

Universidade de São Paulo
Faculdade de Saúde Pública

**Trajетória antropométrica e padrões alimentares: análise
do Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil)**

Mariane de Almeida Alves

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

Área de concentração: Nutrição em Saúde
Pública

Orientadora: Profa. Assoc. Dirce Maria
Lobo Marchioni

São Paulo

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Dedico este trabalho à minha família, por todo suporte e confiança ao longo de toda minha trajetória.

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RESUMO

ALVES, Mariane de Almeida. Trajetória antropométrica e padrões alimentares: análise do Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil). [Tese de doutorado]. São Paulo: Faculdade de Saúde Pública, Universidade de São Paulo, 2023.

Apesar da indiscutível relação entre consumo alimentar e obesidade, ainda há debate na literatura sobre o papel da composição da dieta no aumento da adiposidade. A análise de padrões alimentares é um método que considera a dieta de forma global e pode contribuir para decifrar essa relação entre dieta e aumento de adiposidade. Esta tese teve como objetivo investigar a associação entre padrões alimentares no *baseline* e marcadores de adiposidade ao longo do tempo. Estudo longitudinal que analisou dados do Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil), realizado com indivíduos de 35 a 74 anos de idade (N=15.105). Os dados de consumo alimentar, obtidos no *baseline* por meio de Questionário de Frequência Alimentar (QFA), foram avaliados por três diferentes métodos de análise de padrões alimentares: 1) análise fatorial (AF), 2) *treelet transform* (TT) e 3) *reduced rank regression* (RRR). As associações foram avaliadas por meio de modelos lineares de efeitos mistos. Foi identificado um padrão conveniência marcado pelo consumo de doces e sobremesas, lanches, carne vermelha e processada, e refrigerantes comum aos três métodos aplicados. Além disso, os métodos de AF e TT identificaram um padrão marcado pelo consumo de cereais, frutas, vegetais, peixe e suco de frutas em comum. Adicionalmente o método de AF identificou um padrão tradicional brasileiro marcado pelo consumo de arroz, feijão e carne vermelha, enquanto o método TT identificou um padrão marcado pelo alto consumo de arroz e feijão e baixo consumo de carne vermelha e dos grupos alimentares presentes no padrão conveniência. Neste estudo, o tempo médio de *follow-up* foi de 8,2 anos. Maior adesão ao padrão conveniência, independente do método, foi consistente e significativamente associado com as medidas longitudinais de adiposidade. Maior adesão ao padrão tradicional brasileiro foi associado com aumento de percentual de gordura e índice de massa de gordura ao longo do tempo. Enquanto maior adesão ao padrão marcado pelo alto consumo de arroz e feijão e baixo consumo de carne vermelha foi consistentemente associado com redução dos marcadores de adiposidade ao longo do tempo. Por fim, maior adesão ao padrão prudente identificado pelo método de AF foi associado com redução em todos os marcadores de adiposidade, exceto peso corporal e o mesmo padrão identificado pelo método TT foi associado apenas com aumento de peso corporal ao longo do tempo de *follow-up*. O padrão alimentar derivado pelo método RRR explicando os valores séricos de aminoácido de cadeia ramificada, foi caracterizado pelo alto consumo de

arroz, feijão, carnes vermelha, de frango e processada, refrigerantes, lanches e pasta e simultaneamente caracterizado pelo baixo consumo de cereais e leite. Maior adesão a este padrão alimentar foi consistentemente associado com aumento da adiposidade ao longo do período de *follow-up*. A aplicação de três métodos de análise de padrão alimentar permitiu identificar nuances na composição da dieta que podem explicar o aumento de adiposidade na população.

Palavras-chave: Consumo alimentar. Padrões alimentares. Adiposidade. Epidemiologia nutricional. Estudo longitudinal.

ABSTRACT

ALVES, Mariane de Almeida. Anthropometric trajectory and dietary patterns: analysis of Longitudinal Study of Adult Health (ELSA-Brasil). [Thesis]. São Paulo: “Faculdade de Saúde Pública, Universidade de São Paulo”, 2023.

Despite the well-known relationship between diet and obesity, there is still some debate on how the diet composition influences the adiposity gain. Dietary pattern analysis emerges as an approach to evaluate the diet in a holistic way, considering the interaction between food groups and contributing to disentangle this relation between diet and adiposity. This thesis aimed to investigate the association between baseline dietary patterns and adiposity markers over the follow-up period. Methods: Longitudinal study embedded in the Longitudinal Study of Adult Health (ELSA-Brasil), a multicenter national cohort that recruited individuals aged 35 to 74 years old (N=15,105). Dietary consumption data was assessed at baseline by a food frequency questionnaire (FFQ) and the study population’s dietary patterns were identified using three different statistical methods: 1) factor analysis (FA), 2) treelet transform (TT), 3) reduced rank regression (RRR). The associations between baseline dietary patterns and adiposity were computed using linear mixed-models. Applying three different methods to identify dietary patterns, we found a common dietary pattern characterised by the consumption of sweets and desserts, snacks, red meat, processed meat, and soft drinks, labelled as convenience. Additionally, the FA and TT identified a similar dietary pattern characterised by cereals, fruits, vegetables, fish, and fruit juice, labelled as prudent. FA also identified a dietary pattern characterized by rice, beans, and red meat, representing a traditional Brazilian meal, while TT analysis identified a dietary pattern characterized by high consumption of rice and beans and low consumption of red meat and other groups present in the convenience dietary pattern, labelled as rice and beans. This study had a mean follow-up time of 8.2 years. A convenience dietary pattern was associated with higher adiposity measures over time. The Brazilian traditional dietary pattern was associated with lower weight over the follow-up, however for the body composition measures, this dietary pattern was associated with higher body fat percentage and fat mass index over the follow-up period. The rice and beans dietary pattern, characterized by high consumption of rice and beans combined with low consumption of red meat, was associated with lower levels of adiposity indicators. Lastly, the prudent dietary pattern identified by FA was associated with lower levels of adiposity indicators, except for weight. The same dietary pattern identified by TT analysis was associated with higher weight over the follow-up period. The RRR-dietary pattern explaining the circulating levels of

branched-chain amino acids was characterized by high consumption of rice, beans, red and processed meat, poultry, soft drinks, snacks, pasta, and low consumption of cereals and milk. This RRR-dietary pattern was associated with higher adiposity for all indicators over the follow-up period. Applying three different statistical methods to identify dietary patterns in the same population allowed us to find some nuances in the diet composition and further insights on how the dietary patterns of a population from Brazil may explain the changes on adiposity levels over time.

Keywords: Food consumption. Dietary patterns. Adiposity. Nutritional Epidemiology. Longitudinal study.

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APRESENTAÇÃO

Esta tese está vinculada ao Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil), um estudo nacional e multicêntrico que tem como objetivo principal investigar a incidência e progressão de diabetes e doenças cardiovasculares e seus fatores associados, tanto biológicos quanto sociais, na população adulta brasileira. O ELSA-Brasil é um estudo de coorte prospectivo que recrutou 15.105 servidores públicos de cinco Universidades Federais e um Instituto de Pesquisa dos estados Bahia, Espírito Santo, Minas Gerais, Rio Grande do Sul, São Paulo e Rio de Janeiro.

