the history of HPV infection and the development of virus-related diseases.(13,15). Therefore, the International Agency for Research on Cancer (IARC) emphasizes the necessity for further studies on the interaction between HPV infection and diet, especially with nutrients involved in the one-carbon metabolism.(7)

In addition to single component studies in nutrition, the investigation of the overall diet became an important research field.(16) The dietary pattern analyses generate data that better reproduce the actual dietary consumption by accounting for the cumulative and interactive components of a diet.(16) The reduced rank regression (RRR) method derives dietary patterns by combining food intake that explain as much response variation as possible, that is, nutrient-related disease previously selected based on the evidences.(17) Thus, dietary patterns derived from RRR should more accurately reflect the associations between diet and disease. However, there is a lack of studies on dietary patterns and duration of HPV infection.

The objective of this study was to investigate the relationship between men's dietary patterns, derived using RRR, and duration of HPV infection in men.

Methods

Population and Study Design

The HPV Infection in Men (HIM) study was a continuous prospective study of the natural history of HPV infections in men living in three different cities: Tampa, Florida (USA); Cuernavaca, Morelos (Mexico), and São Paulo, São Paulo (Brazil). The eligibility criteria for participation were: (a) men aged 18–70 years; (b) residents of southern Florida, São Paulo, or Cuernavaca; (c) reported no previous diagnosis of penile or anal cancers; (d) reported no previous diagnosis of genital or anal warts; (e) had not participated in an HPV vaccine study; (f) reported no previous diagnosis of HIV; (g) reported no current penile discharge or burning during urination; (h) were not being treated for sexually transmitted infection; (i) had not been imprisoned or homeless during the past 6 months; (j) had not received drug treatment during the past 6 months; (k) had no plans to relocate in the next 4 years; and (l) were willing to comply with ten scheduled visits every 6 months for 4 years. A full description of cohort procedures has already been published.(4)

These analyses will be restricted to the Brazilian population, who was recruited from a large clinic in São Paulo that was providing genitourinary services, and the general population through publicity in various institutions and media (n=1,412). In Brazil, the enrolment occurred from July 2005 to September 2009. Only men who answered two times the food frequency questionnaire (visits 2 and 6), and those with all HPV DNA detections in the period from visit 2 to visit 6 (5 measures, one every 6 months) were included in this study (n=1,194; 84.6%).

Written informed consent was obtained from all eligible men. Before study initiation, ethical approval was granted by the human-subjects' committees of the University of South Florida, FL, USA, the Ludwig Institute for Cancer Research, São Paulo, Brazil, the Centro de Referencia e Tratamento de Doencas Sexualmente Transmissiveis e AIDS, São Paulo, Brazil, and the National Institute of Public Health of Mexico, Cuernavaca, Mexico, approving all study procedures.

Data Measurement

Duration of HPV infection

Samples of penile and scrotal cells were obtained at each visit every 6 months. Clinical and laboratory measurements were detection of HPV DNA by use of PCR and genotyping.

By use of Dacron (Digene, Gaithersburg, MD, USA) swabs prewetted with saline, three separate specimens were obtained from the coronal sulcus, glans penis, penile shaft, and scrotum, and placed in 450 μL of Specimen Transport Medium, and combined into one sample before DNA extraction. All specimens were stored at –70°C until PCR analyses and genotyping were undertaken.(18) For HPV analyses, DNA was extracted with the Media Kit (QIAGen, Valencia, CA, USA) on a robotic system according to the manufacturer's instructions. DNA was stored at 4°C until use. HPV testing was undertaken by use of PCR for amplification of a fragment of the L1 gene.(19)

Specimens were tested for the presence of HPV by amplification of 30 ng of DNA with the PGMY09/11 L1 consensus primer system.(19) HPV genotyping was done with the linear array method(20) on all samples irrespective of the HPV PCR result (Roche Molecular Diagnostics, Alameda, CA, USA). Only samples that tested positive for β globin (99% at enrolment) were judged to be adequate and included in the analysis. Before genotyping, the amplifycation products were run on 2% agarose gels to visualise a 450 bp band corresponding to HPV amplifycation for identification of samples that might have a HPV type other than the 37 types analysed in the genotyping assay. Samples in which HPV was amplified on PCR, but did not hybridise with a specific HPV type during the genotyping assay (e.g, unclassified infections), were classified as HPV negative.

For each positive test result, consecutive or non-consecutive, for at least one of 37 HPV genotypes (any type of HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108) was considered 6 months of infection duration. Therefore, the duration of any type of HPV infection varies from 0 to 30 months.

Dietary intake

The dietary intake was assessed using a validated 54-item quantitative food frequency questionnaire (FFQ) developed based on a population from the same local where the HIM study was conducted.(21) The participants were asked to recall their frequency of dietary intake over the past year for each food item (from 0 to 10 times a day, week, month or year), and the portion size consumed. Household measures (standard serving sizes) were used to help participants to describe the portion size consumed.

The participants answered the FFQ twice throughout the study (visits 2 and 6). The present analysis included only participants who completed both FFQ. Dietary intakes (grams) were an average across both. Energy and nutrient intakes were calculated using the nutritional composition tables of the Nutrition Data System for Research (NDSR, version 2.0, 2007 – University of Minnesota, Minneapolis). The nutritional information of the NDSR software is based on the food composition databases from the United States Department for Agriculture (USDA). However, the NDSR food content matches from 80-120% with the Brazilian food composition tables in terms of energy and macronutrients.(22) The dietary folate equivalents (DFE) intake was corrected due to differences in the amount of folic acid added to fortified foods (USA and Brazil). The adjustment of the nutrients for energy was performed by the residuals method.(23)

Dietary pattern analysis

The dietary patterns were derived using reduced rank regression (RRR), which is a statistical method that can be used to derive dietary patterns that maximise the variation explained by response variables. These response variables are previously selected on the basis of a hypothesis about the association between diet and the outcome of interest.(17) In this study about duration of HPV infection in men, the nutrients docosahexaenoic acid (DHA),(24) vitamin B6,(25) vitamin B12,(26) and DFE(27) were selected as response variables for deriving the dietary patterns using RRR. These nutrients were selected based on the evidences from the relevant literature on nutrients involved in the one-carbon metabolism that can influence immune function, and then, duration of HPV infection.(28–30)

Forty-one food groups were used as predictors in the RRR analysis. Food items, grouping description, frequency of intake, and the daily amount of intake for each item were presented in **Supplement Table S1**. Using RRR, the number of extracted patterns is dependent on the number of response variables. Thus, intakes (g/d) of all forty-one food groups and the four response variables were used to derive four dietary patterns. Dietary pattern scores were categorised into tertiles.

Socio-demographic and lifestyle factors

The choice of the socio-demographic and lifestyle factors that could influence the duration of HPV infection was based on previous studies,(6) and, also, based on the data collected at the baseline (visit 2) through a computer assisted self-interview questionnaire.(28–31) Therefore, age (18-30, 31-44, and 45-70 years), race (white, black/African American, and Others), education (<12, 12, 13-15, and ≥16 years), family income (≤ US$473.93, US$473.93 ┤ US$1,421.80 and > US$1,421.80), marital status (single/divorced/widowed, and married/cohabiting), smoking status (yes/no), nutritional status (under/normal weight, overweight, and obese), circumcision status (yes/no), number of lifetime female sexual partners (0-1, 2-9, 10-49, ≥50, and refused to answer), number of female partners in past 3 months (none, 1, 2, ≥3, and refused to answer), and number of male anal-sex partners in past 3 months (none, 1, 2, and ≥3) represented the socio-demographic and lifestyle factors considered in this study. Nutritional status was based on the body mass index (BMI), calculated and categorized according to WHO criteria (underweight and normal weight: ≤ 24.9 kg/m2; overweight: 25.0-29.9 kg/m2; and obese: ≥ 30.0 kg/m2).(32)

Statistical analyses

Spearman correlation coefficients were used to investigate correlations between dietary patterns and response variables for duration of HPV infection. Medians (95% confidence intervals) of response variables (nutrients), and the top five food items with direct and inverse associations with the four dietary patterns were presented by tertiles of dietary pattern. Univariate linear regressions tested for trends across tertiles of dietary patterns.

Univariate logistic regressions were used to test for significant associations between socio-demographic and lifestyle factors (independent variables) and adherence to dietary pattern (dependent variable, compared tertile 3 with tertile 1, being tertile 3 the reference group).

Multivariate linear regressions were used to identify the association between the duration of HPV infection (dependent variable) and the dietary pattern scores (independent variables, in tertiles). The variables race, education, family income, marital status, smoking status, nutritional status, circumcision status, number of female partners in past 3 months, and number of male anal-sex partners in past 3 months were selected to be tested in the multivariate models. Univariate linear regression analysis was performed (**Supplement Table S2**) and, from the above variables, the ones that presented p<0.20 were selected to the multivariate models. The final model for each dietary pattern was stablished with a forward-stepwise selection. Age, total number of female partners, and HPV status at enrolment were retained in the final models because these covariates might be important factors in the epidemiology of HPV infection.(6) Also, all models were adjusted by total energy intake.(23)

SAS Studio (SAS Institute) was used to run the RRR analysis. The other analyses were performed using Stata Statistical Software (release 12, 2011, StataCorp LP, College Station, TX).(33) P<0.05 was considered statistically significant.

Results

The explained variations in food intakes was 13.0% and in response variables was 87.5%. As the fourth dietary pattern explained less than 10% of the variation in response variables, it was not further investigated.

Dietary pattern 1

The dietary pattern 1 was positively correlated with vitamin B6 (r_s = 0,75), DHA (0.73), and vitamin B12 (0.68) (**Table 1**). The food items directly associated with this pattern were fish and seafood, leaf and other vegetables, fruits, and natural juice (**Table 2**). Those men with more than 30 years of age, more than 12 years of education, who earned more than US$473.93 as family income, overweighed or obese, with more than nine lifetime female sexual partners (compared to 0-1 female partners) had more chance to highly adhere to the dietary pattern 1 (**Table 3**).

Dietary pattern 2

DFE (0.91), vitamin B6 (0.29), and DHA (0.09) were positively and vitamin B12 (-0.56) was negatively correlated with the dietary pattern 2 (**Table 1**). The food items directly associated with this pattern were fruits, bread, other vegetables, natural juice, and bean (**Table 2**). Those men with more than 30 years of age, and who were black or African American (compared to white people) had more chance to highly adhere to the dietary pattern 2. Whereas, those who earned more than US$1,421.80, smokers, overweighed (compared to under and normal weight), those with more than two female sexual partners in past 3 months, and those who had two male anal-sex partners in past three months (compared to none) had less chance to highly adhere to this pattern (**Table 3**).

Dietary pattern 3

The dietary pattern 3 was positively correlated with vitamin B6 (0,59), vitamin B12 (0.27), and DFE (0.07), and negatively correlated with DHA (-0,37) (**Table 1**). The food items directly associated with this pattern were beef and offal, natural juice, *feijoada* (a traditional Brazilian recipe, black bean stew), fruits, and beer (**Table 2**). Those men who earned more than US$1,421.80, were overweighed or obese, with more than 12 months of duration of HPV infection, and who had a positive HPV status at baseline had more chance to highly adhere to the dietary pattern 3 (**Table 3**). However, those men who were black or African American (compared to white people) had less chance to highly adhere to this dietary pattern.

Dietary patterns and duration of HPV infection

The majority of study participants had between 31-44 years of age (48.9%), with 61% reporting white race, and 37.9% reporting 12 years of education (**Table 3**). Approximately 44.5% earned from US$473.93 to US$1,421.80 per month (family income), 51.9% were either single, divorced or widowed. The majority of participants were non-smokers at the time of the study (82.4%), were classified as under or normal weight (47.5%), reported no circumcision (84.6%), presented HPV infection duration between 0-12 months (38.4%), had between 10-49 female sexual partners in their lives (39.8%), but 1 female partner in the past 3 months (35.7%), reported no male anal-sex partners in past 3 months (87.5%), and tested positive for any HPV infection at study baseline (56.1%) (**Table 3**).

The dietary patterns 1 and 2 had no association with duration of HPV infection (**Table 4**). Whereas, men with a higher adherence to the dietary pattern 3 had on average, an increase from 1.15 to 1.18 months in the duration of HPV infection, compared to those with a lower adherence to this pattern (**Table 4**).

Discussion

This study found three dietary patterns associated with DFE, vitamin B6, vitamin B12 and DHA. A dietary pattern positively correlated to vitamin B6 and vitamin B12, with a weak correlation with DFE, but negatively correlated to DHA was associated with longer duration of HPV infection. This dietary pattern was directly associated with the intake of beef and offal, natural juice, *feijoada*, fruits and beer. Furthermore, those men who adhered more to this pattern earned more on a monthly basis, had excessive weight, self-reported being more white than black or African American, and had a positive HPV status at baseline.

The persistence of HPV infection leads to a permanent genetic alteration, and subsequently, a higher risk of cancer.(7,34,35) The persistence of HPV infection is determined by viral factors, host-related factors (e.g. immune response) and environmental factors (e.g. diet).(36) Inadequate nutritional status of micronutrients may suppress immune functions, which predisposes to new infections and to increased persistence of existing ones. It has been found a protective effect of vitamin C,(11) carotenoids,(12) tocopherols,(12), vitamin B12(13) and folate(14) as HPV cofactors in cervical cancer and precursor lesions.(36,37) However, the nutrients associated with duration of HPV infection remains with inconsistent results, and only a few of them investigated men population.(13,38)

The nutrients involved in one-carbon metabolism, such as folate, vitamin B6 and vitamin B12, are of paramount importance to the biosynthesis of nucleic acid and protein, and consequently to the immune functions, once antibodies and cytokines are built up from amino acids.(27,39) These vitamins, together with other vitamins and trace elements, work in synergy to support the protective activities of the immune cells, acting on the antibody production and cellular immunity.(34) The deficiencies of folate, vitamins B6 and B12 reduce the proportion of circulating T lymphocytes and their proliferation and activity, which, in turn, decreases resistance to infections.(27) However, the biochemical mechanisms through which these deficiencies alter immune response is still unknown.(25–27)

Polyunsaturated fatty acids (PUFAs) seem to interact with the one-carbon metabolism pathway by regulating mRNA expression of enzymes of this metabolism, such as methylenetetrahydrofolate reductase, cystathionine-beta-synthase, and cystathionine-gamma-lyase.(40–43) Then, n-3 PUFA can indirectly affect immune function through its influence on the one-carbon metabolism. Also, the n-3 PUFA might regulates the expression of genes involved in immune cells functioning (genes encoding for proteins involved in cellular responses) and in the production of immune cell-derived mediators (communication between cells).(24,35)

In a previous analysis conducted within the HIM study, no association was observed between dietary intake of carbohydrate, protein, total fat, retinol, lycopene, vitamin A, alfa-carotene, beta-carotene, beta-cryptoxanthin, lutein+zeaxanthin, folate, vitamin D, vitamin E, vitamin B12 and vitamin C and persistent oncogenic-type HPV infection; however, vitamin B12 intake was inversely associated with nononcogenic HPV persistence.(44) Up to now, there is no consistent evidence for the influence of these nutrients status on the history of HPV infection in men.(7,45)

Studies exploring the intake of food groups in association with HPV-related outcomes have been conducted. The high consumption of fruits and vegetables, nuts, and fish seems to be protective, while the low consumption of the same food groups, but also tofu, meat, and yogurt are not protective against HPV persistent infection and cervical intraepithelial neoplasia.(35) In the present study, a dietary pattern positively correlated to vitamin B6, vitamin B12, fruits and beef, poorly correlated to DFE, but negatively correlated to DHA and fish, and with no correlation with vegetables or food sources of DFE was associated with longer duration of HPV infection. These results support the hypothesis that there is a synergy between these nutrients and the importance of a balanced diet instead of isolated nutrients.(35) In this sense, these results contribute to the study of the diet effect on the duration of HPV infection in men.

Alcohol intake is a strong modulator of immune system. Chronic alcohol abuse, acute and moderate alcohol intake can lead to immune deficiency and increased susceptibility to chronic and infectious diseases.(46) A study with 9,230 Korean women identified that alcohol consumption for more than or equal to 2 times a week and drinking beer for more than or equal to 3 glasses per occasion were risk factors to high risk HPV persistence.(47). Among 1,313 men from the US cohort of the HIM study, intake of alcohol was associated with an increased risk for prevalent HPV infections.(48) Despite the fact that alcohol was not a response variable in the present study, beer intake was directly associated with the dietary pattern 3 and may be contributing to the association found between this dietary pattern and duration of HPV infection.

HPV is a major problem for public health due to its high carcinogenic potential.(1) Several countries implemented the prophylactic vaccines to prevent HPV infections, which has been shown to be effective in preventing HPV infections.(49,50) The Brazilian Ministry of Health implemented the tetravalent vaccine in the routine national immunization schedules since 2014.(50) Nowadays, all girls and boys from 9 to 15 years of age and immunosuppressed people from 9 to 26 years of age must be vaccinated.(50) However, the adherence to the vaccination against HPV is still low in Brazil: only 4% of the municipalities reached the target of 80% and half of the municipalities, including São Paulo, achieved less than 50% of the targeted population.(50) Therefore, although vaccination is effective in preventing HPV infection and HPV-related outcomes, people from many developing countries are not having proper access to these services.(35) Whenever possible, policy makers and health professionals should focus on a balanced-diet prevention strategy, prioritizing wide access to healthy food, even before the HPV infection.(35) The World Cancer Research Fund reinforce this recommendation, once the consumption of a variety of whole foods naturally contain various vitamins, minerals, and bioactive compounds.(32)

To the best of our knowledge, this is the first study investigating the association of dietary patterns with HPV infection in men. Therefore, we add important findings to the discussion around the influence of diet on the history of HPV infection, particularly because the results presented here were based on a well-designed cohort study with a large sample size, using the state-of-the-art in dietary pattern analysis. The use of RRR, which is a statistic approach that allows a broadly look at diet (foods, nutrients and the synergy between them) is a strength of this study. The use of other statistics approaches to derive dietary pattern, e.g., principal component analysis, has been criticized because of their poor comparability. Even with the variation in food availability and dietary patterns when compared different populations, dietary patterns with food sources of the same nutrients, as the ones investigated here, can improve the comparability of our results. However, one potential limitation of this study is the use of a FFQ to evaluate the dietary intake, as this instrument may have recall bias of past dietary history. Secondly, the use of foreign food composition tables to derive the dietary intake of nutrients can be a limitation of this study. Unfortunately, the Brazilian food composition tables do not have data on the majority of nutrients of interest. Thirdly, the association of nutrients involved in one-carbon metabolism with duration of HPV infection was limited to the analysis of DFE, vitamin B6, vitamin B12 and DHA. As the number of response variables (nutrients) determine the number of dietary patterns, not all components of the one-carbon metabolism could be included in this analysis. Lastly, dietary intake and its association with health outcomes is a result of many factors, many of which could not be included in this study.

Conclusion

Men’s dietary pattern that provides vitamin B6 and vitamin B12 mainly via beef and offal, natural juice, *feijoada*, fruits, and beer, poorly correlates to DFE, but lacks in DHA, is associated with longer duration of HPV infection. A dietary pattern poor in plant-based foods and fish is probably unbalanced in nutrients involved in one-carbon metabolism. This population might have weaker immune functions, leading to a persistent HPV infection and an increased risk of developing cancer associated to the virus. The biological role that diet plays in HPV infection remains understudied and limited epidemiological data exist, especially among men. Thus, future research need to complement this data in order to fully investigate the relationship between diet and history of HPV infection in men.

Table 1 Explained variation (%) in food intakes and response variables for each DP as assessed using reduced rank regression and correlation coefficient between DP and response variables for duration of HPV infection in men in the HIM Study - São Paulo/Brazil.

DP	Explained variation (%)						Correlation coeficient[a]							
	Food intakes (total)	Responses (total)	DHA	B6	B12	DFE	DHA	p^*	Vitamin B6	p^*	Vitamin B12	p^*	DFE	p^*
DP1	4.57	38.6	66.2	46.0	41.8	0.4	**0.73**	<0.0001	**0.75**	<0.0001	**0.68**	<0.001	0.05	0.1070
DP2	3.34	27.6	67.5	54.9	71.4	70.8	**0.09**	0.0025	**0.29**	<0.0001	**-0.56**	<0.001	**0.91**	<0.0001
DP3	2.55	13.3	96.6	74.5	75.8	71.1	**-0.37**	<0.0001	**0.59**	<0.0001	**0.27**	<0.001	**0.07**	0.0158
DP4	2.54	8.0	97.0	82.6	87.8	82.3	**-0.09**	0.0017	**-0.33**	<0.0001	**0.34**	<0.001	**0.38**	<0.0001
	13.0	87.5												

DP: dietary pattern; DHA: docosahexaenoic acid; DFE: dietary folate equivalents. $^*p$ values of less than 0.05 were regarded as statistically significant. [a]Spearman correlation coeficient.

Table 2 Intakes of response variables and key foods across specific tertiles of dietary pattern for HPV infection in men in the HIM Study - São Paulo/Brazil

Response variables, direct and inverse associations for DP1 to DP4	**Factor loading**	**Tertiles of dietary patterns**						*Ptrend**
		T1		T2		T3		
		Median	95% CI	Median	95% CI	Median	95% CI	
DP1								
Response variables								
DHA	-	0.04	0.03,0.04	0.05	0.05,0.06	0.11	0.10,0.11	<0.001
Vitamin B6	-	1.88	1.86,1.91	2.19	2.17,2.22	2.45	2.42,2.48	<0.001
Vitamin B12	-	4.76	4.65,4.87	5.70	5.57,5.77	6.58	6.47,6.74	<0.001
DFE	-	515.6	504.8,525.6	509.5	502.7,516.8	520.8	512.1,529.6	0.62
Direct associations								
Fish and seafood	0.58	5.1	4.6,5.7	10.3	9.8,11.4	29.5	26.3,31.8	<0.001
Leaf vegetables	0.24	22.8	20.6,25.5	32.8	30.8,35.7	43.6	40.0,47.8	<0.001
Other vegetables	0.23	59.4	55.0,67.8	87.1	80.9,93.1	106.7	97.7,117.4	<0.001
Fruits	0.21	133.9	122.2,149.0	178.3	163.4,200.2	221.2	204.7,237.8	<0.001
Natural juice[a]	0.18	77.5	67.5,103.5	130.0	114.1,144.4	157.5	144.4,180.5	<0.001
Inverse associations								
Butter or margarine (regular)	-0.25	138.3	110.6,159.6	91.6	83.0,110.6	55.3	48.8,62.4	<0.001
Crackers	-0.20	26.0	23.6,30.1	18.3	16.5,20.9	10.5	9.0,11.9	<0.001
Soft drinks (regular)	-0.18	18.8	15.9,19.9	11.3	10.9,14.1	7.9	6.8,9.3	<0.001
Bread	-0.15	61.6	55.8,61.6	50.0	50.0,55.8	50.0	48.6,50.0	<0.001
Bean	-0.13	128.4	116.7,129.3	106.2	98.5,117.0	86.7	80.6,97.3	<0.001

DP: dietary pattern; T: tertile; 95% CI: 95% confidence interval; DHA: docosahexaenoic acid; DFE: dietary folate equivalents. *Linear regression analyses tested for trends across tertiles of DP. [a]Natural juices are made with fresh fruits or frozen fruit pulps, with the addition of water or not.

Table 2 Intakes of response variables and key foods across specific tertiles of dietary pattern for HPV infection in men in the HIM Study - São Paulo/Brazil. (*continued*)

Response variables, direct and inverse associations for DP1 to DP4	**Factor loading**	**Tertiles of dietary patterns**						*Ptrend**
		T1		T2		T3		
		Median	95% CI	Median	95% CI	Median	95% CI	
DP2								
Response variables								
DHA	-	0.05	0.05,0.06	0.06	0.05,0.06	0.06	0.05,0.07	<0.001
Vitamin B6	-	2.09	2.05,2.12	2.17	2.13,2.21	2.35	2.31,2.40	<0.001
Vitamin B12	-	6.36	6.26,6.50	5.61	5.50,5.71	4.86	4.73,4.97	<0.001
DFE	-	451.4	445.9,454.8	516.1	511.9,518.9	589.3	581.3,594.1	<0.001
Direct associations								
Fruits	0.45	120.1	109.8,130.1	181.6	164.3,197.4	280.8	264.1,305.3	<0.001
Bread	0.38	45.1	41.8,48.6	55.6	50.0,55.8	61.6	60.2,72.3	<0.001
Other vegetables	0.30	66.4	61.1,74.0	81.5	74.5,88.0	115.5	107.1,123.3	<0.001
Natural juice[a]	0.30	90.0	75.9,108.3	117.2	108.3,144.4	175.1	152.9,189.0	<0.001
Bean	0.25	82.1	71.5,90.4	113.3	104.8,121.1	129.0	121.3,136.0	<0.001
Inverse associations								
Beef and offal	-0.36	39.2	31.4,47.1	35.3	31.4,39.2	23.4	20.9,29.3	<0.001
Soft drinks (regular)	-0.18	117.1	103.3,138.8	110.6	92.6,130.5	50.3	40.6,55.3	<0.001
Feijoada[b]	-0.17	74.1	72.3,81.3	63.3	58.0,70.6	45.2	43.5,50.8	<0.001
Pork	-0.14	21.4	19.6,23.1	18.7	17.4,21.2	15.6	13.1,17.2	<0.001
Processed meat, sandwiches and snacks	-0.12	121.0	107.6,132.9	102.8	89.7,113.2	74.5	68.6,83.0	<0.001

DP: dietary pattern; T: tertile; 95% CI: 95% confidence interval; DHA: docosahexaenoic acid; DFE: dietary folate equivalents. *Linear regression analyses tested for trends across tertiles of DP. [a]Natural juices are made with fresh fruits or frozen fruit pulps, with the addition of water or not. [b]*Feijoada* (traditional Brazilian recipe): black bean stew.

Table 2 Intakes of response variables and key foods across specific tertiles of dietary pattern for HPV infection in men in the HIM Study - São Paulo/Brazil. (*continued*)

	Factor loading	Tertile of DP						*Ptrend**
		T1		T2		T3		
		Median	95% CI	Median	95% CI	Median	95% CI	
DP3								
Response variables								
DHA	-	0.08	0.07,0.09	0.05	0.05,0.06	0.04	0.04,0.05	<0.001
Vitamin B6	-	1.93	1.89,1.97	2.12	2.09,2.15	2.42	2.39,2.45	<0.001
Vitamin B12	-	5.22	5.11,5.31	5.59	5.45,5.68	6.02	5.85,6.22	<0.001
DFE	-	515.2	503.8,526.0	510.7	502.8,518.5	519.9	512.2,528.9	0.09
Direct associations								
Beef and offal	0.36	50.8	45.1,54.2	58.0	57.8,65.1	74.1	72.5,79.8	<0.001
Natural juice[a]	0.21	89.1	75.9,106.5	124.1	108.3,144.4	153.1	144.4,178.7	<0.001
Feijoada[b]	0.20	29.3	22.6,31.4	31.4	29.3,35.3	41.1	35.3,47.1	<0.001
Fruits	0.19	154.2	139.9,168.4	161.8	148.0,175.6	226.9	207.7,242.2	<0.001
Beer	0.17	41.0	28.9,57.8	78.4	54.2,113.2	101.5	75.2,141.8	<0.001
Inverse associations								
Fish and seafood	-0.53	20.3	16.8,24.4	11.4	9.8,12.1	8.5	6.9,9.3	<0.001
Desserts and sweets	-0.26	52.9	47.5,60.1	40.6	37.2,44.5	32.8	30.1,37.1	<0.001
Crackers	-0.24	24.8	22.8,27.6	16.9	14.3,19.4	12.7	11.1,14.8	<0.001
Butter or margarine (regular)	-0.23	15.6	13.2,17.2	14.0	12.3,15.4	8.2	7.7,10.2	<0.001
Bread	-0.20	56.7	55.8,61.6	51.4	50.0,55.8	50.0	50.0,50.0	<0.001

T: tertile; 95% CI: 95% confidence interval; DP: dietary pattern; DHA: Docosahexaenoic acid; DFE: dietary folate equivalents. *Linear regression analyses tested for trends across tertiles of DP. [a]Natural juices are made with fresh fruits or frozen fruit pulps, with the addition of water or not. [b] *Feijoada* (traditional Brazilian recipe): black bean stew.

Table 3 Socio-demographic and lifestyle factors of men according to their dietary patterns in the HIM Study - São Paulo/Brazil.

Characteristics	Total	DP1	DP2	DP3
	n (%)	OR (95% CI)*		
Age (years)				
18-30	452 (37.9)	1.00	1.00	1.00
31-44	584 (48.9)	**1.91 (1.41,2.59)**	**1.51 (1.11,2.03)**	0.92 (0.68,1.24)
45-70	158 (13.2)	**2.79 (1.75,4.46)**	**2.32 (1.47,3.67)**	0.98 (0.63,1.52)
Race				
White	720 (61.0)	1.00	1.00	1.00
Black or African American	353 (29.9)	0.83 (0.61,1.14)	**1.44 (1.05,1.97)**	**0.60 (0.43,0.83)**
Others[a]	108 (9.1)	1.55 (0.93,2.59)	1.16 (0.71,1.89)	1.01 (0.63,1.62)
Education (years)				
<12	237 (20.0)	1.00	1.00	1.00
12	448 (37.9)	0.85 (0.57,1.26)	0.95 (0.64,1.40)	1.03 (0.70,1.51)
13-15	166 (14.0)	**2.89 (1.76,4.77)**	1.03 (0.63,1.69)	1.21 (0.75,1.96)
≥16	332 (28.1)	**1.72 (1.14,2.59)**	0.77 (0.51,1.16)	1.28 (0.85,1.93)
Family income (US$)[b]				
≤ 473.93	366 (32.6)	1.00	1.00	1.00
473.93 ┤ 1,421.80	499 (44.5)	**1.64 (1.17,2.30)**	0.81 (0.58,1.14)	1.14 (0.82,1.59)
> 1,421.80	257 (22.9)	**5.80 (3.76,8.94)**	**0.42 (0.28,0.62)**	**1.48 (1.00,2.18)**
Marital status				
Single, divorced or widowed	616 (51.9)	1.00	1.00	1.00
Married or cohabiting	570 (48.1)	0.93 (0.70,1.23)	0.95 (0.72,1.25)	1.12 (0.85,1.48)
Current smoker				
No	975 (82.4)	1.00	1.00	1.00
Yes	208 (17.6)	0.75 (0.53,1.08)	**0.61 (0.42,0.88)**	1.06 (0.73,1.52)
Nutritional status[c]				
Under and normal weight	567 (47.5)	1.00	1.00	1.00
Overweight	442 (37.0)	**1.58 (1.16,2.15)**	**0.66 (0.49,0.90)**	**1.68 (1.24,2.29)**
Obese	185 (15.5)	**2.30 (1.52,3.48)**	0.68 (0.45,1.01)	**1.66 (1.10,2.50)**
Circumcision status				
No	1,008 (84.6)	1.00	1.00	1.00
Yes	184 (15.4)	1.23 (0.82,1.84)	1.21 (0.81,1.79)	1.25 (0.85,1.86)
Duration of HPV infection (months)				
0-12	459 (38.4)	1.00	1.00	1.00
18-24	398 (33.3)	0.94 (0.68,1.31)	0.94 (0.67,1.30)	**1.40 (1.01,1.94)**
30	337 (28.2)	1.09 (0.77,1.54)	1.15 (0.82,1.63)	**1.56 (1.10,2.21)**

DP: dietary pattern; T: tertile. *Univariate logistic regression analysis was used to determine if any significant differences existed regarding socio-demographic and lifestyle factors (independent variables) according to the men's adherence to the DP's (dependent variable, T1=0, T2=., and T3=1). Missing data in the variables lifetime female sexual partners (9), race (13), education (11), family income (72), marital status (8), current smoker (11), circumcision status (2), female partners in past 3 months (665), and male anal-sex partners in past 3 months (6). [a]The category 'Others' includes Asian, American Indian or Alaskan Native or Other populations. [b]Family income converted in United States Dollar (US$). The mean of currency rates at the time of the study (July/2005-September/2009) was US$1.00 = R$2.11. [c]BMI; under and normal weight: ≤ 24.9 kg/m2 ; overweight: 25.0-29.9 kg/m2; and obese: ≥ 30.0 kg/m2. Prevalence of underweight in this population was 1.7% (n=20).