Destaca-se que esta tese foi idealizada e desenvolvida para obtenção do título de Doutora em Ciências pelo Programa de Pós-graduação em Nutrição em Saúde Pública da Faculdade de Saúde Pública (FSP) da USP. Durante o período de doutorado foram realizadas atividades de pesquisa como membro do Grupo de Estudos Epidemiológicos e Inovação em Alimentação e Saúde (GEIAS) liderado pela Dra. Dirce Marchioni e atividades de pesquisa como pesquisadora visitante na *Erasmus University Medical Center* como integrante do grupo de pesquisa *Nutrition and Lifestyle Epidemiology* liderado pela Dra. Trudy Voortman. Além disso, foram desenvolvidas atividades de docência durante o Programa de Aperfeiçoamento de Ensino como estagiária nas disciplinas Gestão de Cardápios e Integradora, que fazem parte do curso de Graduação em Nutrição da FSP/USP, sob supervisão da Dra. Dirce Marchioni e Dra. Aline Martins de Carvalho. Além disso, houve a participação como ministrante de diversos curso oferecidos pelo Laboratório de Apoio a Pesquisa Epidemiológicas em Avaliação do Consumo Alimentar. Também foram realizadas atividades de construção e gerenciamento de banco de dados de consumo alimentar coletados por meio do instrumento Recordatório de 24 horas utilizando o software GlobalDiet.

Nesta tese buscou-se estudar a associação entre padrões alimentares derivados por métodos *a posteriori* e híbrido com mudanças nas medidas de adiposidade ao longo do período de follow-up do estudo ELSA-Brasil. O primeiro capítulo apresenta o referencial teórico que fundamental o estudo, trazendo a cenário epidemiológico global e brasileiro da obesidade, a relação entre a dieta e este desfecho e as particularidades quanto a aplicação de cada um dos métodos para identificação de padrões alimentares.

No segundo capítulo são apresentados os objetivos da tese. Estes objetivos foram respondidos em três manuscritos elaborados durante o período de doutorado. O terceiro capítulo apresenta os métodos e procedimentos aplicados no desenvolvimento desta tese.

No quarto capítulo são apresentados os resultados e discussão dos achados desta tese. No subcapítulo 4.1 está apresentado o primeiro artigo da tese “*Different statistical methods identify similar population-specific dietary patterns: an analysis of ELSA-Brasil*”. Este artigo descreve os padrões alimentares identificados pelos métodos análise fatorial, *treelet transform* e *reduced rank regression* na população do ELSA-Brasil e discute as similaridades e diferenças na aplicação de cada um destes métodos. Este estudo está publicado no periódico *British Journal of Nutrition*. Os resultados preliminares oriundos destas análises também foram apresentados no 11º Congresso Brasileiro de Epidemiologia, realizado virtualmente, nas modalidades pôster e apresentação oral.

O subcapítulo 4.2 apresenta o segundo manuscrito resultante desta tese: “*Dietary patterns and adiposity over time – Analysis of the ELSA-Brasil*”. Este manuscrito investigou a associação entre os padrões alimentares previamente identificados com indicadores de adiposidade medidos ao longo do tempo, incluindo peso corporal, circunferência da cintura, índice de massa corporal, percentual de gordura corporal e índice de massa de gordura. Este manuscrito está submetido para avaliação no periódico *European Journal of Nutrition*.

No subcapítulo 4.3 desta tese está apresentado o manuscrito “*RRR-dietary pattern explaining circulating branched-chain amino acids is associated with increased levels of adiposity over time in Brazilian adults*”. Neste manuscrito foram utilizados os dados de metabolômica disponíveis no ELSA-Brasil e explorou-se a hipótese de que padrões alimentares relacionados com valores séricos aumentados de aminoácidos de cadeia ramificada estariam associados com adiposidade. Este manuscrito será submetido ao periódico *Nutrition*.

O quinto e último capítulo desta tese consiste nas considerações finais do estudo desenvolvido, considerando as implicações dos achados para a pesquisa em epidemiologia nutricional e para as políticas de alimentação, nutrição e saúde pública.

1 INTRODUÇÃO

Uma crescente base de evidências têm demonstrado a importância da dieta para saúde das populações e para a prevenção de doenças crônicas não transmissíveis (DCNT). Comportamentos alimentares não saudáveis são considerados como um dos principais fatores de risco ligados a carga global de doenças e mortalidade ^{1; 2}. Diante deste cenário global, a Assembleia Geral das Nações Unidas definiu em 2016 a Década de Ação sobre a Nutrição (2016-2025), incluindo todas as formas de má nutrição como desafio global para atingir os Objetivos do Desenvolvimento Sustentável ³ e a Organização Mundial da Saúde (OMS) reconheceu que uma dieta adequada é um dos principais fatores que podem contribuir com a redução de 25% na mortalidade causada por DCNT como diabetes, doenças cardiovasculares, câncer e obesidade ⁴.

O controle do aumento do excesso de peso da população se caracteriza como um dos mais importantes desafios para a saúde pública, visto que a prevalência mundial de sobrepeso e obesidade duplicou entre os anos de 1980 e 2015 ⁵, comprometendo o alcance do objetivo da OMS de deter o crescimento da obesidade até 2025 ⁶. Dados do *Global Burden of Disease Study* 2015 (GBD 2015) apontam que 1,9 bilhões de adultos apresentam excesso de peso (considerando as prevalências de sobrepeso e obesidade) e, entre estes indivíduos, 609 milhões são classificados com obesidade, representando aproximadamente um terço da população global ^{5; 7}.

A presença de sobrepeso e obesidade tem sido observada em todas as faixas etárias e em ambos os sexos. Entretanto, as maiores prevalências de obesidade são observadas entre mulheres e, em ambos os sexos, os maiores valores de prevalência são observados entre a população de 50 a 65 anos de idade ⁷. Em relação as regiões geográficas, os continentes Americano e Europeu lideram com os maiores valores de prevalência de obesidade de 28,3% e 22,9%, respectivamente ⁷. Segundo os dados do Sistema de Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico (VIGITEL), o Brasil vem acompanhando essa tendência mundial. Os resultados do VIGITEL 2017 revelaram um crescimento de 26,3% do excesso de peso nos últimos dez anos ⁸. Este mesmo inquérito realizado no ano de 2021 revelou que mais da metade (57,2%) da população adulta vivendo nas capitais brasileiras apresentava excesso de peso, enquanto a prevalência de obesidade foi de 22,4% ⁹.