Table 3 Socio-demographic and lifestyle factors of men according to their dietary patterns in the HIM Study - São Paulo/Brazil. (*continued*)

Characteristics	**Total** 1,194 (100%) n (%)	**OR (IC 95%)***	**OR (IC 95%)***	**OR (IC 95%)***
Lifetime female sexual partners				
0-1	178 (15.0)	1.00	1.00	1.00
2-9	326 (27.5)	1.12 (0.72,1.75)	0.70 (0.45,1.10)	1.31 (0.84,2.06)
10-49	472 (39.8)	**1.83 (1.21,2.75)**	0.71 (0.47,1.09)	1.33 (0.87,2.04)
≥50	96 (8.1)	**2.69 (1.50,4.82)**	0.89 (0.48,1.65)	1.35 (0.73,2.48)
Refused to answer	113 (9.5)	1.41 (0.80,2.48)	0.75 (0.43,1.33)	0.97 (0.54,1.74)
Female partners in past 3 months				
None	154 (29.1)	1.00	1.00	1.00
1	189 (35.7)	0.83 (0.49,1.41)	0.73 (0.44,1.22)	1.19 (0.72,1.99)
2	80 (15.1)	0.95 (0.50,1.82)	0.96 (0.48,1.91)	1.19 (0.60,2.37)
≥3	83 (15.7)	1.90 (0.98,3.68)	**0.38 (0.20,0.74)**	1.56 (0.77,3.16)
Refused to answer	23 (4.4)	0.66 (0.24,1.82)	0.89 (0.33,2.42)	0.91 (0.33,2.50)
Male anal-sex partners in past 3 months				
None	1,040 (87.5)	1.00	1.00	1.00
1	56 (4.7)	0.83 (0.43,1.61)	1.07 (0.56,2.06)	1.31 (0.65,2.61)
2	37 (3.1)	0.98 (0.43,2.20)	**0.32 (0.13,0.82)**	1.03 (0.46,2.33)
≥3	55 (4.6)	0.71 (0.39,1.31)	1.02 (0.52,1.97)	1.51 (0.73,3.10)
HPV status at baseline				
No	524 (43.9)	1.00	1.00	1.00
Yes	670 (56.1)	1.15 (0.87,1.52)	1.14 (0.86,1.51)	**1.40 (1.06,1.86)**

DP: dietary pattern; T: tertile. *Univariate logistic regression analysis was used to determine if any significant differences existed regarding socio-demographic and lifestyle factors (independent variables) according to the men's adherence to the DP's (dependent variable, T1=0, T2=., and T3=1). Missing data in the variables lifetime female sexual partners (9), race (13), education (11), family income (72), marital status (8), current smoker (11), circumcision status (2), female partners in past 3 months (665), and male anal-sex partners in past 3 months (6).

Table 4 Adjusted associations of men's dietary patterns with duration of HPV infection in the HIM study - Sao Paulo/Brazil‡

	Duration of HPV infection[a]
	β (95%CI)
Dietary patterns	
Dietary pattern 1	
T1	1.00
T2	-0.18 (-1.26,0.89)
T3	-0.18 (-1.25,0.90)
Dietary pattern 2	
T1	1.00
T2	0.00 (-1.07,1.07)
T3	-0.08 (-1.14,0.99)
Dietary pattern 3[b]	
T1	1.00
T2	**1.18 (0.11,2.24)**
T3	**1.15 (0.09,2.21)**

HPV: human papilloma virus; 95% CI: 95% confidence interval. ‡All four models presented $p<0.0001$ and were adjusted by age (years), number of lifetime female sexual partners, HPV status at baseline, and total energy. Loss of 9 individuals in the models because of missing data in the variable lifetime female sexual partners. *p* values of less than 0.05 were regarded as statistically significant. [a]Duration of HPV infection, the dependent variable in these univariate linear regression models, vary from 0 to 30 months (mean=17.9 months (SD: 10.8), median: 18 months (IQR: 6-30)). [b]*p-trend*: 0.033

Supplement Table S1 Grouping description, its frequency of intake and daily amount of intake of men in the HIM Study - São Paulo/Brazil.

Food items	Intake (%)	Daily amount of intake among consumers (g/day) Median (95% CI)
FFQ items		
Soups	92.9	15.1 (13.6,15.7)
Beef and offal	98.3	63.1 (58.0,65.3)
Chicken	97.9	27.9 (27.9,30.5)
Fish and seafood	96.0	12.2 (11.4,13.3)
Whole milk	84.8	119.9 (111.5,126.8)
Low-fat milk	17.1	77.2 (77.2,79.2)
Skimmed milk	11.3	77.2 (77.2,81.4)
Yogurt (whole milk)	44.3	15.3 (15.3,18.9)
Yogurt with flavor (whole milk)	73.9	22.1 (18.7,27.8)
Mozzarella cheese	95.2	8.6 (8.4,9.0)
White cheese	82.5	3.7 (3.5,4.4)
Egg	97.7	20.6 (18.4,20.6)
Bean	99.5	105.7 (104.4,112.2)
Feijoada[a]	90.5	35.3 (35.0,39.2)
Rice	99.9	163.5 (161.0,163.5)
French fries, cassava (fried)	95.3	7.6 (7.0,8.2)
Potato salad, with vegetables and mayonnaise	87.4	10.3 (9.0,11.3)
Cassava or corn (flour)	92.5	8.2 (6.9,8.3)
Oil (for salad dressing)	96.0	3.6 (3.5,3.9)
Mayonnaise, salad dressing	57.7	1.8 (1.4,2.3)
Natural juice[b]	95.9	130.0 (116.8,144.4)
Artificial juice[c]	91.3	7.4 (7.0,8.1)
Coffee/tea (unsweetened)	46.1	67.4 (62.4,71.4)
Coffee/tea (sweetened)	81.9	200.2 (200.2,214.5)
Soft drinks (regular)	89.7	110.6 (95.9,110.6)
Soft drinks (diet/light)	17.5	42.9 (42.0,63.1)
Beer	78.6	133.7 (116.8,170.5)
Bread	99.9	51.8 (50.0,55.8)
Butter or margarine (regular)	88.1	14.3 (14.0,15.6)
Butter or margarine (light)	26.5	10.9 (9.4,10.9)
Chocolate milk (powder)	73.2	4.0 (3.9,4.4)
Sugar, honey, jam	88.7	9.0 (7.8,9.6)
Grouped FFQ items		
Pasta[d]	99.3	64.1 (61.1,67.0)
Processed meat, sandwiches and snacks[e]	99.9	98.5 (90.8,105.9)
Potato, cassava, polenta[f]	98.7	9.6 (9.1,10.0)
Pork[g]	96.8	19.2 (18.2,20.4)
Leaf vegetables[h]	99.3	32.8 (31.3,34.6)
Other vegetables[i]	99.4	85.6 (82.3,90.3)
Fruits[j]	99.6	176.0 (168.2,185.7)
Crackers[k]	94.3	18.8 (17.5,20.9)
Desserts and sweets[l]	99.6	41.3 (38.6,43.8)

95%CI - 95% confidence interval; FFQ – food frequency questionnaire. [d]Pasta with or without meat sauce, gnocchi, lasagna. [e]Hamburger, chicken nuggets, meatball, lunchmeats - ham, mortadella and salami; sandwiches - hot dog, hamburger; fried snacks, baked savory, pizza, and savory pancakes. [f]Potato or cassava (boiled or roasted), polenta. [g]Pork (loin and chops), pork sausage. [h]Lettuce, spinach, arugula, watercress, cabbage, chard, cauliflower, broccoli. [i]Tomato, carrot, eggplant, cucumber, zucchini, and chayote. [j]Orange, tangerine, banana, apple, pear, papaya, melon, watermelon. [k]Savory and sweetened crackers. [l]Cakes (with or without filling), chocolate, and bakery desserts - sweet pies, pudding, mousse.

Supplement Table S2 Unadjusted associations of men's dietary patterns, socio-demographic and lifestyle factors with duration of HPV infection in the HIM study - Sao Paulo/Brazil.

Men's dietary patterns, socio-demographic and lifestyle factors	Duration of HPV infection[a]	
	β (95% CI)	*p**
DP1		
T1	1.00	0.3889
T2	1.03 (-0.47,2.54)	
T3	0.68 (-0.83,2.18)	
DP2		
T1	1.00	0.9194
T2	0.27 (-1.23,1.78)	
T3	0.27 (-1.23,1.78)	
DP3		
T1	1.00	**0.0024**
T2	2.00 (0.50,3.50)	
T3	2.52 (1.02,4.01)	
Age (years)		
18-30	1.00	0.6112
31-44	-0.65 (-1.98,0.68)	
45-70	-0.60 (-2.56,1.37)	
Lifetime female sexual partners		
0-1	1.00	**<0.0001**
2-9	1.55 (-0.34,3.43)	
10-49	7.35 (5.58,9.13)	
≥50	9.16 (6.60,11.72)	
Refused to answer	6.33 (3.90,8.76)	
HPV status at baseline		
No	1.00	**<0.0001**
Yes	15.16 (14.27,16.05)	
Race		
White	1.00	0.6862
Black or African American	-0.51 (-1.89,0.87)	
Others[b]	-0.69 (-2.89,1.50)	
Education (years)		
<12	1.00	0.4553
12	0.20 (-1.51,1.90)	
13-15	0.93 (-1.21,3.08)	
≥16	-0.66 (-2.46,1.14)	

HPV: human papilloma virus; 95% CI: 95% confidence interval. **p* values of less than 0.05 were regarded as statistically significant. Missing data in the variables lifetime female sexual partners (9), race (13), education (11), family income (72), marital status (8), current smoker (11), circumcision status (2), female partners in past 3 months (665), and male anal-sex partners in past 3 months (6). [a]Duration of HPV infection, the dependent variable in these univariate linear regression models, vary from 0 to 30 months (mean=17.9 months (SD: 10.8), median: 18 months (IQR: 6-30)). [b]The category 'Others' includes Asian, American Indian or Alaskan Native or Other populations.

Supplement Table S2 Unadjusted associations of men's dietary patterns, socio-demographic and lifestyle factors with duration of HPV infection in the HIM study - Sao Paulo/Brazil. (*continued*)

Men's dietary patterns, socio-demographic and lifestyle factors	Duration of HPV infection[a]	
	β (95% CI)	*p**
Family income (US$)[c]		
≤ 473.93	1.00	**0.0482**
473.93 ┤ 1,421.80	1.83 (0.37,3.28)	
> 1,421.80	1.05 (-0.67,2.76)	
Marital status		
Single, divorced or widowed	1.00	**0.0001**
Married or cohabiting	-2.45 (-3.67,-1.23)	
Current smoker		
No	1.00	**<0.0001**
Yes	3.38 (1.77,4.98)	
Nutritional status[d]		
Under and normal weight	1.00	0.6013
Overweight	0 (-1.35,1.34)	
Obese	-0.87 (-2.67,0.92)	
Circumcision status		
No	1.00	0.2783
Yes	-0.94 (-2.64,0.76)	
Female partners in past 3 months		
None	1.00	**<0.0001**
1	2.67 (0.47,4.88)	
2	3.63 (0.83,6.43)	
≥3	7.98 (5.21,10.75)	
Refused to answer	2.95 (-1.59,7.49)	
Male anal-sex partners in past 3 months		
None	1.00	0.7131
1	-0.24 (-3.15,2.67)	
2	1.54 (-2.01,5.09)	
≥3	-1.12 (-4.05,1.81)	

HPV: human papilloma virus; 95% CI: 95% confidence interval. **p* values of less than 0.05 were regarded as statistically significant. Missing data in the variables lifetime female sexual partners (9), race (13), education (11), family income (72), marital status (8), current smoker (11), circumcision status (2), female partners in past 3 months (665), and male anal-sex partners in past 3 months (6). [a]Duration of HPV infection, the dependent variable in these univariate linear regression models, vary from 0 to 30 months (mean=17.9 months (SD: 10.8), median: 18 months (IQR: 6-30)). [c]Family income converted in United States Dollar (US$). The mean of currency rates at the time of the study (July/2005-September/2009) was US$1.00 = R$2.11. [d]BMI; under and normal weight: ≤ 24.9 kg/m^2 ; overweight: 25.0-29.9 kg/m2; and obese: ≥ 30.0 kg/m^2. Prevalence of underweight in this population was 1.7% (n=20).

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4.4 QUARTO MANUSCRITO - *DIETARY PATTERN INFLUENCES HOMOCYSTEINE LEVEL MORE THAN SPECIFIC NUTRIENTS: A STRUCTURAL EQUATION MODEL APPROACH*

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Abstract

Purpose: A structural equation model (SEM) was used to test multiple and simultaneous relationships between socio-demographic factors, dietary patterns, biochemical levels of folate, vitamin B12, docosahexaenoic acid (DHA), and its effects on homocysteine (Hcy) level. **Methods**: Socio-demographic and lifestyle characteristics, blood sample, anthropometric measurements and a food frequency questionnaire (FFQ) was obtained from 281 individuals of ISA-Capital study (Sao Paulo, Brazil). The dietary patterns (DP) were estimated using factor analysis with principal component's estimation based on the frequency of daily intake derived from the 38-item FFQ. The SEM considered a theoretical model where the DP were expected to be directly associated to Hcy level, and indirectly via biochemical levels of folate, vitamin B12 and DHA. The variables sex, age, ethnicity and MTHFR C677T polymorphism were included in the model. **Results**: The Prudent DP (-0.12, p=0.04) had a negative effect, while MTHFR C677T polymorphism (0.16, p=0.01), age (0.22, p<0.01), and being man (0.16, p=0.01) had a positive effect on Hcy level. There were no indirect effects of any dietary patterns on Hcy level, neither via folate, vitamin B12, nor DHA. DHA was negatively associated with the Modern DP (-0.12, p=0.04) and positively associated with the Prudent DP (0.19, p<0.01). **Conclusions**: The DP mainly composed of fruits and vegetables, natural juices, potato/cassava/cooked cornmeal, fish, and chicken, was negatively associated to the Hcy level in this population. These findings support the role of a healthy dietary pattern in health outcomes, rather than promoting specific foods or nutrients, for policy-based health promotion strategies.

Keywords: one-carbon metabolism; folate; homocysteine; MTHFR; structural equation model

Introduction

Elevated homocysteine, i.e. hyperhomocysteinaemia (HHcy), has been associated with an increased risk of cardiovascular and cerebrovascular diseases,[1] such as atherosclerosis, Alzheimer's disease, and dementia.[2] Some types of cancer and bone fracture have also been associated with high levels of Hcy.[2] The definition of HHcy differs between studies, but in the general population its prevalence varies from 5% to 10%,[3] and can achieve from 30% to 40% among elderly.[4, 5] Genetic factors (polymorphism of enzymes involved in one-carbon metabolism), physiological factors (age and sex), use of medications known to affect one-carbon metabolism (folate, vitamins B6 and B12 antagonists, and sex steroids), clinical conditions (folate, vitamins B6 and B12 deficiencies and renal failure), and lifestyle factors (smoking habit and intake of alcohol, coffee, folate and vitamins B2, B6 and B12) are among the factors who contribute most to the Hcy level.[6]

The relationship between Hcy concentration and intakes of folate, vitamins B6 and B12 is well established, being low Hcy level considered a sensitive marker for the nutritional adequacy of these vitamins.[7, 8] Polyunsaturated fatty acids (PUFAs) seem to interact with the one-carbon metabolism pathway and also have being associated with decreased Hcy level.[9, 10] Intervention studies verified that n-3 PUFA could decrease plasma Hcy.[9, 10] A recent meta-analysis including eleven placebo-controlled trials suggested that n-3 PUFA supplementation decreased plasma Hcy concentration.[11] The biological mechanism behind this association might be by PUFAs regulating mRNA expression of enzymes involved in one-carbon metabolism, such as methylenetetrahydrofolate reductase (MTHFR), cystathionine-beta-synthase (CBS), and cystathionine-gamma-lyase (CSE).[12, 13] However, this relationship needs to be better elucidated.

More recently, studies exploring the intake of foods and the overall diet emerged as an important research field complementary to single component studies in nutrition.[14] Some studies explored the association of Hcy with foods and dietary patterns. The increased intake of breakfast cereals, fruits and vegetables was associated with higher folate and lower Hcy concentrations in elderly participants from Framingham Heart Study, independently of sex.[15] In the Hordaland Homocysteine Study, the intake of vegetables, fruits, cereals, eggs, fish and milk, and non-processed meats was inversely associated with Hcy concentration.[16] On the other hand, people who adhered to a refined cereals dietary pattern were more likely to have higher Hcy concentrations in an urban Chinese population.[17] In Brazil, one study showed that the dietary intake of fruits, vegetables and beans, i.e. natural folate-rich foods, was not associated with plasma Hcy level in adolescents living in Sao Paulo.[18] There are no data on whether interactions between habitual dietary intake, biochemical levels of vitamins and PUFA, and polymorphisms of one-carbon metabolism influencing Hcy level in adult populations.

In the current study we aimed to extend this research by testing an integrated theoretical model in predicting Hcy levels. Considering the dietary behavior can influence Hcy levels, a structural equation modelling method was used to test: (1) which nutrients from one-carbon metabolism pathway are most determining for Hcy level; (2) the association of PUFA with Hcy level; and (3) how far is the relationship between dietary patterns and Hcy level and how far mediated by vitamins and PUFA biochemical levels.

Methods

Study population and design

This study analyzed data from the cross-sectional population-based study Health Survey of the City of São Paulo (ISA-Capital study), which was designed to collect health and nutritional information, life conditions and health service use. The participants were selected in a two-stage cluster sampling of census tracts and households to obtain a probabilistic sample of individuals living in permanent homes located in the urban area of the city of São Paulo-Brazil. Details about study design can be found in a previous publications.[19] Between 2008 and 2009, a total of 1,162 adults (20 to 59 years old) were interviewed at their homes and answered a structured questionnaire about socio-demographic and lifestyle factors. In a second wave, occurred between 2010 and 2011, the participants provided blood sample, had their blood pressure and anthropometric measurements assessed during a home visit. One week before, a food frequency questionnaire (FFQ) was answered by phone interviewing. For the present analysis, all adults with no missing data for the variables evaluated were included (n=281) (**Figure 1**).