Os efeitos adversos à saúde decorrentes do desenvolvimento de sobrepeso e obesidade são bem estabelecidos na literatura e, por isso, as elevadas prevalências observadas representam um importante problema de saúde pública. A ocorrência de obesidade está relacionada com o aumento no risco de desenvolvimento de uma vasta lista de doenças, como diabetes tipo 2 ¹⁰, hipertensão, dislipidemia, esteatose hepática não alcoólica e doença renal ¹¹, doença cardiovascular ¹² e diversos tipos de câncer ¹³. Além de promover pior estado de saúde físico nos indivíduos afetados por esta doença ^{11;14}, as consequências do excesso de peso na população têm efeitos negativos na qualidade de vida, produtividade, e nos custos dos sistemas de saúde. Nos Estados Unidos da América, se estima que os custos de saúde com um único indivíduo com obesidade cheguem U\$1.901,00 por ano ¹⁵, enquanto na Europa os custos diretos e indiretos relacionados ao excesso de peso representam um valor equivalente a até 0,67% do produto interno bruto ¹⁶. Estudo realizado no Brasil, mostrou que os custos de saúde diretamente relacionados com obesidade chegaram a U\$269 milhões no ano de 2011, representando 59% das internações hospitalares e 40,8% dos procedimentos ambulatoriais realizados no país ¹⁷.

Segundo a Organização Mundial da Saúde, sobrepeso e obesidade são definidos como o acúmulo excessivo ou anormal de gordura corporal que pode causar danos à saúde ¹⁸. O Índice de Massa Corporal (IMC) é um parâmetro amplamente utilizado em estudos populacionais para a classificação do estado nutricional de indivíduos adultos independente do sexo e idade. O IMC é calculado por meio da razão entre o peso (quilogramas) e a altura (metros) ao quadrado, sendo os indivíduos com valor de $IMC \geq 25\text{kg/m}^2$ classificados com sobrepeso e aqueles com valor de $IMC \geq 30\text{kg/m}^2$ classificados com obesidade ¹⁸.

Ainda que o IMC seja um indicador de adiposidade corporal amplamente utilizado em estudos epidemiológicos, ele é incapaz de fornecer medidas pontuais de adiposidade, como a adiposidade abdominal além de não ser capaz de diferenciar entre massa magra e massa de gordura corporal. Já a circunferência da cintura é uma medida antropométrica simples que reflete a obesidade central e a distribuição da gordura corporal ¹⁹. Esta medida destacou-se por estar fortemente associada a mortalidade por todas as causas ^{20; 21; 22} e mortalidade por doença cardiovascular ^{23; 24}. Assim, a circunferência da cintura permite uma avaliação mais precisa de riscos à saúde caracterizados pelo aumento do IMC e pode ser utilizada para classificação de risco cardiometabólico em indivíduos com obesidade ²⁵. Adicionalmente, as medidas de composição corporal também informam quanto ao risco cardiovascular e de mortalidade de

forma superior quando comparadas ao IMC ^{26; 27}. O alto custo e necessidade de infraestrutura específica para sua mensuração das medidas de composição corporal muitas vezes inviabilizam sua aplicação em grandes estudos populacionais. Desta forma, a compreensão dos fatores que estão associados às mudanças nestas medidas antropométricas e de composição corporal é de grande importância em estudos populacionais.

O desenvolvimento de obesidade é causado por múltiplos fatores que podem ser influenciados por características individuais ou ainda características sociais, ambientais e comportamentais. Dentre estes fatores, a relação entre o consumo alimentar e seus efeitos na saúde humana, incluindo o desenvolvimento de obesidade e outras DCNT, vem sendo amplamente explorada pela epidemiologia nutricional ^{28; 29; 30; 31; 32; 33}. Estes estudos têm contribuído com importantes achados, como a análise conduzida por Afshin e colaboradores que mostrou que o consumo inadequado de fatores dietéticos como grãos integrais, frutas e sódio foram responsáveis por mais de 50% das mortes mundiais relacionadas com câncer, doença cardiovascular e diabetes ¹, reforçando o relevante papel da alimentação na saúde da população.

A investigação da existente relação entre dieta e desfechos de saúde é uma das prioridades dos estudos da área de epidemiologia nutricional. Tradicionalmente, estes estudos têm focado na análise de alimentos e nutrientes específicos e, apesar destes estudos terem apresentado importantes contribuições, eles estão sujeitos a limitação de não considerar a complexidade da dieta. Sabendo que as pessoas se alimentam em refeições que apresentam variada combinação de alimentos, surge a necessidade do estudo da dieta de forma holística, levando em consideração a complexidade da interação entre os alimentos e nutrientes ^{34; 35}.

Neste contexto, a análise de padrões alimentares surge como um método alternativo e complementar em estudos de investigação da relação entre dieta e desfechos de saúde ^{35; 36; 37}. Na análise de padrões, o consumo alimentar será caracterizado por meio do comportamento alimentar, proporcionando uma avaliação mais próxima do mundo real, onde alimentos e nutrientes são consumidos de forma combinada e seus efeitos podem ser investigados por meio de um padrão de consumo ³⁴.

Até o momento, estudos de padrões alimentares têm sido desenvolvidos por duas diferentes abordagens: métodos baseados em hipóteses ou métodos de análise exploratória de dados. Na

definição de padrões baseados por hipóteses, também chamada *a priori*, são utilizados índices de avaliação da dieta baseados em conceitos de alimentação saudável, diretrizes e recomendações nutricionais definidos por meio de evidências científicas prévia, possibilitando uma avaliação da adesão a uma determinada recomendação nutricional ³⁸. Já nas análises de abordagem exploratória dos dados, também chamada *a posteriori*, são aplicados métodos estatísticos de redução de dados que levam em consideração a correlação entre os alimentos consumidos. Desta forma são identificados padrões alimentares por meio dos dados originais de consumo alimentar referentes à população de estudo ³⁹.

A análise de padrões alimentares *a posteriori* pode ainda ser subdividida em dois tipos: análise de dados independentes do desfecho e análise de dados dependentes do desfecho. Entre alguns exemplos dos métodos independentes do desfecho estão as análises de cluster, fatorial e *treelet transform*. Já em análises denominadas como dependente do desfecho os métodos *reduced rank regression* (RRR) and *partial least squares* são frequentemente aplicados ^{40; 41; 42}.

Análise fatorial é um termo genérico que inclui tanto a análise de componentes principais como a análise de fator comum, sendo a análise fatorial pelo método de componentes principais amplamente utilizada em estudos de padrões alimentares. Por ser uma técnica de redução de dados, a análise fatorial é capaz reduzir uma grande dimensão de dados a um conjunto relativamente menor. Cada conjunto de variáveis criado é denominado como fator latente (ou, neste caso, padrão alimentar) e representa as dimensões, ou seja, as relações entre as variáveis originais. Assim, grupos de alimentos pertencentes a um mesmo fator são considerados como altamente correlacionados e, na análise de dados de consumo alimentar, podemos interpretar como alimentos que são consumidos juntos ⁴³. Feito isso, é possível realizar qualquer correlação ou análise de regressão para investigar associações entre os padrões alimentares encontrados e desfechos de saúde que interessem ao pesquisador.