Blood samples

Participants had blood samples taken after 12-hour fasting by skilled nursing professionals using standard procedures. Approximately 20ml of blood were collected from each participant in dried and EDTA (ethylenediaminetetraacetic acid) vacutainers. The tubes were immediately wrapped in aluminum foil to avoid photodegradation of biomarkers. After a maximum of two hours maintained in a polystyrene box with ice packs during transportation, the blood samples were centrifuged at 3,000 rpm for 15 minutes and processed into serum and plasma aliquots. All aliquots were stored at -80°C for further biochemical analysis, excepting for part of the plasma aliquot in EDTA tube used for DNA extraction.

Assessment of exposures

Dietary intake: food frequency questionnaire and dietary pattern analysis

The dietary intake of the population studied was evaluated using a 38-item FFQ (interview-based) where the participants were asked about the frequency of their consumption over the past year. In an attempt to improve the accuracy of diet effect on Hcy level, dietary patterns were estimated using factor analysis with principal component's estimation and were based on the frequency of daily intake derived from the FFQ.[14] The dietary pattern analysis were performed using Stata Statistical Software (release 12, 2011, StataCorp LP, College Station, TX).[20] In a correlation matrix, the food item 'cassava flour, farofa, oat and tapioca' did not present correlation with any other item and was not included in the dietary pattern analysis. To identify the number of dietary patterns to be retained, the eigenvalue >1.0 criterion, the Scree test analysis, and the interpretation of each factor were considered, resulting in three dietary patterns. Varimax orthogonal rotation was performed to maximize the factor loadings assuming nonrelated factors. Rotated factor loadings > 0.20 or < -0.20 were considered to significantly contribute to a pattern. We excluded 'pork' (factor1=0.11, factor2=0.10, and factor3=-0.13), 'beef jerky' (factor1=0.06, factor2=0.03, and factor3=-0.04), 'smoked meat' (factor1=-0.03, factor2=0.03, and factor3=-0.05), 'artificial juices' (factor1=0.03, factor2=0.01, and factor3=-0.01), and 'distilled alcoholic beverage' (factor1=0.07, factor2=0.03, and factor3=-0.16) from the final analysis because they did not load on any of the retained factors.

Nutritional status: biochemical levels of folate, vitamin B12, and docosahexaenoic acid (DHA)

Plasma 5-methyltetrahydrofolate (5-mTHF) and circulating folic acid (unmetabolized folic acid) measurements were determined in the Vitamin Metabolism Laboratory/Jean Mayer USDA HNRCA at Tufts University by the affinity-HPLC method with electrochemical detection[21] with the modifications to measure the different form of the vitamins in the blood.[22] Total folate (pmol/mL) was the sum of 5-mTHF and circulating folic acid. Vitamin B12 was assayed using the Access Immunoassay Systems® which is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of vitamin B12 levels in human serum and plasma.[23] Total plasma lipids were extracted using a mixture of methanol and chloroform chromatographic solution (2:1, vol/vol), and fatty acids (FA) were converted to FA methyl esters using a modified sodium methoxide method.[24] Then, the FA profile was measured using flame-ionization gas chromatography on a device (SHIMADZU, CG-2010, Kyoto, Japan) equipped with a DB-FFAP capillary column (15 m x 0.100 mm x 0.10 μm [J&W Scientific from Agilent Technologies, Folsom, CA, USA]). The PUFA docosahexaenoic acid (DHA) was quantified as the area under the peak and results expressed as percentages of the total area of all FA peaks.[25] Mean inter-assay coefficients of variation were 3.4% for total folate, 8.5% for vitamin B12, 8.8% for DHA, and 4.0% for Hcy.

Total folate (pmol/mL), vitamin B12 (pg/ml), and DHA (% 20:6 n-3) were treated as continuous variables.

Assessment of outcome

The biochemical level of Hcy was considered the outcome in this study. The Hcy level was assessed by chemiluminescent microparticle immunoassay (CMIA) using commercials Homocysteine Reagent Kits from Abbott Diagnostics Division.[26] All assays were performed according to manufacturers' instructions.

Covariates

Sex (male/female), age (years), years of education (<10 years of education/≥ 10 years of education), ethnicity (self-reported skin color divided in white/non-white), smoking habit (yes/no), alcohol intake (yes/no), body mass index (BMI, under and normal weight/overweighed/obese), physical activity (The International Physical Activity Questionnaires – IPAC long version; insufficiently active/active/very active), and C677T polymorphism for methylenetetrahydrofolate reductase (MTHFR) (yes/no) were used to describe the population.

BMI was calculated using the weight and height measured at the second wave visit and was then categorized in accordance with WHO criteria.[27] DNA was extracted and isolated using a salt out method.[28] The amount was determined using the NanoDrop® 1000 Spectrophotometer (Wilmington, DE, USA). The polymorphisms were genotyped by allele-specific polymerase chain reaction amplification.[29] Lastly, the MTHFR C677T polymorphism (rs1801133) was analyzed. The MTHFR C677T polymorphism was categorized in two categories: MTHFR 677TT genotype (homozygous for mutation) plus MTHFR 677CT (heterozygous for mutation) genotype versus MTHFR 677CC genotype (wild type).

Statistical Analysis

Homocysteine (µmol/mL) was divided into tertiles according to sex and the third tertile was recoded as 1 as an indicator of possible increased cardiovascular risk compared to the first and second tertiles (recoded as 0). Kruskal-Wallis tests for continuous variable and chi-square tests for categorical variable were implemented to identify significant differences among groups with different levels of Hcy (1st/2nd tertiles versus the 3rd tertile) according to socio-demographic and lifestyle factors, nutritional status, MTHFR C677T polymorphism, vitamins and DHA levels.

The population was divided in above or below the median biochemical levels of total folate and DHA. Kruskal-Wallis tests were applied to identify differences in Hcy level (continuous) according to levels of folate and DHA.

These analyzes were performed using Stata Statistical Software (release 12, 2011, StataCorp LP, College Station, TX).[20]

Structural Equation Model

Structural equation models (SEM) provide estimates of the strength of all the hypothesized relationships between variables in a theoretical model, both directly from one variable to another and via other variables positioned between the other two.[30] For the current analysis, the SEM considered a conceptually derived model specifying the relationships among the exposure variables, the covariate variables and the outcome (understanding complex patterns of interrelationships among them) (**Figure 2**). The dietary patterns were expected to be related to Hcy level via biochemical levels of nutrients involved in one-carbon metabolism and DHA under the hypothesis that dietary pattern can influence the biochemical levels of these nutrients and, consequently, the Hcy level. Direct effects of dietary patterns on Hcy level were estimated as well, assuming that unknown nutrients, components and its interaction can influence Hcy level. DHA was expected to influence Hcy levels due to its potential effect on the regulation of the MTHFR.[31] The variables sex, age, ethnicity and MTHFR C677T polymorphism were controlled in the model (**Figure 2**).

All continuous variables were standardized (component scores of dietary patterns, total folate, vitamin B12, DHA, homocysteine and age). The standardized coefficient expresses the effect of a predictor on the outcome variable in standard deviation units. This allows comparisons between the effects of predictors measured using different scales. There was no substantial multicollinearity (> 0.70 or < -0.70) between variables, checked with a correlation matrix. We estimated goodness of fit using the root mean square error of approximation (RMSEA), where 90% confidence interval upper limit smaller than 0.08 were considered indicative of poor-fitting models.[32, 33] High values of Comparative Fit Index (CFI) and Tucker Lewis index (TLI) (greater than 0.90) were considered as indicative of a good-fitting model.

Modification index tests were evaluated in order to identify significant correlations between measurement error terms (i.e. residuals) of variables that would improve model fit. Only plausible correlations were allowed.[30] Double-headed path were added between DHA and vitamin B12, because of large residual variances between them. Correlations between error terms of the three dietary patterns were also included, considering that errors for two or more dietary patterns may be correlated.

The conceptually derived model was tested in R software (R Core Team 2016) using lavaan package.[34]

Results

Over one-half (52.0%) of the participants had a MTHFR C677T polymorphism, 63.3% were women, 55.0% had less than 10 years of formal education, 58% self-reported white skin color, 47.3% had under or normal weight, 74.7% were non-smokers, 51.6% reported non-intake of alcoholic beverages, and 40.7% were insufficiently active (**Table 1**). The median Hcy level of this population was 7.8 µmol/mL (95% Confidence Interval (CI) = 7.5,8.3) and 5.3% had HHcy, considering a cut off of 15 µmol/mL.[5] The group with higher Hcy levels (3rd tertile, p<0.001) were older (p=0.004), were more likely to self-report non-white skin color (p=0.016), presented more frequently the MTHFR C677T polymorphism (p=0.002), and had lower biochemical levels of folate (p=0.011) and vitamin B12 (p<0.001) when compared with the 1st/2nd tertiles of Hcy level (**Table 1**).

Dietary patterns

The retained dietary patterns accounted for 26% of the variance in food intake. The highest factor loadings and previous dietary pattern studies within the ISA-Capital study were considered to name the patterns resulting in Modern, Traditional, and Prudent dietary patterns. Frankfurter, cake, potato/cassava/cooked cornmeal (fried and boiled), hamburger, beer, soft drink, pizza, egg (boiled or fried), lunchmeats (ham/mortadella), pasta/lasagna/gnocchi, crackers, desserts and sweets, fried snacks, and cheese significantly contributed to the Modern dietary pattern. The Traditional dietary pattern had the main contribution of butter/margarine, bread, milk, yogurt, bean, wine, rice, natural juice (freshly squeezed), sausage (pork/chicken), coffee (sweetened), hamburger (processed meat), baked savory, and beef. Vegetables (green leafy vegetables and others), fish, fruits, chicken, natural juice (freshly squeezed), and potato/cassava/cooked cornmeal (boiled or roasted) significantly contributed to constitute the Prudent dietary pattern. (**Table 2**).

Differences in Hcy levels according to MTHFR C677T polymorphism, folate and DHA levels

The median level of Hcy was higher in those with polymorphism (8.4 µmol/mL, IC95%=7.9,9.1) in comparison with those without this polymorphism (7.4 µmol/mL, IC95%=7.1,7.7) (p=0.001) (data not shown in table).

The median biochemical level of the population was 26.2 pmol/mL for total folate and 2.0 (% 20:6 n-3) for DHA, and these values were considered to categorize the population in above or below the median for each of the nutrients. Considering those with total folate level > 26.2 pmol/mL, the median Hcy level was higher for those with MTHFR C677T polymorphism when compared with those without this polymorphism (7.2 vs 8.0 µmol/mL; p=0.018) (data not shown in table). The difference in the median Hcy level was not statistically different among those with and without MTHFR C677T polymorphism when the individuals presented not only total folate level > 26.2 pmol/mL, but also DHA level > 2.0 (% 20:6 n-3) (7.2 vs 7.3 µmol/mL; p=0.172) (**Figure 3**).

Hcy levels by exposure variables: structural equation model

The goodness-of-fit indices indicated an acceptable fit of the SEM (RMSEA: 0.027; 90%CI 0.000,0.063; CFI: 0.968; TLI: 0.908). The Prudent dietary pattern had a negative direct effect on level of Hcy (-0.12, p=0.04) and positive direct effect on level of DHA (0.19, p=0.001). Folate, vitamin B12 and DHA biochemical levels did not mediate the relationship between dietary patterns and Hcy (*p* values varied from 0.27 to 0.92) and no other indirect effect was found in any of the presented relationships. Neither folate (-0.02, p=0.80), vitamin B12 (0.01, p=0.92), nor DHA (-0.07, P=0.24) had a direct effect on level of Hcy. Yet, the levels of folate were negatively influenced by the presence of MTHFR C677T polymorphism (-0.19, p<0.01). The model adjustments revealed a positive association of age (0.21, p<0.01) and negative association of male sex (-0.12, p=0.03) with the Prudent dietary pattern. Sex was also positively associated with the Traditional dietary pattern (0.19, p<0.01). Non-white ethnicity (0.12, p=0.03) and male sex (0.17, p<0.01) were positively and age (-0.22, p<0.01) was negatively associated with the Modern dietary pattern. Age (0.22, p<0.01), sex (0.16, p=0.01), and MTHFR C677T polymorphism (0.16, p=0.01) had a positive direct effect on Hcy levels (**Figure 4**).

Discussion

In this study, the Prudent dietary pattern had a negative direct effect, while MTHFR C677T polymorphism, age, and being man had a positive direct effect on Hcy level. In contrast to what was expected, the SEM showed no indirect effect of any dietary patterns on Hcy level, neither via folate, vitamin B12, nor DHA. DHA was negatively associated with the Modern dietary pattern and was positively associated with the Prudent dietary pattern.

Hcy is a sulfur amino acid that, when in high levels, is associated with increased total and cardiovascular mortality and with a sort of negative outcomes in health, such as, hypertension,[35] stroke, dementia and Alzheimer's disease,[36] schizophrenia,[37] depression,[38] bone fracture, preeclampsia and maybe neural tube defects.[2] Elevated Hcy level has a potential neurotoxic effect and also may lead to endothelial dysfunction, smooth cell proliferation and vascular calcification, ending in arteriosclerotic vessel wall degeneration, which, taken together, could led to the development of cardiovascular and cerebrovascular diseases.[1, 38, 39] However, if the Hcy is a risk factor or a biomarker in the relationships presented above remains to be determined.[2]

Folate, vitamins B6 and B12 are co-factors in one-carbon metabolism and essential to balance the Hcy levels. In one of the remethylation metabolic pathways, Hcy acquires a methyl group from N-5-methyl-tetrahydrofolate (MTHF) to form methionine in a vitamin B12-dependent reaction, where MTHFR is essential.[2] A considerable proportion of methionine is then activated by ATP to form S-adenosylmethionine (SAM), a universal methyl donor.[2] A reduction in MTHFR activity, as occurs in the MTHFR C677T polymorphisms, is associated with decreased plasma folate and increased plasma Hcy concentrations.[40, 41] The results of the present study reinforce these associations: the MTHFR C677T polymorphism (C:T/T:T) was negatively associated with folate and positively associated with Hcy concentrations. In a study conducted in Sao Paulo, the Hcy concentrations increased in the homozygous and heterozygous for MTHFR C677T polymorphism (C:T/T:T) compared to the wild-type genotype (CC) in all populations, and differences between the Hcy concentrations according to the MTHFR C677T polymorphism were observed independently of folate level.[40]

Some studies indicated that n-3 PUFA, especially DHA, is interlinked with the one-carbon metabolism.[42–44] Kulkarni, et al. (2011)[45] suggested that the metil groups donated by SAM in the methylation pathway are used to convert phosphatidylethanolamine-DHA to phosphatidylcholine-DHA, necessary step to delivery DHA from the liver to the plasma and distribution to peripheral tissues. Thus, low concentrations of DHA may result in excess methyl group availability for other trans-methylation reactions.[45] Another possible mechanism is the modulation effect of PUFA on MTHFR polymorphisms increasing its activity.[11–13] However, the mechanism by which PUFA influences one-carbon metabolism is not yet fully understood.[11] Despite the fact that homocysteine concentrations are no longer different among those with or without MTHFR C677T polymorphism by the interaction of folate and DHA (**Figure 3**), the DHA concentration was not associated to the Hcy concentration in the SEM (**Figure 4**).