A RRR é uma das análises utilizadas para derivar padrões alimentares que também está baseada em redução de dados, porém combina a utilização de conhecimento prévio, semelhante ao que acontece nos métodos de análise de padrões alimentares *a priori*. Para a condução da RRR é necessário definir uma variável resposta que está relacionada com o desfecho de interesse e por isso o objetivo deste método difere da análise fatorial. Enquanto a análise fatorial procura explicar a maior variabilidade do conjunto de dados original (variáveis de consumo alimentar),

a RRR irá identificar os padrões alimentares que melhor explicam a variabilidade das variáveis resposta selecionadas. A variável resposta pode ser definida como um nutriente ou conjunto de nutrientes, um biomarcador, sendo estes dois os mais utilizados na literatura, ou ainda um contaminante, medidas antropométricas ou outras informações de interesse do pesquisador. A seleção das variáveis resposta que serão incluídas na análise é uma etapa crucial para que o objetivo seja alcançado com sucesso ⁴⁰. Desta forma, a RRR possibilita que o pesquisador estabeleça uma melhor relação entre a análise exploratória dos dados e o desfecho de saúde de interesse. Como a RRR utiliza tanto a análise exploratória de um conjunto de dados quanto uma hipótese baseada em conhecimento prévio, ela é considerada como um método híbrido que busca combinar as vantagens destas duas formas de abordagem ⁴⁰.

Recentemente, foi proposta uma nova análise para aplicação em técnicas de redução de dados. Diante das dificuldades de interpretação dos dados provenientes, principalmente, da análise fatorial, pesquisadores propuseram o método denominado *treelet transform* ⁴⁴. Este método tem como principal objetivo unir as vantagens da utilização da análise fatorial, gerando fatores altamente não correlacionados e que explicam maior parte possível da variabilidade dos dados originais, com as vantagens da análise de cluster que permite melhor interpretação de cada um dos fatores retidos nas análises ⁴⁵. O método *treelet transform* busca combinar de forma refinada elementos da análise de agrupamento hierárquico com elementos dos métodos de redução de dados, gerando a identificação de fatores que possuem alto grau de disparidade entre si ⁴⁵. Essa característica também é capaz de permitir a melhor interpretação de possíveis associações entre os padrões alimentares e os desfechos estudados.

Como já bem estabelecido na literatura, os métodos de padrões alimentares por análises exploratórias podem diferir substancialmente dependendo do país, cultura, etnia e hábitos alimentares da população em que estão sendo aplicados ^{46; 47; 48}. No entanto, pouco estudos investigaram a aplicação de diferentes métodos para a identificação de padrões alimentares em uma mesma população de estudo ^{49; 50; 51; 52; 53}, especialmente em estudos conduzidos com a população brasileira ⁵⁴. Este estudo se propôs a investigar os padrões alimentares da população brasileira participantes do Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil) usando três diferentes métodos estatísticos com o intuito de identificar as particularidades de cada método e com isso avançar na compreensão da relação entre dieta e desfechos de saúde.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Identificar padrões alimentares por meio de três diferentes métodos estatísticos e investigar como se associam com medidas de adiposidade na população brasileira participante do Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil).

2.2 OBJETIVOS ESPECÍFICOS

- Comparar os padrões alimentares dos participantes do ELSA-Brasil identificados por meio de três diferentes métodos estatísticos;
- Estimar a associação entre os padrões alimentares e medidas longitudinais de adiposidade;
- Estimar a associação entre padrões alimentares relacionados à biomarcadores de obesidade e medidas de adiposidade ao longo do tempo.

3 MATERIAL E MÉTODOS

3.1 INSERÇÃO DO ESTUDO

Este projeto de tese está vinculado ao Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil), um estudo nacional e multicêntrico que tem como principal objetivo investigar a incidência e progressão de diabetes e doenças cardiovasculares e seus fatores biológicos e sociais na população adulta brasileira. O ELSA-Brasil é um estudo de coorte conduzido com 15.105 servidores públicos de cinco Universidades Federais dos estados da Bahia, Espírito Santo, Minas Gerais, Rio Grande do Sul e São Paulo e um Instituto de Pesquisa (Fundação Oswaldo Cruz) no estado do Rio de Janeiro, abrangendo três regiões geográficas do Brasil (Sul, Sudeste e Nordeste) ⁵⁵. O ELSA-Brasil é financiado pelo Ministério da Saúde – Departamento de Ciência e Tecnologia, Ministério da Ciência Tecnologia e Inovação, Financiadora de Inovação e Pesquisa (FINEP) e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) com os editais: 01060010.00-RS, 01060212.00-BA, 01060300.00-ES, 01060278.00-MG, 01060115.00-SP, 01060071.00-RJ.

3.2 AMOSTRA E AMOSTRAGEM

O tamanho da amostra do estudo ELSA-Brasil foi estimado com base nos dois principais desfechos de interesse – diabetes tipo 2 e infarto do miocárdio. Considerando o desconhecimento da incidência de diabetes na população brasileira, os pesquisadores do ELSA-Brasil estimaram um acumulado de três anos de incidência de 1,4%. Em seguida, por meio dos valores de *alpha* de 5%, poder estatístico de 80%, prevalência de exposição de 20% e risco relativo de 2,0, foi obtido um tamanho de amostra de aproximadamente 6.400 indivíduos. Este tamanho de amostra permitiria a identificação de um número adequado de incidência de infarto do miocárdio, uma vez que se espera que a incidência de infarto do miocárdio, baseado em dados de mortalidade, seja maior que a de diabetes. Com o objetivo de realizar análises específicas por gênero e considerando possíveis perdas ao longo do seguimento, os pesquisadores definiram o número de 15.000 indivíduos como uma amostra desejável ⁵⁵.

Foram recrutados todos os servidores públicos, ativos e aposentados, das instituições públicas anteriormente mencionadas com idade entre 35 e 74 anos. Foram definidos como critérios de exclusão a ocorrência de algum dos seguintes episódios: gestação, intenção de deixar o trabalho na instituição em um futuro próximo, severo prejuízo cognitivo ou de comunicação e, quando aposentado, residir fora da região metropolitana referente ao centro de pesquisa de condução do estudo ⁵⁵. Para a segunda e terceira onda do estudo, todos os participantes (excluindo indivíduos que morreram entre os períodos de seguimento) foram convidados a retornar para entrevistas e exames de acordo com a data de entrada no *baseline* ⁵⁶. No total, participaram da segunda etapa do estudo 14.014 (92,8%) indivíduos e na terceira onda participaram 12.636 indivíduos (83,7%).

3.3 COLETA DE DADOS

A coleta de dados de *baseline* do estudo foi realizada entre os anos de 2008 e 2010. A segunda onda do estudo teve sua coleta de dados entre 2012 e 2013, e a terceira onda teve início em 2017 e foi finalizada em 2020. Para definição do modelo de aplicação dos questionários que seria realizado (mediada por entrevistador ou auto administrado) foi realizado um estudo preliminar com 144 indivíduos, sendo 24 indivíduos de cada centro de investigação do ELSA. Desta forma foi possível realizar comparações entre as respostas essenciais, pesquisa de opinião quanto a preferência dos participantes e sistematização das observações dos aplicadores. Com base na avaliação destes resultados optou-se pela realização de entrevistas presenciais ⁵⁷.