Successfully, nutritional interventions with B-complex vitamins supplementation has been implemented to reduce total Hcy levels, excepting for vitamin B6 supplementation that presented controversial results.[5] More recently, a meta-analysis study using placebo-controlled trials identified that supplementation of n-3 PUFA also decreases Hcy concentration.[11] Experimental studies observed beneficial effects of a combined, but not individual, vitamin B12 and n-3 PUFA supplementation in improving pregnancy outcomes and cardiometabolic markers in the offspring,[46] and may play a role in reducing oxidative stress and inflammation in preeclampsia.[47]

Despite the fact that micronutrients supplementation reduces the Hcy level, there is no evidence supporting that this reduction is associated to cardiovascular risk and/or all-cause mortality reduction.[5, 48] In contrast, higher adherence to dietary patterns characterized by regular consumption of fruits, vegetables, legumes, whole grains, nuts, low-fat dairy, and fish, and low consumption of red and processed meat, sugar sweetened foods and drinks are associated with decreased risk of cardiovascular disease.[14] To understand a population dietary pattern, and its effects as a health outcome exposure, the complexity and interactively relation existing between nutrients and bioactive foods constituents, in a cumulative or opposition way, must be considered.[49, 50] The study of dietary patterns could capture the synergistic action and the true dietary intake metabolic response.[49, 50]

In this context, some researchers have been investigating associations between dietary patterns and Hcy levels. Overall, dietary patterns constituted by high intake of refined cereals, fried potatoes, and read meats are associated with high Hcy and low vitamin B12 concentrations, while dietary patterns high in fruits, vegetables and whole grains are associated with lowest Hcy and higher plasma folate.[17, 51, 52] The adherence to a Prudent dietary pattern, composed of fruits and vegetables (green leafy vegetables and others), potato/cassava/cooked cornmeal (boiled or roasted), natural juice (freshly squeezed), chicken, and fish was negatively associated with Hcy concentrations in this study. The same dietary pattern was associated with higher DHA levels, while the Modern dietary pattern was associated with lower DHA levels. The hypothesis is that a varied food pattern, with a complex combination of micronutrients and bioactive food constituents is that, in fact, can be associated to better health outcomes, including Hcy levels.[50]

Another analysis conducted in the ISA-Capital study with adults (n=417) derived dietary patterns through exploratory structural equation modelling using two non-consecutive 24h dietary recall. They found three dietary patterns, similar to what was derived in this study. The Prudent dietary pattern, characterized by the intake of vegetables, olive oil, natural seasonings, fruits, low-fat and skimmed milk, whole grains, juices, fish and poultry; and the Traditional dietary pattern, characterized by the intake of rice and beans, red meats, sugar, eggs, butter and margarine, and whole milk dietary patterns were negatively associated with metabolic cardiovascular risk factors among adults.[53]

A publication that also integrates the ISA-Capital study with 1,102 individuals aged 20 years or older derived similar three dietary patterns through exploratory principal component factor analysis using two non-consecutive 24-h dietary recall. There was a higher probability of adherence to the Prudent dietary pattern from female and elderly individuals, and lower probability in individuals who reported non-white ethnicity.[54] In this study, older women adhered more to the Prudent dietary pattern, while men, youngers and those classified as non-white ethnicity adhered more to the Modern dietary pattern, in accordance with previous studies about the determinants of dietary intake.[55–59] Remarkably, the socio-demographic characteristics not only determine the dietary intake of a population, but mainly its health conditions.[60] In the Framingham Heart Study cohort (1989-1990), with 1,041 elderly (67 to 96 years), the median Hcy concentration was 11.6 µmol/L, was higher in men than in women and increased with age.[4] In the present study, similar Hcy concentrations was found among those individuals in the 3rd tertile of Hcy levels, with the same association with sex and age. Overall, male and older individuals were independently and positively associated to Hcy concentrations in the SEM. The lack of oestrogen in men (that is protective to Hcy concentrations) and the decreased kidney function among elderly can partially explain the higher levels of Hcy in populations with these characteristics.[4]

Most of studies concerned with describing the relationship between an outcome and one or more exploratory variables use regression methods.[61] However, the use of SEM brings a higher-level perspective to the analysis, when compared with more standard techniques such as ANOVA and multiple regression.[30] As can be seen in this study, the SEM starts from a theory, by specifying a model representing plausible relationships between variables.[30] During the SEM analysis the goal is to have a model that makes theoretical sense, is parsimonious and has acceptable correspondence to the data.[30] This analysis provides estimates of model parameters for hypothesized effects, a set of logical implications of the model, and the degree to which the testable implications of the model are supported by the data.[30] The use of SEM has been more frequently seen in social sciences, and only few studies published in the nutrition field used this approach. In this sense, the present study extends the research in nutrition by investigating multiple and simultaneous relationships among socio-demographic factors, diet and Hcy levels, supporting the role of a healthy dietary pattern in preventing disease.

The present study has numerous strengths, including the use of statistical approaches that allowed a broadly look at diet, nutritional status and their relationship with homocysteine levels. Yet, the nutritional status of folate, vitamin B12 and DHA were based on biochemical measurements, a more precise method than the dietary assessment based on individual's report. In addition, the use of data on polymorphism allowed a more accurate evaluation of the factors associated to Hcy level. In order to improve the interpretability of our results, we followed the methodological steps described in the STROBE guidelines.[62] However, there are a number of limitations that should be acknowledged. Firstly, the sample used for the current analysis differs from the original sample of the ISA-Capital study, and it is not possible to performing inferences for the general population. However, no statistical differences were found between the original sample (n=1,162) and the sample of this study (n=281) with regard to age (p=0.22, Mann-Whitney U test), sex (0.09, Chi-square test) and ethnicity (p=0.64, Chi-square test). Secondly, the dietary intake was evaluated using a FFQ, which is a method that rely on memory and may be biased. Despite the limitations of FFQ, similar results were found in previous studies conducted within the same population, even they using a different method to estimate the dietary intake (24h dietary recall). The choice for using the FFQ instead of the 24h dietary recall is because the FFQ was answered in the same day that the blood samples were taken for the biochemical analysis of Hcy, folate, vitamin B12 and DHA; compared to a 2-year interval between the two 24-hour recalls collection. Thirdly, the factor analysis applied to the dietary information has been criticized because of its subjective nature, once the authors decide about the food grouping, the number of factors, the methods of rotation and also about the labels of the patterns. Moreover, the associations of dietary patterns with the socio-demographic factors and the biochemical levels of nutrients indicated the extracted dietary patterns potentially reflect the actual dietary habit. Lastly, not all components of the one-carbon metabolism could be evaluated and included in the SEM, e.g., other B-complex vitamins and polymorphisms. Still, dietary intake and health/disease are the product of different factors and interactions, many of which could not be accomplished in this study. Thus, future research need to complement this data in order to fully investigate the relationship between the social determinants of health and Hcy levels.

Conclusions

The use of a structural equation model in this study allowed identifying that the biochemical levels of folate, vitamin B12 and DHA were not as significant as the adherence to a Prudent dietary pattern was in determining Hcy concentration. The dietary pattern mainly composed of vegetables (green leafy vegetables and others), fruits, natural juices, potato/cassava/cooked cornmeal (boiled or roasted), fish, and chicken, had negative direct effects on the Hcy level of this population. These results support the role of a healthy dietary pattern in health outcomes, rather than promoting specific foods or nutrients, for policy-based health promotion strategies.

Figure 1 Flowchart of sample selection, ISA-Capital study, Sao Paulo/Brazil, 2008/2010

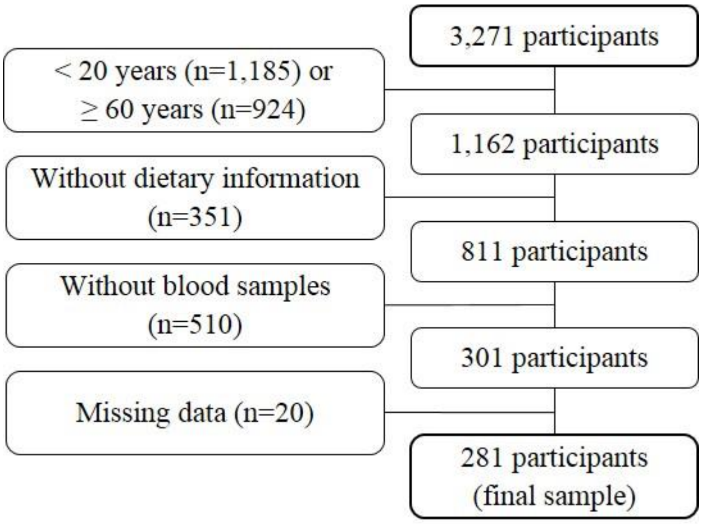
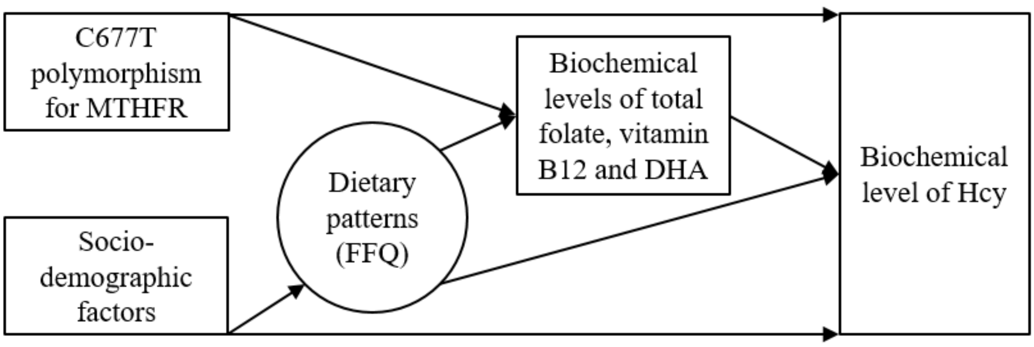


Figure 2 Theoretical model specifying the relationships among the exposure variables, the covariate variables and the outcome, ISA-Capital study, Sao Paulo/Brazil, 2008/2010



FFQ: Food frequency questionnaire; MTHFR: methylenetetrahydrofolate reductase; DHA: docosahexaenoic acid; Hcy: homocysteine

Table 1 Descriptive statistics according to homocysteine plasma level, ISA-Capital study, Sao Paulo/Brazil, 2008/2010.

Characteristics	**Total**	**Homocysteine**		*p**
		$1^{st}/2^{nd}$ tertiles	3^{rd} tertile	
	281 (100%)	189 (67%)	92 (33%)	
	n (%)	n (%)	n (%)	
Sex (male)	103 (36.7)	69 (36.5)	34 (37.0)	0.942
≥ 10 years of formal education	125 (45.0)	80 (42.8)	45 (49.5)	0.294
Ethnicity (non-white self-reported skin colour)[a]	118 (42.0)	70 (37.0)	48 (52.2)	**0.016**
Nutritional status[b]				0.791
Under or normal weight	132 (47.3)	89 (47.3)	43 (47.3)	
Overweighed	86 (30.8)	56 (29.8)	30 (33.0)	
Obese	61 (21.9)	43 (22.9)	18 (19.8)	
Smoking habit (yes)	71 (25.3)	49 (25.9)	22 (23.9)	0.910
Alcohol intake (yes)	136 (48.4)	91 (48.2)	45 (48.9)	0.904
Physical activity				0.951
Insufficiently active	114 (40.7)	77 (41.0)	37 (40.2)	
Active	100 (35.7)	66 (35.1)	34 (37.0)	
Very active	66 (23.6)	45 (23.9)	21 (22.8)	
MTHFR C677T polymorphism (C:T/T:T)	146 (52.0)	82 (45.5)	60 (65.2)	**0.002**
	Median (95% CI)	Median (95% CI)	Median (95% CI)	
Age (years)	41 (37,43)	37 (34,41)	44 (41,48)	**0.004**
Homocysteine (µmol/mL)	7.8 (7.5,8.3)	6.9 (6.6,7.2)	11.2 (10.6,12.2)	**<0.001**
Total folate (pmol/mL)	26.3 (24.4,28.1)	28.3 (25.8,31.3)	23.6 (20.0,25.6)	**0.011**
Vitamin B12 (pg/ml)	276 (262,292)	294 (272,313)	244 (215,275)	**<0.001**
DHA (% 20:6 n-3)	2.01 (1.95,1.11)	2.02 (1.95,2.12)	1.97 (1.84,2.16)	0.137

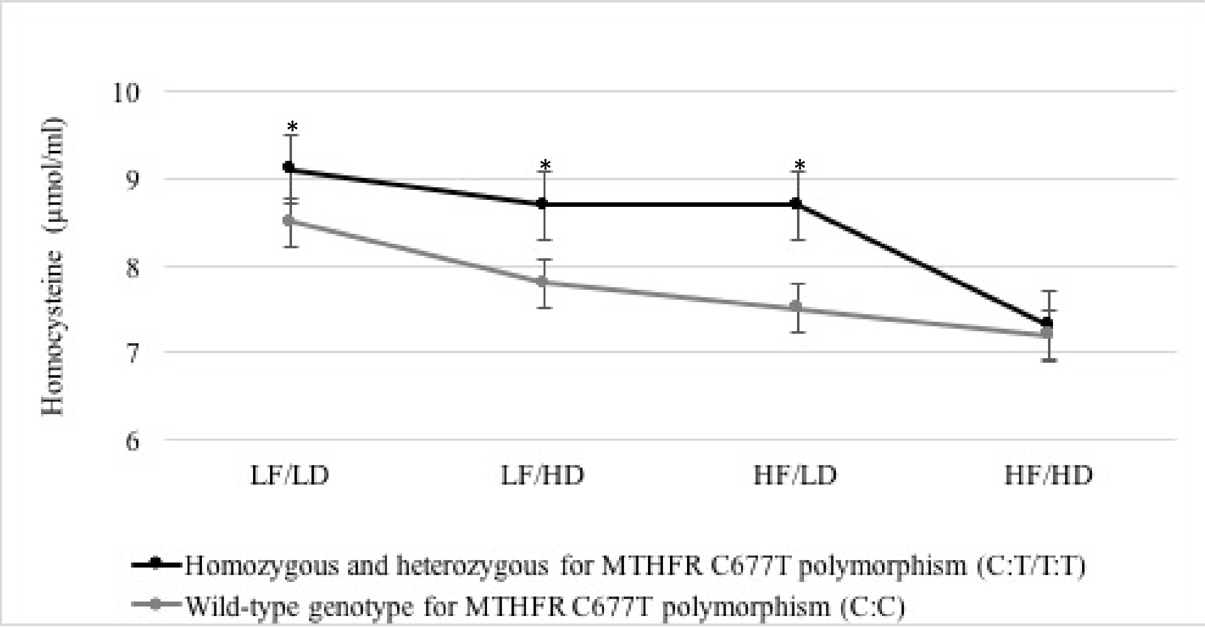
MTHFR: methylenetetrahydrofolate reductase; 95% CI: 95% confidence interval; DHA: docosahexaenoic acid. *Kruskal-Wallis tests (continuous variable) or chi-square tests (categorical variable) were used to determine if any significant differences existed amongst the groups (p<0.05; there is difference). [a]Non-white skin colour includes: mixed-race/brown skin (33.6%), black (6.7%), yellow/Asian (1.1%) and indigenous (0.4%). [b]BMI; underweight and normal weight: ≤ 24.9 kg/m^2 ; overweight: 25.0-29.9 kg/m^2; and obese: ≥ 30.0 kg/m^2

Table 2 Factor loadings according to the dietary patterns of adults, ISA-Capital study, Sao Paulo/Brazil, 2008/2010.

Food items	Modern (0.11)*	Traditional (0.08)*	Prudent (0.07)*
Frankfurter	**0.80**	-0.03	0.00
Cake (simple or filled)	**0.75**	-0.06	0.07
Potato/cassava/cooked cornmeal (fried)	**0.61**	0.09	0.10
Potato/cassava/cooked cornmeal (boiled or roasted)	**0.59**	-0.18	**0.28**
Hamburger (processed meat)	**0.56**	**0.24**	-0.11
Beer	**0.45**	0.10	0.05
Soft drink	**0.44**	-0.02	-0.20
Pizza	**0.39**	0.16	-0.15
Egg (boiled or fried)	**0.38**	0.03	0.17
Lunchmeats (ham/mortadella)	**0.36**	0.02	-0.05
Pasta, lasagne, gnocchi	**0.31**	0.04	0.08
Crackers (with or without filling)	**0.28**	0.09	-0.10
Desserts and sweets	**0.25**	-0.02	-0.20
Fried snacks	**0.21**	0.16	-0.11
Cheese	**0.21**	-0.03	0.12
Butter or margarine (regular and salted)	-0.05	**0.74**	-0.02
Bread	-0.04	**0.74**	0.02
Milk	-0.02	**0.41**	0.18
Yogurt	0.10	**0.39**	0.10
Bean	0.00	**0.39**	-0.10
Wine	0.02	**0.38**	0.04
Rice	-0.03	**0.34**	0.06
Natural juice (freshly squeezed)	0.04	**0.31**	**0.28**
Sausage (pork/chicken)	0.13	**0.28**	-0.11
Coffee (sweetened)	0.05	**0.26**	-0.02
Baked savoury	0.18	**0.23**	-0.14
Beef	0.15	**0.22**	0.14
Vegetable (others)	0.02	0.09	**0.81**
Vegetable (green leafy)	0.02	0.03	**0.81**
Fish	0.00	0.03	**0.46**
Fruit	0.13	0.13	**0.37**
Chicken	-0.09	-0.11	**0.33**

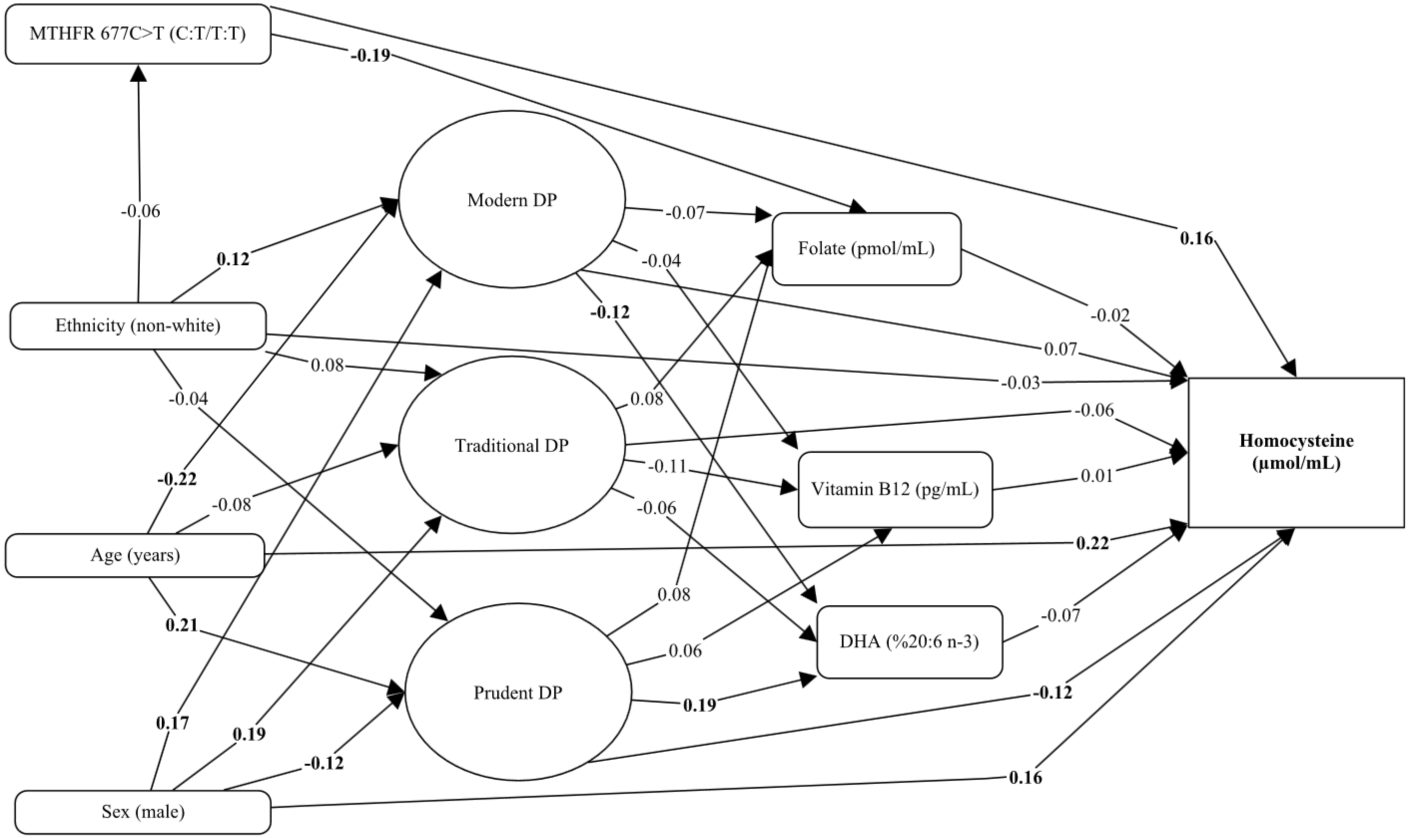
*Percentage of accumulated variance explained. In bold are the rotated factor loadings ≥0.20 or ≤-0.20. The food item 'cassava flour, *farofa*, oat and tapioca' did not present correlation with any other item and was not included in the dietary pattern analysis. [a]The food items ‘pork’, ‘beef jerky’, ‘smoked meat’, ‘artificial juices’, and ‘distilled alcoholic beverage were excluded from the final analysis because they did not load on any of the retained factors.

Figure 3 Homocysteine level (mean and SE) according to MTHFR C677T polymorphism, total folate and DHA, ISA-Capital study, Sao Paulo/Brazil, 2008/2010



SE: standard error; MTHFR: methylenetetrahydrofolate reductase; DHA: docosahexaenoic acid; LF: low folate; HF: high folate; LD: low DHA; HD: high DHA. High folate: ≥26.3 pmol/mL for total folate (median value); high DHA: ≥ 2.0 (% 20:6 n-3) for DHA (median value). *Kruskal-Wallis tests were applied to identify differences in homocysteine level (continuous) according to MTHFR C677T polymorphism, total folate and DHA (*p<0.05; there is difference).

Figure 4 Associations between dietary factors and homocysteine level using structural equation model, ISA-Capital study, Sao Paulo/Brazil, 2008/2010



MTHFR: methylenetetrahydrofolate reductase; DHA: docosahexaenoic acid; DP: dietary pattern. The dietary pattern factor loadings, and the levels of folate, vitamin B12, DHA, and homocysteine were kept as continuous variables in this analysis. Numbers in bold represent statistically significant associations (p<0.05). Correlations terms were added between DHA and vitamin B12 (0.16, p=0.01), and also between the dietary patterns (Modern-Traditional: -0.07, p=0.22; Modern-Prudent: 0.09, p=0.14; Prudent-Traditional: 0.05, p=0.38), but the double arrows were omitted in the figure to facilitate the visualization.

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5 CONSIDERAÇÕES FINAIS

O estudo da alimentação como variável de exposição para diferentes desfechos em diferentes contextos se mostra de grande relevância. Ainda, o estudo dos padrões alimentares permite um olhar mais amplo sobre o consumo alimentar, explicitando a combinação das escolhas alimentares, evidenciando a cultura alimentar de uma determinada população, e permitindo o estudo dos determinantes dessas escolhas.

Esta tese teve como objetivo principal colaborar para o melhor entendimento da relação entre padrões alimentares, nutrientes do metabolismo do folato e homocisteína e três desfechos em saúde: medidas antropométricas do recém-nascido, infecção por HPV em homens e concentrações de homocisteína em adultos.

Observou-se que aderir à um padrão alimentar composto principalmente por alimentos industrializados como carnes processadas, sanduíches, *snacks*, doces, e bebidas adoçadas, ricos em gordura, e pobres em carboidrato e micronutrientes como cálcio, vitamina D e folato no período pré-gestacional esteve associado a ter um filho pequeno ao nascer. Isso significa que algumas crianças deixam de atingir o seu potencial máximo de crescimento em virtude de um fator modificável, que é a alimentação materna. O início de vida em desvantagem torna essas crianças mais suscetíveis à mortalidade, maior morbidade e consequências adversas funcionais ao longo da vida. As mulheres que aderem mais à esse padrão alimentar foram as mais jovens, com escolaridade mais alta (porém ainda baixa, ≥ 8 anos de escolaridade), que trabalhavam formalmente, e que nasceram na região Sudeste do Brasil. Isso significa que para a interrupção desse ciclo de pobreza e desvantagens, as intervenções e políticas públicas na área de alimentação e nutrição devem ser priorizadas.

Não somente para o desenvolvimento fetal, mas a manutenção de um padrão alimentar desbalanceado em alimentos e nutrientes também altera negativamente a história da infecção por HPV em homens. Observou-se neste estudo que um padrão alimentar fracamente correlacionado ao folato, fortemente às vitaminas B6 e B12, principalmente via carne de boi e miúdos, suco natural, feijoada, frutas e cerveja, mas negativamente correlacionado ao DHA, se associou à maior duração da infecção por HPV em homens. Os homens que aderiram mais à esse padrão foram aqueles com maior renda familiar, com excesso de peso e que tiveram teste positivo para infecção por HPV na primeira visita do estudo. A alimentação pouco variada, pobre em micronutrientes, pode influenciar as funções imunológicas, aumentando não somente a vulnerabilidade à novas infecções, mas também a persistência de infecções já existentes. No caso do HPV, a persistência da infecção está diretamente associada ao desenvolvimento de verrugas e de lesões neoplásicas. Dessa forma, a adesão à um padrão alimentar variado e rico em micronutrientes pode ser um fator importante para a diminuição do tempo de infecções por HPV em homens.

Um padrão alimentar variado parece influenciar as concentrações de homocisteína em adultos. Este estudo mostrou que a maior adesão a um padrão alimentar composto de verduras e legumes, frutas, sucos naturais, batata e mandioca cozida, polenta, peixe e frango esteve associado à diminuição das concentrações de homocisteína em adultos, mais do que as concentrações bioquímicas de folato, vitamina B12 e DHA. Concentrações bioquímicas de DHA, idade e ser mulher estiveram diretamente associados à esse padrão alimentar. Altas concentrações de homocisteína são observadas em doenças cardiovasculares e cerebrovasculares, entretanto permanece indeterminado se a homocisteína é um fator de risco ou marcador para essas doenças. O fato é que intervenções com suplementação de nutrientes como folato, vitamina B12 e ômega-3 mostraram reduzir as concentrações de homocisteína, mas a redução dessas concentrações não esteve associada a menor risco cardiovascular ou mortalidade. Em contrapartida, maior adesão à padrões alimentares ricos em frutas, verduras, legumes, grãos integrais, nozes, laticínios com baixo teor de gordura e peixe, e baixo consumo de carne vermelha e processada, alimentos e bebidas açucarados está associada à diminuição do risco cardiovascular. Os resultados deste estudo corroboram com a literatura e reforçam a sinergia existente entre micronutrientes e compostos bioquímicos, naturalmente presentes na matriz alimentar. O impacto positivo da alimentação sobre a saúde se dá por meio de um conjunto de alimentos e nutrientes que interagem entre si, e não por meio de um nutriente em específico.

O estudo dos AGPI, mais especificamente o DHA, como um fator associado ao metabolismo do folato e homocisteína é relativamente novo e se mostrou importante para as relações estudadas. Não é possível afirmar que o efeito do DHA sobre os desfechos estudados se dá por meio do metabolismo do folato e homocisteína, mas estudos prévios sugerem mecanismos plausíveis que embasam a inclusão do DHA neste estudo.

Esta tese inova no uso de técnicas estatísticas para a derivação dos padrões alimentares, como análise fatorial por componentes principais e *reduced rank regression* (RRR), e modelos para a estimativa da relação entre alimentação e desfechos, utilizando modelo de equações estruturais. O uso de medidas bioquímicas de vitaminas e DHA e polimorfismo do metabolismo do folato e homocisteína são pontos fortes do estudo. Ainda, diferentes estudos realizados na cidade de São Paulo foram combinados para um olhar amplo sobre as relações entre alimentação e desfechos de importância em saúde. Como os dados de todos os estudos já haviam sido coletados, não houve necessidade de nenhum financiamento adicional para o desenvolvimento dessa pesquisa, o que vale ser ressaltado diante do contexto de escassez de recursos em que se encontra a universidade pública e a pesquisa no Brasil.

O mesmo tipo de instrumento (QFA) foi utilizado para avaliação do consumo alimentar dos três estudos abordados aqui. O QFA é um instrumento que depende da memória do respondente e pode apresentar erros de medida, sendo o seu uso, então, considerado uma limitação deste estudo. Outro limitante a ser ressaltado é a não utilização de concentrações bioquímicas dos nutrientes em interesse, uma medida de maior acurácia do estado nutricional, nos estudos sobre medidas antropométricas do recém-nascido e infecção por HPV. Ainda, o metabolismo do folato e homocisteína é bastante complexo e muitas vitaminas participam das suas reações, não tendo sido possível incluir todas elas nas análises apresentadas nesta tese. Além disso, as amostras dos estudos ProcriAr e HIM foram selecionadas por conveniência. Para as análises dos dados do estudo ISA-Capital, de amostragem aleatória e representativa da população da cidade de São Paulo, foram utilizados dados de uma sub-amostra, porém não foram observadas diferenças estatísticas entre a amostra principal e a sub-amostra com relação a idade, sexo e raça.

Concluindo, os padrões alimentares de diferentes populações da cidade de São Paulo influenciam as medidas antropométricas do recém-nascido, a duração da infecção por HPV em homens e as concentrações de homocisteína em adultos. Os resultados desta tese reforçam a importância de estudos sobre alimentação e nutrição que considerem não somente nutrientes, mas principalmente o consumo de alimentos e suas combinações, servindo como base para a elaboração de estratégias e políticas públicas de promoção à saúde.

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ANEXOS

Anexo 1 – Parecer do Comitê de Ética em Pesquisa da Faculdade de Saúde Pública da Universidade de São Paulo (COEP/FSP) referente ao projeto de pesquisa de doutorado

FACULDADE DE SAÚDE PÚBLICA DA UNIVERSIDADE DE SÃO PAULO

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Evidências sobre a relação entre metabolismo do folato e homocisteína e três desfechos de importância em saúde: baixo peso ao nascer, remissão da infecção por HPV em homens e fatores de risco para doenças cardiovasculares

Pesquisador: Juliana Araujo Teixeira

Área Temática:

Versão: 1

CAAE: 53093916.9.0000.5421

Instituição Proponente: Faculdade de Saúde Pública da Universidade de São Paulo - FSP/USP

Patrocinador Principal: FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 1.501.677

Apresentação do Projeto:

O projeto propõe análise de dados secundários provenientes de três estudos (Estudo ProcriAr, Estudo HIM e Estudo ISA-Capital 2008) para análise da relação entre nutrientes envolvidos na via metabólica do folato e da homocisteína e três desfechos de importância em saúde: baixo peso ao nascer, remissão da infecção por HPV em homens e fatores de risco para doenças cardiovasculares.

Objetivo da Pesquisa:

Investigar a relação entre nutrientes envolvidos na via metabólica do folato e da homocisteína e baixo peso ao nascer, remissão da infecção por HPV em homens e fatores de risco para doenças cardiovasculares (DCV).

Avaliação dos Riscos e Benefícios:

Riscos mínimos, tendo em vista utilização de dados secundários, provenientes de bancos de dados já coletados no âmbito de outros três estudos. Em termos de benefícios, há possibilidade de gerar informação para consenso sobre relação entre consumo de nutrientes envolvidos no metabolismo do folato e homocisteína, bem como para prática clínica devido ao potencial de intervenção.

Endereço: Av. Doutor Arnaldo, 715
Bairro: Cerqueira Cesar **CEP:** 01.246-904
UF: SP **Município:** SAO PAULO
Telefone: (11)3061-7779 **Fax:** (11)3061-7779 **E-mail:** coep@fsp.usp.br

Comentários e Considerações sobre a Pesquisa:

A pesquisa apresenta-se bem delineada e bem descrita, sendo investigação de relevância em termos de saúde pública.

Considerações sobre os Termos de apresentação obrigatória:

Termos de Consentimento Livre e Esclarecido, assim como termos de anuência das instituições envolvidas, apresentam-se adequados ao propósito do estudo.

Recomendações:

Aprovação do projeto.

Conclusões ou Pendências e Lista de Inadequações:

Nada a declarar.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_480122.pdf	02/02/2016 14:02:22		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_ProcriAr.pdf	02/02/2016 13:56:27	Juliana Araujo Teixeira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_HIM.pdf	02/02/2016 11:51:50	Juliana Araujo Teixeira	Aceito
Folha de Rosto	folha_de_rosto_enviada.pdf	02/02/2016 11:43:09	Juliana Araujo Teixeira	Aceito
Declaração de Pesquisadores	Declaracao_de_anuencia_Estudo_ISA.pdf	13/01/2016 14:06:50	Juliana Araujo Teixeira	Aceito
Outros	CEP_SMS_PROCRIAR.pdf	11/01/2016 11:30:20	Juliana Araujo Teixeira	Aceito
Outros	CEP_HC_PROCRIAR.pdf	11/01/2016 11:29:57	Juliana Araujo Teixeira	Aceito
Outros	CEP_FSP_ISA_Capital.pdf	11/01/2016 11:29:36	Juliana Araujo Teixeira	Aceito
Outros	CEP_CRT_HIM.pdf	11/01/2016 11:28:43	Juliana Araujo Teixeira	Aceito
Declaração de Pesquisadores	Declaracao_de_anuencia_Estudo_ProcriAr.pdf	11/01/2016 10:57:03	Juliana Araujo Teixeira	Aceito

Declaração de Pesquisadores	Declaracao_de_anuencia_Estudo_HIM.pdf	11/01/2016 10:56:14	Juliana Araujo Teixeira	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_09mar15_Qualificação_FINAL.pdf	10/03/2015 17:24:12		Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO PAULO, 14 de Abril de 2016

Assinado por:
Maria Regina Alves Cardoso
(Coordenador)

Anexo 2 – Publicação do primeiro manuscrito

Teixeira JA, Castro TG, Grant CC, Wall CR, Castro ALDS, Francisco RPV, Vieira SE, Saldiva SRDM, Marchioni DM. Dietary patterns are influenced by socio-demographic conditions of women in childbearing age: a cohort study of pregnant women. BMC Public Health. 2018 Mar 1;18(1):301. doi: 10.1186/s12889-018-5184-4. https://bmcpublichealth.biomedcentral.com/track/pdf/10.1186/s12889-018-5184-4

Teixeira et al. BMC Public Health (2018) 18:301
https://doi.org/10.1186/s12889-018-5184-4

BMC Public Health

RESEARCH ARTICLE Open Access

Dietary patterns are influenced by socio-demographic conditions of women in childbearing age: a cohort study of pregnant women

Juliana Araujo Teixeira[1*], Teresa Gontijo Castro[2,3], Cameron C. Grant[2,3,4], Clare R. Wall[2,5], Ana Lúcia da Silva Castro[6], Rossana Pulcineli Vieira Francisco[6], Sandra Elisabete Vieira[7], Silvia Regina Dias Medici Saldiva[8] and Dirce Maria Marchioni[1]

Abstract

Background: Women's health during their reproductive years and whilst pregnant has implications for their children's health, both in utero and during childhood. Associations of women's pre-pregnancy dietary patterns (DP) with maternal socio-demographic characteristics and nutrient intake were investigated in ProcriAr cohort study in São Paulo/Brazil, 2012.