Os questionários utilizados no ELSA-Brasil envolvem a aplicação de diversos tipos de instrumentos para contemplar as diferentes informações coletadas pelos pesquisadores. Em função da extensão do questionário ele foi aplicado em duas fases distintas, de 40 minutos e duas horas, respectivamente. A aplicação destes questionários permitiu a obtenção de informações contidas nos seguintes eixos temáticos: características sociodemográficas, história médica pregressa, história ocupacional, história familiar de doenças, história reprodutiva, acesso ao sistema de saúde, fatores psicossociais, história de peso e de imagem corporal, consumo alimentar, tabagismo, consumo de álcool, atividade física, medicação, função cognitiva e saúde mental. Como critérios de decisão para a repetição de temas abordados na primeira onda foram considerados a avaliação da frequência e intensidade de modificação das exposições e a necessidade de atualização do status da exposição ⁵⁷.

3.3.1 Dados de consumo alimentar

No ELSA-Brasil os dados de consumo alimentar foram coletados por meio de um Questionário de Frequência Alimentar (QFA). O instrumento utilizado foi uma adaptação de um QFA já existente desenvolvido para a população do Rio de Janeiro ⁵⁸. Para adaptação deste questionário à população do ELSA-Brasil foi realizado um estudo prévio com a aplicação de um Recordatório de 24h (R24h) em cada centro de pesquisa. Estes R24h foram aplicados em indivíduos com características de idade, sexo e escolaridade semelhantes aos participantes do ELSA-Brasil e por meio das informações obtidas foi possível incluir até dois itens alimentares regionais de cada centro de investigação. Como exemplo pode ser mencionado a inclusão de chimarrão, acarajé, pão de queijo, cuscuz paulista e moqueca capixaba ⁵⁷.

O instrumento final aplicado no estudo foi um QFA semi-quantitativo contendo 114 itens alimentares. No momento da entrevista o participante era questionado quanto a frequência de consumo dos itens alimentares relativa a uma porção padrão nos últimos 12 meses. Os pesquisadores de campo disponibilizavam utensílios de cozinha para auxiliar os participantes na descrição das medidas caseiras consumidas ^{57; 59}.

O estudo de validação do QFA final foi realizado por meio da escolha aleatória de participantes do estudo contatados de abril a maio de 2009. Os participantes do estudo de validação responderam ao QFA no momento inicial e final do estudo e também foi solicitado o preenchimento de três registros alimentares em momentos distintos. Os valores de correlação intraclasse obtidos no estudo de validação variaram entre 0,20 e 0,72 para cálcio e selênio, respectivamente. Por fim, os dados de consumo alimentar foram obtidos por meio de entrevista presencial realizada por pesquisadores de campo treinados durante a fase de entrevistas em que era solicitada a presença dos participantes no centro de investigação do ELSA ^{57; 59}.

3.3.2 Dados antropométricos

Os dados de peso foram aferidos utilizando balança da marca Toledo® com capacidade máxima de 200 kg. A altura foi aferida por meio de estadiômetro da marca Seca® apropriado para

aferição de medidas antropométricas com propósito de pesquisa científica. A circunferência da cintura foi aferida no ponto médio entre a borda inferior da costela e a crista ilíaca na linha axilar média conforme definido por Lohman ⁶⁰. Todos os equipamentos e seus respectivos procedimentos de instalação e calibração foram padronizados para todos os centros de investigação envolvidos no ELSA-Brasil ⁵⁶. Todas as medidas antropométricas mencionadas acima foram aferidas tanto no *baseline* quanto na segunda e terceira onda do estudo, permitindo a avaliação das mudanças antropométricas ao longo do seguimento.

3.3.3 Dados de composição corporal

As medidas de composição corporal foram realizadas na segunda e terceira onda do estudo por meio de bioimpedância segmental com oito eletrodos (InBody 230; InBodyCo., Seoul, Korea) capazes de distinguir a massa de tronco e membros. Para a realização do exame os participantes estavam em jejum de pelo menos oito horas e foram orientados a não beber café ou fazer atividade física nas 24 horas prévias ao exame. Além disso, no momento de realizar o exame foi solicitado que os participantes removessem seu acessório metálicos (brincos, anéis, óculos, etc.) e nos minutos prévios ao exame foram orientados a esvaziar suas bexigas. O exame de bioimpedância não foi realizado em participantes com marca-passo ou próteses metálicas ⁶¹. Percentual de gordura corporal e o índice de massa de gordura foram utilizados como marcadores de adiposidade. O índice de massa de gordura foi calculado dividindo massa total de gordura (kg) pela altura ao quadrado (m²).

3.3.4 Dados bioquímicos

Os valores de níveis séricos de aminoácidos de cadeia ramificada (BCAA) circulantes foram analisados por meio de ressonância nuclear magnética utilizando o kit *LP4 NMR MetaboProfile™ Analysis* ⁶², no laboratório norte americano *LipoScience/LabCorp Global Research Services (GRS)*. A quantificação dos aminoácidos valina, leucina e isoleucina foi realizada por meio de algoritmos matemáticos, permitindo sua quantificação de forma acurada. Foi utilizada a soma final dos três componentes gerando a quantificação de BCAA.

3.3.5 Dados sociodemográficos

Para obtenção dos dados sociodemográficos dos participantes foi realizada aplicação de questionários nas duas fases de entrevistas. Nesta etapa foram coletadas informações referentes a idade, sexo, cor da pele, local e características de moradia, composição familiar e histórico de migração, escolaridade e histórico ocupacional (do participante e seu respectivo cônjuge), renda familiar, religião, escolaridade dos pais e condições de vida na infância ⁵⁷.

3.4 ANÁLISE DOS DADOS

3.4.1 Análise dos dados de consumo alimentar

Esta tese analisou os dados de consumo alimentar por meio da identificação de padrões alimentares. Devido a extensa lista de alimentos presente no QFA, foi realizado um agrupamento de alimentos baseado na similaridade de composição nutricional e uso culinário dos itens, além de considerar a matriz de correlação entre os alimentos. Por fim, os grupos de alimentos foram analisados por gramas de consumo. Foram aplicados três métodos estatísticos de identificação de padrões alimentares, descritos a seguir.

3.4.1.1 Análise Fatorial

A análise fatorial pelo método de componentes principais tem sido o método mais utilizado nos estudos de epidemiologia nutricional. O consumo em gramas dos grupos alimentares foi utilizado para a identificação dos padrões alimentares. Para verificar a adequação da aplicação da análise fatorial aos dados de consumo alimentar foram realizados os testes de *Kaiser-Meier-Olkin* (KMO) e de esfericidade de *Bartlett*, considerando como aceitáveis os valores de 0,50 e $p < 0,05$, respectivamente ⁶³. Como critérios para a retenção de fatores foram considerados *eigenvalue* > 1 , análise do gráfico *screeplot* e a interpretabilidade dos fatores. Os fatores obtidos foram submetidos à rotação ortogonal *varimax* com o objetivo de simplificar a interpretação dos dados, maximizando a maiores cargas fatoriais e minimizando as mais baixas. Para determinar os grupos de alimentos pertencentes a cada padrão alimentar foi estabelecido o valor de carga fatorial ≥ 0.30 ou ≤ -0.30 como ponto de corte. Os fatores retidos foram interpretados e nomeados de acordo com os alimentos que apresentaram carga fatorial mais expressiva no fator.

3.4.1.2 *Reduced Rank Regression*

Partindo do mesmo agrupamento de alimentos utilizado na análise fatorial, foi aplicada a análise *Reduced Rank Regression* (RRR). Esta análise foi utilizada em dois artigos com variáveis respostas distintas. Primeiro, foram utilizadas como variáveis resposta a densidade energética (kcal/100 g), densidade de fibra (g/1000 kcal) e gordura total (g/dia) ajustada pela ingestão de energia total pelo método do resíduo. No terceiro manuscrito, esta análise foi utilizada com os valores circulantes de aminoácidos de cadeia ramificada (BCAA) como variável resposta. Para determinar os grupos de alimentos pertencentes a cada padrão alimentar identificado pelo método RRR foi estabelecido o valor de carga fatorial ≥ 0.20 ou ≤ -0.20 como ponto de corte.

3.4.1.3 *Treelet Transform*

A análise *Treelet Transform* (TT) foi aplicada de acordo com o procedimento descrito por Gorst-Rasmussen ⁴⁵. Assim como ocorre na análise fatorial, a *Treelet Transform* é conduzida na matriz de correlação dos grupos de alimentos. A definição dos padrões alimentares que serão retidos foi baseada nas técnicas de agrupamentos hierárquicos, onde o ponto de corte é definido pela análise do dendograma (*cluster tree*) gerado.

Ao fim de cada uma das análises de identificação dos padrões alimentares, o escore dos indivíduos em cada um dos padrões foi utilizado para avaliar a adesão ao padrão alimentar. Esta foi a variável de exposição utilizada nos modelos de associação entre os padrões alimentares e os desfechos de adiposidade ao longo do tempo.

3.4.2 Análise dos dados antropométricos e de composição corporal

Os dados antropométricos peso, índice de massa corporal e circunferência da cintura circunferência da cintura foram analisados de forma contínua. Foram utilizadas as medidas antropométricas aferidas no *baseline* e na segunda e terceira onda do estudo. O mesmo foi definido para os dados de composição corporal. As medidas de percentual de gordura corporal e o índice de massa de gordura estavam disponíveis em dois pontos do estudo (segunda e terceira onda).

3.4.3 Análise dos dados sociodemográficos

Os dados sociodemográficos e de estilo de vida foram utilizados como variáveis de ajuste nos modelos de associação. Foram utilizadas as variáveis sexo (masculino e feminino), idade (em anos completos), escolaridade (ensino fundamental incompleto, ensino fundamental completo, ensino médio completo, ensino superior), atividade física e tabagismo.

3.4.4 Análises estatísticas

Foram realizadas análises descritivas de média e desvio padrão ou mediana e intervalo interquartil de acordo com as características das variáveis contínuas e distribuição da frequência com seus respectivos intervalos de confiança de 95% (IC95%) para as variáveis categóricas. Para as análises de associação entre a variável independente (score do padrão alimentar) e a variável dependente (indicadores de adiposidade), foram aplicados modelos lineares mistos. Visto que em estudos longitudinais as medidas do desfecho não apresentam independência, a utilização de modelos mistos é adequada por considerar a correlação existente entre as medidas repetidas de cada indivíduo. As análises estatísticas referentes à análise RRR foram realizadas no *software SAS® Studio 3.8 University Edition*. Todas as outras análises estatísticas conduzidas neste estudo foram realizadas nos *softwares Stata®* versão 14.0 e R versão 4.1.1 (*R Foundation for Statistical Computing, Vienna, Austria*).

3.5 CONSIDERAÇÕES ÉTICAS

O Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil) foi submetido e aprovado pelos comitês de ética de cada um dos centros de pesquisa participante. Além disso, por ser um estudo de natureza multicêntrica, o protocolo de pesquisa também foi aprovado pelo Comitê Nacional de Ética em Pesquisa. Todos os participantes assinaram termo de consentimento aceitando em participar do estudo e também foi solicitada autorização dos participantes para o armazenamento de material biológico coletado durante o estudo ⁵⁵. Esta tese foi submetida e aprovada pela Plataforma Brasil junto ao Comitê de Ética em Pesquisa da Faculdade de Saúde Pública da Universidade de São Paulo (FSP/USP).

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4 RESULTADOS E DISCUSSÃO

Neste capítulo estão apresentados os resultados e discussão dos achados desta tese no formato de artigos. As subseções a seguir estão os artigos apresentados na formatação de acordo com o período a qual foi publicado, submetido ou ainda será submetido.

4.1 DIFERENTES MÉTODOS ESTATÍSTICOS IDENTIFICAM PADRÕES ALIMENTARES SIMILARES QUANDO APLICADOS NA MESMA POPULAÇÃO

Este subcapítulo apresenta o artigo “*Different statistical methods identify similar population-specific dietary patterns: an analysis of ELSA-Brasil*” de autoria de Mariane de Almeida Alves, Maria del Carmen Bisi Molina, Maria de Jesus Mendes da Fonseca, Paulo andrade Lotufo, Isabela Martins Benseñor e Dirce Maria Lobo Marchioni. O artigo foi aceito para publicação no periódico *British Journal of Nutrition* (comprovante em anexo).

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Different statistical methods identify similar population-specific dietary patterns: an analysis of ELSA-Brasil

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ABSTRACT

In recent decades, different data-driven approaches have emerged to identify dietary patterns (DPs) and little is discussed about how these methods are able to capture diet complexity within the same population. This study aimed to apply three statistical methods to identify the DP of the Longitudinal Study of Adult Health (ELSA-Brasil) population and evaluate the similarities and differences between them. Dietary data were assessed at baseline in the ELSA-Brasil study using a food frequency questionnaire (FFQ). DPs were identified by applying three statistical methods: (1) factor analysis (FA), (2) treelet transform (TT), and (3) reduced rank regression (RRR). The characteristics of individuals classified in the last tertile of each DP were compared. Cross-classification and Pearson's correlation coefficients were assessed to evaluate the agreement between the DP of the three methods. A similar convenience DP was identified for all three methods. FA and TT also identified a similar prudent DP and a DP highly loaded for the food groups rice and beans. Individuals classified in the third tertile of similar DPs of each method presented similar sociodemographic and nutrient intake characteristics. Regarding the cross-classification, prudent DP from FA and TT presented a higher level of agreement (75%), while convenience DP from TT and RRR presented the lowest agreement (44.8%). The different statistical methods were able to capture the populations' DPs in a similar way while highlighting the importance of the particularities of each method.

Keywords: nutritional epidemiology, dietary patterns, factor analysis; reduced rank regression; treelet transform

Introduction

The study of the relationship between diet and health outcomes is a central issue in nutritional epidemiology research. Traditionally, these studies have focused on specific foods and nutrients and, although they have brought important contributions, these studies present the limitation of not considering diet complexity(1). In real life, people eat meals with a variety of foods and nutrients that may be interactive or synergistic(2; 3). In this context, dietary patterns (DPs) analysis has emerged as a complementary method(2), where food consumption is characterized in a holistic way and may better inform the comprehensive effect of diet on health outcomes(1; 4).