Methods: The DPs of 454 women were investigated by principal component factor analysis, using dietary information from a validated 110-item food frequency questionnaire. Multiple linear regression models identified independent associations between DPs and maternal socio-demographic characteristics and Spearman's correlation determined associations between DPs and nutrients intake.

Results: Participants' mean age was 26.1 years (standard deviation = 6.3), 10.3% had more than 8 years of formal education, 30% were migrants from outside of the Southeast of Brazil, 48% were employed, 13% were smokers, and 51% were overweight/obese. Four DPs were derived: 'Lentils, whole grains and soups,' 'Snacks, sandwiches, sweets and soft drinks,' 'Seasoned vegetables and lean meats,' and 'Sweetened juices, bread and butter, rice and beans'. The 'Lentils, whole grains and soups' score was positively related to maternal age, being non-smoker and born in the South, North or Midwest of Brazil. The 'Snacks, sandwiches, sweets and soft drinks' score was positively related to higher maternal education, and negatively related to age, lack of formal work and being born in the Northeast region. The 'Seasoned vegetables and lean meats' score was positively related to higher maternal education. The 'Sweetened juices, bread and butter, rice and beans' score was positively related to unemployment and to no family history of hypertension, and negatively related to maternal overweight and obesity. Dietary intake of fruits and vegetables, foods that require preparation, nutrients from one-carbon metabolism, protein, iron, calcium and vitamin D were correlated with the 'Seasoned vegetables and lean meats'. Dietary intake of sugar-sweetened and alcoholic beverages, industrialized and takeaway foods, and foods rich in sugar, energy, fat, and synthetic folate were correlated with the 'Snacks, sandwiches, sweets and soft drinks'.
(Continued on next page)

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Anexo 3 – Relatório de estágio em pesquisa no exterior: University of Auckland, Nova Zelândia

UNIVERSITY OF SÃO PAULO
SCHOOL OF PUBLIC HEALTH

Folic acid supplementation and the developmental origins of health and disease: a comparison between birth cohort studies in Brazil and New Zealand

JULIANA ARAUJO TEIXEIRA
FAPESP process n°: 2016/15356-3 – BEPE scholarship

Final scientific report submitted to the São Paulo Research Foundation regarding the completed Research Internship at University of Auckland, New Zealand

Supervisor (NZ): Prof. Dr. Cameron C. Grant
Co-supervisor (NZ): Dr. Teresa G. Castro
Co-supervisor (NZ): Prof. Dr. Clare Wall

Supervisor (BR): Prof. Dirce Maria L. Marchioni

São Paulo
2018

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1 INITIAL WORK PLAN AND SCHEDULE OF COMPLETION

Table 1. Initial work plan of the research internship abroad

Stages of the research	Months											
	J	F	M	A	M	J	J	A	S	O	N	D
Database organization	■	■										
Literature review	■	■	■	■	■	■	■	■	■	■		
Comparison folic acid supplementation BR and NZ		■	■									
Analysis folic acid supplementation and child health outcomes BR and NZ				■	■	■	■	■	■			
Presentation of results and discussion of their implication					■	■		■	■	■		
Drafting of scientific papers and abstracts				■	■	■	■	■	■	■	■	■
Congress participation				■ a						■ b		

[a] 15th World Congress on Public Health, Melbourne, Australia, 3-7 April, 2017

[b] 10th World Congress on Developmental Origins of Health and Disease. Lifecourse Health and Disease. Observations, experiments and interventions. Rotterdam, The Netherlands, 15-19 October, 2017.

The research internship happened in accordance with the initial plan, with activities from January to December, 2017. The main alteration was the non-attendance to the 10th World Congress on Developmental Origins of Health and Disease due to insufficient financial support. This internship represented the joint efforts of researchers from School of Public Health, University of São Paulo in Brazil and from Center for Longitudinal Research – *He Ara Ki Mua*, The University of Auckland in New Zealand.

2 ACTIVITIES DEVELOPED DURING THE RESEARCH INTERNSHIP

2.1 THE COMPREHENSION OF THE NEW ZEALAND STUDY

The Growing Up in New Zealand study is made up of around 7,000 children who were recruited from all expected births in the Auckland, Counties-Manukau and Waikato District Health Board regions between 25 April 2009 and 25 March 2010. In total, 6,822 pregnant women and 4,401 of their partners were recruited into the main cohort. The ethnicity and socio-demographic characteristics of the children and families in the cohort are broadly generalisable to those of children being born in New Zealand today. The size and diversity of the cohort is one of its key strengths and an important feature to making the information collected as useful as possible for policy development and research.

The folic acid supplement use and the intake of food rich in natural folate, fortified or suitable for fortification with folic acid among pregnant women in NZ were presented in two papers developed during the internship in an attempt to better understand the study and the country.

2.1.1 Determinants of folic acid supplement use outside national recommendations for pregnant women: results from the Growing Up in New Zealand study

Teixeira JA, Castro TG, Wall CR, Marchioni DM, Berry S, Morton SM, Grant CC. Determinants of folic acid supplement use outside national recommendations for pregnant women: results from the Growing Up in New Zealand cohort study. Public Health Nutr. 2018 Apr 30:1-10. doi: 10.1017/S1368980018000836.

ABSTRACT

OBJECTIVE: To evaluate the sociodemographic and lifestyle factors associated with insufficient and excessive use of folic acid supplements (FAS) among pregnant women.

DESIGN: A pregnancy cohort to which multinomial logistic regression models were applied to identify factors associated with duration and dose of FAS use.

SETTING: The Growing Up in New Zealand child study, which enrolled pregnant women whose children were born in 2009-2010.

SUBJECTS: Pregnant women (n 6822) enrolled into a nationally generalizable cohort.

RESULTS: Ninety-two per cent of pregnant women were not taking FAS according to the national recommendation (4 weeks before until 12 weeks after conception), with 69 % taking insufficient FAS and 57 % extending FAS use past 13 weeks' gestation. The factors associated with extended use differed from those associated with insufficient use. Consistent with published literature, the relative risks of insufficient use were increased for younger women, those with less education, of non-European ethnicities, unemployed, who smoked cigarettes, whose pregnancy was unplanned or who had older children, or were living in more deprived households. In contrast, the relative risks of extended use were increased for women of higher socio-economic status or for whom this was their first pregnancy and decreased for women of Pacific v. European ethnicity.

CONCLUSIONS: In New Zealand, current use of FAS during pregnancy potentially exposes pregnant women and their unborn children to too little or too much folic acid. Further policy development is necessary to reduce current socio-economic inequities in the use of FAS.

KEYWORDS: Folic acid supplementation; Growing Up in New Zealand; Health behaviour; Pregnancy; Public health

2.1.2 Effects of different scenarios of folic acid food fortification on the dietary folate intake of a multi-ethnic pregnant women population: evidence from Growing Up in New Zealand

Teixeira JA, Castro TG, Wall CR, Marchioni DM, Berry S, Morton SM, Grant CC. Effects of different scenarios of folic acid food fortification on the dietary folate intake of a multi-ethnic pregnant women population: evidence from Growing Up in New Zealand. [Under review]

ABSTRACT

OBJECTIVE: To simulate the effects of different scenarios of folic acid fortification of food on dietary folate equivalents (DFE) intake in an ethnically diverse sample of pregnant women.

DESIGN: A 44-item food frequency questionnaire was used to evaluate the dietary intake of the population. The DFE intakes were estimated for different scenarios of food fortification with folic acid: (1) voluntary-fortification-2009; (2) increased-voluntary-fortification-2016; (3) simulated-bread-mandatory-fortification-2016; and (4) simulated-grains-and-rice-mandatory-fortification-2016.

SETTING: The Growing Up in New Zealand child study, which enrolled pregnant women whose children were born in 2009-2010.

SUBJECTS: Pregnant women (n=5,664) enrolled into a nationally population generalizable cohort.

RESULTS: The participants identified themselves as European (56.0%), Asian (14.2%), Māori (13.2%), Pacific (12.8%) or other ethnicities (3.8%). Bread, breakfast cereals, and yeast spread were the main food sources of DFE in the two voluntary fortification scenarios. In those scenarios, green leafy vegetables, bread and breakfast cereals were the main contributors of DFE intake for Asians. In descending order, the proportions of different ethnicities in the lowest tertile of DFE intake for the four fortification scenarios were: Asians (39-60%), Others (41-44%), Europeans (31-37%), Pacific (23-26%) and Māori (23-27%). However, in comparisons within each ethnic group across the scenarios of food fortification with folic acid, differences were observed only with the simulated-grains-and-rice-mandatory-fortification-2016 scenario.

CONCLUSIONS: If fortification with folic acid was mandatory for grains and rice in New Zealand, intakes of DFE would be more evenly distributed among different ethnicities and it would be easier for public health authorities to implement this health policy effectively.

KEYWORDS: Folate; Food fortification; Maternal nutrition; Maternal and child health; Growing Up in New Zealand.

2.2 THE COMPARISON BETWEEN BIRTH COHORT STUDIES

2.2.1 Women's folic acid supplement use and folate intake during pregnancy in two birth cohort studies: Brazil and New Zealand

These analyses has been conducted by Juliana A. Teixeira,[1,2] Teresa Castro,[2] Clare R. Wall,[2] Dirce Maria Marchioni,[1] Sarah Berry[2], Susan M. B. Morton,[2] Cameron C. Grant[2] ([1]Department of Nutrition, School of Public Health, University of Sao Paulo, Brazil; [2]The Centre for Longitudinal Research – He Ara ki Mua, University of Auckland, New Zealand.

The presented analysis aimed to compare the women's folic acid supplement use and folate intake during pregnancy in two birth cohort studies: the *ProcriAr* study in Brazil (BR) and the *Growing Up in New Zealand* study in New Zealand (NZ).

The ProcriAr study (The Influence of Nutritional Factors and Urban Air Pollutants on Children's Respiratory Health: A Cohort Study in Pregnant Women) was conducted in São Paulo, Brazil. All women with positive pregnancy tests between March 2011 and December 2013 in three primary health care units were invited to participate (n=454). Use of folic acid supplements (FAS) in the pre-pregnancy period was self-reported by the women in the food frequency questionnaire (FFQ) during the enrolment interview in the first trimester of their pregnancy. A quantitative 110-item FFQ was used to assess the usual dietary intake of the population, and referred to the previous 12 months.

Growing Up in New Zealand is a nationally representative birth cohort study that has a large ethnically diverse sample of pregnant women from New Zealand (NZ) (n=5,489). The use of folic acid supplements (FAS) was evaluated in the 3 months before becoming pregnant and during pregnancy. The dietary intake was assessed using a semi-quantitative 44-item FFQ which referred to the previous four weeks.

In the ProcriAr study, only 4.2% (n=19) of the pregnant women used FAS according to the recommendation in the pre-pregnancy period. In the GUiNZ 34.2% (n=1,878) of the pregnant women used FAS according to the recommendation in the pre-pregnancy period. Some characteristics of pregnant women who did not use FAS as recommended before pregnancy is similar comparing the ProcriAr study with the GUiNZ study. They belonged to minorities in terms of ethnicity or race, have similar and high prevalence of excessive weight, lack of social support (marital status), are more unemployed, and have unplanned pregnancies (**Table 2**).

Only 6.6% of the pregnant women in the ProcriAr study consumed ≥3 servings/day of total bread. The determinants of consuming this amount of bread daily were be unemployed (RR=3.15; 95% Confidence Interval=1.38,7.19) and in a subsequent pregnancy (2.25; 1.06,4.80). Almost 40% of pregnant women in the GUiNZ study consumed ≥3 servings/day of total bread. The determinants of consuming this quantity of bread daily were not only being unemployed (1.20; 1.12,1.29) and in a subsequent pregnancy (1.25; 1.16,1.34), but also self-prioritised Maori (1.49; 1.37,1.62), or Pacific (1.77; 1.64,1.91) ethnicities (compared with European), being less than 20 years of age (1.21; 1.07,1.35) (compared with 20-29 years of age), and those who during pregnancy continued (1.15; 1.06,1.26) or stopped (1.13; 1.03,1.23) smoking cigarettes (compared with non-smokers). Asian ethnicity was negatively associated with the dietary intake of ≥3 servings/day of total bread (0.79; 0.70,0.90) (**Table 3**).

The pregnant women who did not use FAS as recommended before pregnancy belong to ethnic minorities, regardless of living in Brazil or New Zealand. Brazil already fortifies the wheat and maize flours with folic acid, but New Zealand does not. The public health policy of food fortification with folic acid should be considered by New Zealand in order to improve the population folate intake.

Table 2. Characteristics of pregnant women according to recommended use of folic acid supplements before pregnancy in the cohort studies GUiNZ in New Zealand (2008) and ProcriAr in Sao Paulo/Brazil (2012)‡

Characteristics			ProcriAr in Sao Paulo/Brazil		GUiNZ in New Zealand		Difference between studies among groups who did not take FAS as recommended
			Recommended use of FAS	Did not take FAS as recommended	Recommended use of FAS	Did not take FAS as recommended	
			19 (4.2)	435 (95.8)	1,878 (34.2)	3,611 (65.8)	
			% (SE)	% (SE)	% (SE)	% (SE)	*p**
Self-prioritised ethnicity or race[1]	**ProcriAr**	**GUiNZ**					
	White	European	42.1 (11.6)	39.5 (2.3)	77.7 (1.0)	46.6 (0.8)	0.005**
	Others	Maori	57.9 (11.6)	60.5 (2.3)	4.8 (0.5)	17.0 (0.6)	
		Pacific			2.2 (0.3)	17.2 (0.6)	
		Asian			11.5 (0.7)	15.5 (0.6)	
		Others			3.8 (0.4)	3.7 (0.3)	
Age (years)	<20		21.1 (9.6)	18.2 (1.9)	0.4 (0.2)	6.6 (0.4)	<0.0001
	20-29		47.4 (11.8)	54.7 (2.4)	23.2 (1.0)	46.8 (0.8)	
	>29		31.6 (11.0)	27.1 (2.1)	76.4 (1.0)	46.6 (0.8)	
Level of education[2]	No secondary school qualification		52.6 (11.8)	54.3 (2.4)	1.7 (0.3)	8.2 (0.5)	<0.0001
	≥ Secondary school qualification		47.4 (11.8)	45.7 (2.4)	98.3 (0.3)	91.8 (0.5)	
BMI[3]	≤ 24.9 kg/m2		36.8 (11.4)	50.1 (2.4)	66.0 (1.1)	56.1 (0.9)	0.002
	25.0 - 29.9 kg/m2		52.6 (11.8)	30.6 (2.2)	21.9 (1.0)	23.0 (0.8)	
	≥ 30.0 kg/m2		10.5 (7.2)	19.3 (1.9)	12.1 (0.8)	20.9 (0.7)	
Marital status[4]	Without formal partner		31.6 (11.0)	**40.6 (2.4)**	19.2 (0.9)	**45.0 (0.8)**	0.078
	With formal partner		68.4 (11.0)	**59.4 (2.4)**	80.8 (0.9)	**55.0 (0.8)**	
Employment[5]	Yes		63.2 (11.4)	**51.0 (2.4)**	69.4 (1.1)	**48.5 (0.8)**	0.327
	No		36.8 (11.4)	**49.0 (2.4)**	30.6 (1.1)	**51.5 (0.8)**	
Parity	First born		52.6 (11.8)	49.2 (2.4)	47.4 (1.2)	40.3 (0.8)	<0.0001
	Subsequent		47.4 (11.8)	50.8 (2.4)	52.6 (1.2)	59.7 (0.8)	
Pregnancy planned	Yes		36.8 (11.4)	33.3 (2.3)	92.5 (0.6)	46.9 (0.8)	<0.0001
	No		63.2 (11.4)	66.7 (2.3)	7.5 (0.6)	53.1 (0.8)	
Smoking pattern[6]	Continue smoking		10.5 (7.2)	13.6 (1.6)	2.0 (0.3)	12.9 (0.6)	<0.0001
	Stopped smoking		21.1 (9.6)	20.5 (1.9)	4.3 (0.5)	12.6 (0.6)	
	Non-smokers		68.4 (11.0)	65.9 (2.3)	93.7 (0.6)	74.5 (0.7)	

FAS: folic acid supplements; %: percentages; SE: standard error. *p-values were calculated by chi-squared test. No statistically difference was detected in the ProcriAr study between groups of folic acid use (p>0.05 for all covariates). There were statistically differences detected for all covariates in the GUiNZ study when compared by use of folic acid supplements.**comparison between European/White vs Others. [1]In the Procriar study, non-white includes black, *parda* and other. *Parda* race means a mixed-race, brown skin. In the GUiNZ study, others include Middle Eastern, Latin American, African and others ethnicities. [2]In the Procriar study, the category no secondary school qualification includes those with less than 8 years of formal education and the category ≥ secondary school qualification includes those with 8 years or more of formal education. [3]Based on Body Mass Index (BMI); underweight and eutrophic: ≤24.9 kg/m2; Overweight: 25.0-29.9 kg/m2; and Obese: ≥ 30.0 kg/m2. [4]In the ProcriAr study, the category without formal partner includes those single, divorced or widower, and the category with formal partner includes married or in common law marriage. In the GUiNZ study, the category without formal partner includes no relationship, dating or cohabiting and the category with formal partner includes married or civil union. [5]In the GUiNZ study, the category no employed includes not in workforce, unemployed or student. [6]In the Procriar study smoking patterns refers the first trimester of pregnancy and in the GUiNZ study refers to pre/during pregnancy.