DPs studies have been conducted using three different approaches. A priori methods, such as index and scores, which use prior scientific knowledge on diet-disease associations, a posteriori methods that are entirely based on dietary data within a certain study population, and hybrid methods, which combine both data on food intake in a population and pre-existing knowledge on diet-disease relationships(5; 6). The last two approaches are considered data-driven methods, because they entirely depend on the data at hand and identify population-specific dietary patterns.

A range of statistical analyses can be applied to data-driven methods to identify dietary patterns in a population. Factor analysis (FA) is the most widely applied technique(4), which evaluates the correlation matrix of food consumption data and identifies the latent factors that most explain the original data variance(7). FA requires some subjective decisions throughout the analytical process, and interpretation of the final factors can be complicated because they are a linear combination of all original dietary data(8). To address this limitation, a new statistical method, called treelet transform, was proposed by Gorst-Rasmussen(9).

Treelet transform (TT) has been recently applied in nutritional epidemiology to identify dietary patterns as a method that combines the strengths of factor and hierarchical cluster analyses(9; 10). Similar to FA, TT is also based on the correlation matrix of food items; however, the constructs identified are composed of a small number of food items, adding sparsity, and substantive meaning and interpretation of dietary patterns(9; 10). To date, very few studies have applied this method to study dietary patterns(3; 8; 9; 11; 12)

The main hybrid approach applied in nutritional epidemiology is reduced rank regression (RRR). This analysis aims to directly relate the data-driven steps of pattern identification to an outcome of interest by identifying linear functions of food groups that can explain as much

variation as possible of a set of outcome related variables (intermediate variables). These intermediate variables must be related to an outcome of interest, and it may be nutrients or biomarkers, both commonly applied in these studies(13; 14)

As is well known, data-driven approaches differ substantially depending on the country, culture or ethnicity of different study populations(15; 16; 17). However, little is known about how these different methods behave when applied to the same population. Considering that the data-driven approach is applied to capture diet complexity, can different methods describe the population's diet complexity similarly? Thus, the aim of this study was to compare these three different statistical methods to identify dietary patterns in the Longitudinal Study of Adult Health (ELSA-Brasil) population and evaluate their similarities and differences in describing population-specific dietary patterns.

Methods

Study participants

ELSA-Brasil is an ongoing cohort study that recruited 15,105 active and retired civil servants, aged 35-74 years, from five universities and one research institute located in three Brazilian macro-regions (southeast, northeast, and south). Baseline examinations were performed in 2008-2010. Detailed information regarding the study sample and design has been described previously(18). This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the all institutional review boards involved (Fundação Oswaldo Cruz, Universidade Federal da Bahia, Universidade Federal do Espírito Santo, Universidade Federal de Minas Gerais, Universidade Federal do Rio Grande do Sul and Universidade de São Paulo). Written informed consent was obtained from all subjects.

For this study, only participants who had complete dietary data and complete anthropometric measurements at baseline and follow-up were considered. Individuals with misreported energy intake were excluded. Misreporting of energy intake was defined following the procedures defined by McCroy et al.(19), where the agreement between the reported energy intake (rEI) and predicted total energy expenditure (pTEE) was evaluated for each individual considering age, weight, height, and sex. To calculate pTEE, the equation proposed by Vinken et al.(20) was applied. This validated equation was developed based on data from free-living individuals aged 18-81 years evaluated by the double-labeled water (DLW) method. The cut-off point was defined as the ± 2 SD of the agreement between the rEI and pTEE based on the proposal of

Black et al.(21). This cut-off point takes into account the within-person coefficient of variation (CV) of energy intake (23%), the CV of the technical measurement error of the DLW plus the biological variation in total energy expenditure (TEE) (8.2 %) and the CV of the prediction error of the TEE by Vinken's equation (17.7 %). In this sample, the ± 2 SD was $\pm 60\%$, therefore, individuals were classified as under-reporters if they presented an energy intake agreement of less than 40% (n = 137; 52.6% BMI < 30kg/m²; 50.4 % male) and over-reporters if they presented an energy intake agreement greater than 160% (n = 934; 88.65% BMI < 30kg/m²; 43.0% male). The final sample comprised 12,816 participants (Figure 1).

Dietary data

Dietary data were assessed at baseline using a validated food frequency questionnaire (FFQ) composed of 114 food items. The FFQ was validated in relation to the nutrient intake of three food records. The intraclass correlation coefficient (ICC) ranged from 0.22 to 0.72 for selenium and calcium, respectively(22). The FFQ was applied by trained interviewers to evaluate the participant's diet in the last 12 months regarding three sections: (1) food products/food preparations; (2) measures of consumed products; and (3) consumption frequencies with eight response options including 'more than 3 times a day', '2 to 3 times a day', 'once a day', '5 to 6 times a week', '2 to 4 times a week', 'once a week', '1 to 3 times a month', and 'never/rarely'(22; 23).

The food items in the FFQ were classified into 25 food groups: rice, cereals, bread, fruits, vegetables, beans, milk, nuts, sweets and desserts, tubers, pasta, snacks, eggs, cheese, butter/margarine, red meat, poultry, processed meat, fish, soft drinks, juice, coffee, beer, wine, and distilled beverages (Supplementary Table S1). The food group classification was based on the similarity of nutrient profiles and eating occasion.

Dietary patterns

Dietary patterns at baseline were identified using three statistical methods: factor analysis (FA), treelet transform (TT), and reduced rank regression (RRR). All of these three dimension-reduction methods are comparable in identifying a population's dietary patterns because they work by aggregating food groups based on a correlation matrix; however, they present different aims, assumptions, and decisions to take into account. The consumption in grams of the 25 food groups were the input variables for all three methods.

Factor Analysis

Factor analysis (FA) is a widely applied method in nutritional epidemiology studies for identifying DPs. This dimension-reduction method aims to explain as much variance as possible of the original data through latent variables (factors) that reveal the intrinsic structure of the data(1). Principal component factor analysis was applied in this study. Kaiser-Meyer-Olkin (KMO) and Bartlett's tests were performed to verify whether our data were suitable for FA. The identified factors were orthogonally rotated using the varimax procedure to achieve a simpler structure with greater interpretability. The criteria used to retain the factors were eigenvalues > 1 , identification of a breakpoint in the scree plot, and interpretability. Food groups with factor loadings ≥ 0.30 or ≤ -0.30 were considered relevant and characteristic of the dietary patterns, and factors were labelled according to these food groups.

Treelet Transform

A treelet transform (TT) analysis was conducted as proposed by Gorst-Rasmussen(9; 10). TT combines the statistical principles of cluster and principal component analysis (PCA), leading to sparsity in factor loadings by creating components with food groups that present factor loadings exactly zero. This procedure results in fewer food items included in each factor when compared to FA, thereby, facilitating the researcher's interpretation of each DP. The treelets are constructed as follows: between all of the original variables, the algorithm identifies the two variables with the largest correlation and then performs a PCA on them. These two variables are replaced by a sum factor, and this procedure is repeated until all original variables have joined the hierarchical cluster tree. To select the treelets that provide greater variance, 10-fold cross-validation was applied to find the optimal cut level of the cluster tree, as proposed by Lee et al.(24). To assess the sensitivity of the selected cut level, TT analysis was repeated at ± 3 levels of the optimal level. To verify the stability of the identified treelets the tstab procedure was performed through 100 bootstrap replications in subsamples of 80% of the original data(10).