Table 3. Associations between maternal sociodemographic and lifestyle factors and intake of bread‡

Characteristics			ProcriAr in Sao Paulo/Brazil				GUiNZ in New Zealand			
			Total bread (≥3 servings/day) 30 (6.6%)				**Total bread (≥3 servings/day)** 2,322 (39.7%)			
			Unadjusted	*p*	Adjusted model	*p*	Unadjusted	*p*	Adjusted model	*p*
Self-prioritised ethnicity or race[1]	**GUiNZ**	**ProcriAr**								
	European	White	1.00				1.00		1.00	
	Maori	Non-white	0.75 (0.37;1.49)	0.410			1.71 (1.59;1.85)	**<0.001**	1.49 (1.37;1.62)	**<0.001**
	Pacific						2.00 (1.86;2.14)	**<0.001**	1.77 (1.64;1.91)	**<0.001**
	Asian						0.78 (0.69;0.88)	**<0.001**	0.79 (0.70;0.90)	**<0.001**
	Others1						0.90 (0.72;1.11)	0.311	0.89 (0.72;1.09)	0.254
Age (years)	<20		0.84 (0.32;2.18)	0.716			1.37 (1.22;1.53)	**<0.001**	1.21 (1.07;1.35)	**0.002**
	20-29		1.00				1.00		1.00	
	>29		0.78 (0.34;1.83)	0.573			0.87 (0.81;0.93)	**<0.001**	0.98 (0.92;1.05)	0.537
Level of education[2]	No secondary school qualification		1.19 (0.59;2.37)	0.627			1.59 (1.46;1.73)	**<0.001**		
	≥ Secondary school qualification		1.00				1.00			
BMI[3]	≤ 24.9 kg/m2		1.00				1.00			
	25.0 - 29.9 kg/m2		0.92 (0.42;2.03)	0.840			1.12 (1.02;1.22)	**0.014**		
	≥ 30.0 kg/m2		0.81 (0.31;2.15)	0.673			1.45 (1.33;1.57)	**<0.001**		
Marital status[4]	Without formal partner		1.12 (0.56;2.25)	0.748			1.28 (1.20;1.36)	**<0.001**		
	With formal partner		1.00				1.00			
Employment[5]	Yes		1.00		1.00		1.00		1.00	
	No		3.15 (1.38;7.19)	**0.006**	3.15 (1.38;7.19)	**0.006**	1.46 (1.37;1.55)	**<0.001**	1.20 (1.12;1.29)	**<0.001**
Parity	First born		1.00		1.00		1.00		1.00	
	Subsequent		2.26 (1.06;4.83)	**0.035**	2.25 (1.06;4.8)	**0.035**	1.34 (1.25;1.43)	**<0.001**	1.25 (1.16;1.34)	**<0.001**
Pregnancy planned	Yes		1.00				1.00			
	No		0.86 (0.40;1.82)	0.689			1.38 (1.29;1.46)	**<0.001**		
Smoking pattern[6]	Continue smoking		0.93 (0.31;2.79)	0.894			1.58 (1.46;1.71)	**<0.001**	1.15 (1.06;1.26)	**0.001**
	Stopped smoking		0.74 (0.29;1.92)	0.540			1.35 (1.23;1.48)	**<0.001**	1.13 (1.03;1.23)	**0.010**
	Non-smokers		1.00				1.00		1.00	

‡ Values are RRs (95% confidence interval). Two-sided significance was determined at p<0.05. 1In the Procriar study, non-white includes black, parda and other. Parda race means a mixed-race, brown skin. In the GUiNZ study, others include Middle Eastern, Latin American, African and others ethnicities. 2In the Procriar study, the category no secondary school qualification includes those with less than 8 years of formal education and the category ≥ secondary school qualification includes those with 8 years or more of formal education. 3Based on Body Mass Index (BMI); underweight and eutrophic: ≤24.9 kg/m2; Overweight: 25.0-29.9 kg/m2; and Obese: ≥ 30.0 kg/m2. 4In the ProcriAr study, the category without formal partner includes those single, divorced or widower, and the category with formal partner includes married or in common law marriage. In the GUiNZ study, the category without formal partner includes no relationship, dating or cohabiting and the category with formal partner includes married or civil union. 5In the GUiNZ study, the category no employed includes not in workforce, unemployed or student. 6In the Procriar study smoking patterns refers the first trimester of pregnancy and in the GUiNZ study refers to pre/during pregnancy.

2.3 OTHER ACTIVITIES DEVELOPED IN THIS PERIOD

2.3.1 Participation in the 8-year data collection wave

Participation in the development of the training and the actual training for interviewers for the 8-year anthropometric data collection of GUiNZ study.

2.3.2 Presentation for the GUiNZ team meeting

The paper "Determinants of folic acid supplement use outside national recommendations for pregnant women: results from the Growing Up in New Zealand study" was presented in the GUiNZ research team meeting on July 3rd, 2017.

2.3.3 Presentation for undergraduate students

The paper "Determinants of folic acid supplement use outside national recommendations for pregnant women: results from the Growing Up in New Zealand study" was presented on March 27th, 2017, in the Life Cycle Nutrition course ministered to undergraduate students from the School of Population Health, under responsibility of Prof. Helen Eyles.

2.4 SCIENTIFIC EVENTS

2.4.1 15th World Congress on Public Health

The digital poster "Fortified flours are the major food source of folate in a population of reproductive age women: the ProcriAr cohort study in Brazil" was orally presented at the 15th World Congress on Public Health held in Melbourne, Australia, from 3 to 7 April 2017.

2.4.2 69th Annual Scientific Meeting of the Paediatric Society of New Zealand

The abstract "Determinants of insufficient and extended folic acid supplement use during pregnancy: results from the Growing Up in New Zealand study" was orally presented at the 69th Annual Scientific Meeting of the Paediatric Society of New Zealand held in Christchurch, New Zealand, from the 14 to 16 November 2017.

2.5 COURSES

Observership in the Health and Public Policy course (POPLHLTH 718) administered to post-graduate students at the School of Population Health, from March to June 2017, under responsibility of Prof. Tim Tenbensel.

2.6 INTERNATIONAL PARTNESHIP

Abstract orally presented at the 68th Annual Scientific Meeting of the Paediatric Society of New Zealand held in Tauranga, New Zealand: CASTRO T; GRANT C; WALL C; WELCH M; MARKS E; FLEMING CJ; GILCHRIST C; TEIXEIRA JA; BANDARA D; BERRY S; MORTON S. Breastfeeding duration among a nationally representative multi-ethnic sample of New Zealand children. 2016.

Abstract orally presented at the 15th World Congress on Public Health held in Melbourne, Australia: CASTRO T; GRANT C; WALL C; WELCH M; TEIXEIRA JA; MARKS E; FLEMING CJ; GILCHRIST C; BANDARA D; BERRY S; MORTON S. Misclassification of reported exclusive breastfeeding duration within the Growing Up in New Zealand cohort. 2017.

Manuscript published in the New Zealand Medical Journal: CASTRO TG; GRANT C; WALL C; WELCH M; MARKS E; COURTNEY JF; TEIXEIRA JA; BANDARA D; BERRY S; MORTON SM. Breastfeeding indicators among a nationally representative multi-ethnic sample of New Zealand children. New Zealand Medical Journal (Online), 2017.

Manuscript in submission process: CASTRO TG; GILCHRIST CA; TEIXEIRA JA; WALL C; MARCHIONI DML; BANDARA D; WELCH M; MARKS E; COURTNEY JF; BERRY S; MORTON SM; GRANT C. Bias due to overestimation of duration of exclusive breastfeeding in child cohort studies: evidence from the *Growing Up in New Zealand* study

3 EXIT LETTER

THE UNIVERSITY OF AUCKLAND NEW ZEALAND

MEDICAL AND HEALTH SCIENCES

17th February 2018

To São Paulo Research Foundation,
São Paulo
Brazil

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I am writing to confirm that Juliana Teixeira was a visiting scholar at the University of Auckland from 31st January to 15th December 2017. I was responsible for Juliana's supervision during the time that she was in New Zealand. Supervision was also provided by Dr. Teresa Castro, who was Professor Adjunto III at the Federal University of Minas Gerais in Belo Horizonte-MG Brazil, prior to taking up her current academic appointment at the University of Auckland in 2014. During her time in New Zealand Juliana was hosted within the Centre for Longitudinal Research - He Ara Ki Mua, in the School of Population Health at the University of Auckland. I am the Deputy Director of this research centre.

During her time in New Zealand Juliana focused her research activities on maternal nutrition during pregnancy and specifically on folate intake and folic acid supplementation. She completed several substantial pieces of work. In these projects, Juliana described the:

Dietary patterns and socio-demographic conditions of women in childbearing age who were participants of the ProcriAr Cohort Study in São Paulo/Brazil. The manuscript which reports the findings from this project has been accepted for publication in BMC Public Health.

Determinants of inappropriate folic acid supplement use during pregnancy among women enrolled in the New Zealand's contemporary child cohort study 'Growing Up in New Zealand'. Into this study were enrolled 6822 pregnant women with the cohort of 6853 children born in 2009-10 being a nationally generalizable cohort that includes 11% of the children born in New Zealand during the 1 year recruitment period.[1 2] Juliana presented the findings from this project at the Annual Scientific meeting of the Paediatric Society of New Zealand which was held in Christchurch, New Zealand from November 14th to 16th 2017. The manuscript which reports the findings from this project has been submitted for publication in Public Health Nutrition.

Effects of different scenarios of folic acid food fortification on the dietary folate intake of a multi-ethnic pregnant women population. This project was also completed in the Growing Up in New Zealand cohort study and a draft manuscript reporting the findings has been prepared and is currently being reviewed by co-authors prior to being submitted for publication in the peer reviewed literature.

In addition, Juliana made a number of significant contributions to the Growing Up in New Zealand study. She participated in the 8-year anthropometric data collection wave and in the preparation of the training and actual training of interviewers in the collection of anthropometric data. She contributed to a New Zealand government funded project on infant feeding in New Zealand and adherence to the national food and nutrition guidelines.

During the time that Juliana was in New Zealand, the New Zealand Ministry of Health requested an evaluation of the latest scientific evidence on the health benefits and risks of folic acid, and commissioned this report jointly from the Office of the Prime Minister's Chief Science Advisor and the Royal Society Te Apārangi. I am a member of the panel preparing this report. The projects that Juliana completed have been an invaluable resource to this panel's deliberations and have helped to inform the findings which we will report to the Ministry of Health.

Above and beyond any of these tangible outputs it has been an absolute delight to have Juliana spend time with us in New Zealand. Juliana is a very intelligent, hard working, pleasant, polite and engaging person and has been a wonderful ambassador for Brazil. She has outstanding academic potential and I feel very fortunate to have had the opportunity to work with her and learn from her. Juliana has expressed a willingness to continue the collaboration with the Growing Up in New Zealand study and intends to develop some post-doctorate studies using comparative data from Brazil and New Zealand. I greatly look forward to being able to participate in this ongoing collaboration.

Yours faithfully,

Prof. Cameron Grant FRACP PhD
Head of Department
Paediatrics: Child & Youth Health
Professor in Paediatrics
The University of Auckland
Paediatrician, Starship Children's Health
Park Road, Auckland, New Zealand

References

1. Morton SM, Atatoa Carr PE, Grant CC, et al. Cohort profile: Growing Up in New Zealand. *Int J Epidemiol* 2013;42(1):65-75. doi: http://dx.doi.org/10.1093/ije/dyr206

2. Morton SM, Ramke J, Kinloch J, et al. Growing Up in New Zealand cohort alignment with all New Zealand births. *Aust N Z J Public Health* 2015;39(1):82-7. doi: http://dx.doi.org/10.1111/1753-6405.12220

CURRÍCULO LATTES

Dirce Maria Lobo Marchioni

Bolsista de Produtividade em Pesquisa do CNPq - Nível 1D

Endereço para acessar este CV: http://lattes.cnpq.br/9059164202721558
Última atualização do currículo em 05/05/2018

Possui graduação em Nutrição pela Universidade de São Paulo (1985), mestrado em Saúde Pública pela Universidade de São Paulo (1999) e doutorado em Saúde Pública pela Universidade de São Paulo (2003) e pós doutorado no Imperial College London. Atualmente é pesquisador e professor Associado da Universidade de São Paulo. Tem experiência na área de Nutrição, com ênfase em Consumo Alimentar, atuando principalmente nos seguintes temas: consumo alimentar, dieta, recomendações dietéticas, consumo de alimentos e estudos epidemiológicos. **(Texto informado pelo autor)**

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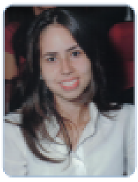
1999 - 2003	Doutorado em Saúde Pública (Conceito CAPES 6). Universidade de São Paulo, USP, Brasil. com **período sanduíche** em International Agency For Research On Cancer (Orientador: Paulo Boffetta). Título: Fatores dietéticos e câncer oral: um estudo caso-controle no Município de São Paulo, Ano de obtenção: 2003. Orientador: Regina Mara Fisberg. Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil. Palavras-chave: câncer oral; consumo de alimentos. Grande área: Ciências da Saúde Setores de atividade: Nutrição e Alimentação; Cuidado À Saúde das Populações Humanas.
1996 - 1999	Mestrado em Saúde Pública (Conceito CAPES 6). Universidade de São Paulo, USP, Brasil. Título: Alimentação no primeiro ano de vida: prevalência de consumo de alimetnos em dois Centros de Saúdo no Município de São Paulo,Ano de Obtenção: 1999. Orientador: Sonia Buongermino de Souza. Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil. Palavras-chave: alimentação do lactente; aleitamento materno; desmame; suplementação alimentar. Grande área: Ciências da Saúde Setores de atividade: Nutrição e Alimentação; Cuidado À Saúde das Populações Humanas.
1980 - 1985	Graduação em Nutrição. Universidade de São Paulo, USP, Brasil.

Pós-doutorado e Livre-docência

2011



Juliana Araujo Teixeira

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Nutricionista graduada pela Faculdade de Saúde Pública da Universidade de São Paulo (FSP-USP). Mestre em Nutrição em Saúde Publica pelo programa de Pós Graduação da FSP-USP. Atualmente é doutoranda em Nutrição em Saúde Pública pela mesma instituição e possui experiência na área de Saúde Coletiva, com ênfase em Epidemiologia Nutricional. Atua principalmente com os temas: avaliação do consumo alimentar, validação, reprodutibilidade e calibração de instrumentos de medida do consumo alimentar, alimentação materna e saúde materno-infantil. teixeira_ja@usp.br **(Texto informado pelo autor)**

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2014	Doutorado em andamento em Nutrição em Saúde Pública. Faculdade de Saúde Pública - USP, FSP-USP, Brasil. com **período sanduíche** em The University of Auckland (Orientador: Cameron C. Grant (coorient. Teresa G. de Castro, Clare Wall)). Título: Evidências sobre a relação entre metabolismo do folato e homocisteína e três desfechos de importância em saúde: baixo peso ao nascer, persistência da infecção por HPV em homens e fatores de risco para doenças cardiovasculares, Orientador: Dirce Maria Lobo Marchioni. Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP, Brasil.
2007 - 2009	Mestrado em Nutrição em Saúde Pública. Faculdade de Saúde Pública - USP, FSP-USP, Brasil. Título: Validade, reprodutibilidade e calibração do questionário quantitativo de freqüência alimentar brasileiro utilizado no estudo ?História Natural da Infecção por HPV em Homens: o Estudo HIM,Ano de Obtenção: 2009. Orientador: Dirce Maria Lobo Marchioni. Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP, Brasil. Palavras-chave: avaliação do consumo alimentar; questionário de frequência alimentar; validação e reprodutibilidade; estudos de calibração. Grande área: Ciências da Saúde
2002 - 2006	Graduação em Nutrição. Faculdade de Saúde Pública - USP, FSP-USP, Brasil.

Formação Complementar

2018 - 2018	Harvard-Brazil Collaborative Public Health Field Course. (Carga horária: 112h). Harvard School of Public Health, HSPH, Estados Unidos.
2018 - 2018	Confundimento e mediação em análise de dados longitudinais. (Carga horária: 20h). Faculdade de Saúde Pública - USP, FSP-USP, Brasil.
2015 - 2015	