Reduced Rank Regression

Reduced rank regression (RRR) analysis combines a data-driven approach with prior knowledge related to an outcome. In this model, the food groups are entered as predictor variables. A crucial step of this method is the selection of intermediate variables that are known to be related to the outcome of interest(13; 14). Unlike FA, RRR aims to explain as much variance as possible of these chosen intermediate variables. In this study, we built a priori knowledge based on obesity as an outcome of interest, selecting as intermediate variables the

following nutrients or derived from nutrients variables: energy density (Kcal/100 g), fibre density (g/1000 kcal), and total fat (g/day) adjusted for total energy intake using the residual method(25). These intermediate variables were chosen based on the World Health Organization report, which brings the evidence that energy density, fibre density and fat intake are linked with obesity risk(26) and previous published studies that has used these intermediate variables when assessing dietary patterns relate to obesity(27; 28; 29). In RRR analyses, food groups with factor loadings ≥ 0.20 or ≤ -0.20 were considered relevant and characteristic of the dietary patterns. In RRR analysis, the number of intermediate variables is a condition for the number of derived dietary patterns, and the interpretability was the criterion applied to retain the DP.

A dietary pattern score was calculated for all DPs identified for each method. This variable indicates the individual-level weights associated with each dietary pattern and theoretically represents the adherence to a dietary pattern.

Statistical analyses

To enable the comparison between individuals' characteristics according to the level of adherence to each dietary pattern of the three methods, the individual's dietary pattern scores were classified into tertiles. Sociodemographic characteristics and nutritional profile of the individuals classified in the third tertile were described as means or proportions. To evaluate the agreement of individuals' classification on tertiles in similar dietary patterns of different methods, we performed a cross-classification analysis, and Pearson's correlation coefficients between dietary patterns scores were also obtained.

Statistical analyses were performed using Stata® (Statistical Software for Professionals, College Station, TX), version 14.2(30) and only the RRR analyses were performed using SAS® Studio version 3.8 (SAS Institute Inc.).

Results

Three DPs were identified in the FA (Table 1). The first DP, labelled as convenience, was highly positively loaded with the food groups sweets and desserts, pasta, snacks, eggs, cheese, butter/margarine, red meat, processed meat, and soft drinks. The second DP, labelled as Brazilian traditional, was highly positively loaded with rice, beans, and poultry, and was highly negatively loaded with cereals, nuts, cheese, and wine. The third DP, labelled as prudent, was highly positively loaded with cereals, fruits, vegetables, tubers, fish, and juice. All three FA-derived DPs explained 26.9% of the variance in the original dietary data. The results for the

KMO and Bartlett's tests were 0.73 and $p < 0.001$, respectively, indicating that the sample was suitable for FA.

The 10-fold cross-validation performed in the TT analysis indicated that 19 was the optimal cut level of cluster three. Repeated analysis at ± 3 levels resulted in DPs with similar characteristics. The same was observed using the *tstab* procedure, in which the DPs obtained for subsamples were similar to the total sample, indicating good stability in the TT analysis. The first TT-derived DP, labelled as convenience, was positively loaded with rice, bread, beans, sweets and desserts, tubers, pasta, snacks, eggs, butter/margarine, red meat, poultry, processed meat, and soft drinks. The second DP, labelled as prudent, was positively loaded with cereals, fruits, vegetables, nuts, cheese, fish, and juice. The third DP, labelled as rice and beans, was positively loaded with rice and beans, and negatively loaded with sweets and desserts, pasta, snacks, eggs, red meat, processed meat, and soft drinks. These three TT-derived DP explained 22.2% of the variance in the original dietary data (Table 2).

RRR analysis derived three DPs (Supplementary Table S2) and according to interpretability, only the first dietary pattern was retained. The RRR-derived DP, labelled as convenience, was positively loaded with sweets and desserts, snacks, butter/margarine, red meat, processed meat, and soft drinks, and was negatively loaded with fruits, vegetables, and beans. This dietary pattern explained 5.8% of the variance in the original dietary data and 48.7% of the variance in the intermediate variables (Table 3).

The characteristics of the total population and individuals classified in the third tertile of each DP are presented in Table 4. Most of the individuals with high adherence to the convenience DP of the three methods were younger, mostly men and smokers when compared to the total population. These individuals presented the highest mean for energy density and percentage of kilocalories from total fat and saturated fat, while presenting the lower mean of fibre density. Individuals classified in the third tertile of the prudent DP of the FA and TT methods were slightly older, most of them were females and non-smokers, and presented a lower mean for energy density. Most of the individuals classified in the third tertile of the traditional Brazilian and rice and beans DPs were men and non-white individuals when compared to the total population. These individuals had the lowest consumption of kilocalories from animal protein and saturated fat. High adherence to the traditional Brazilian dietary pattern was characterised by the largest percentage of smokers, and individuals with the highest adherence to rice and beans DP had the largest mean for fibre density and percentage of kilocalories from

carbohydrates. The food groups' mean consumption of the individuals classified on the third tertile of each DP are presented in the Supplementary Table S3.

The agreement of individuals' classification in a similar DP of the three different methods is presented in Table 5. The prudent DP from FA and TT presented the highest level of agreement and the opposite was observed between the convenience DP from TT and RRR, with the lowest level of agreement. Pearson's correlation coefficients for convenience DP scores from FA and TT, FA and RRR, and TT and RRR were $r = 0.83$; $p < 0.001$, $r = 0.63$; $p < 0.001$, and $r = 0.39$; $p < 0.001$, respectively. The correlation between the prudent DP scores from FA and TT was $r = 0.90$; $p < 0.001$, and the traditional Brazilian and rice beans DP scores had a correlation coefficient of $r = 0.66$, $p < 0.001$.

Discussion

None of the three different statistical methods identified an identical DP, however, a similar convenience DP was observed in the three methods as the first one, e.g., the one that most explained the variance in the original data. The convenience DP shared the food groups sweets and desserts, snacks, butter and margarine, red meat, processed meat, and soft drinks. The FA and TT identified a prudent DP with cereals, fruits, vegetables, fish, and fruit juice as common food groups, and a traditional Brazilian (FA) and rice and beans (TT) dietary patterns highly loaded for the food groups' rice and beans. Even though there were differences in food groups and factor loadings within the identified DP, reflecting the particularities of each method, we could see that, independent of the method applied, these analyses were able to capture the population's diet in a similar way.

There are some peculiarities worthy of highlighting. The RRR-convenience DP was not only related to higher consumption of unhealthy food groups but was also related to lower consumption of the food groups fruits, vegetables, and beans - representing the opposite of prudent and traditional Brazilian/rice and beans DP identified by the other two methods. Similarly, the TT-convenience DP presented low but positive loadings for the food groups rice and beans, indicating the influence of traditional Brazilian foods on this DP. These particularities may explain the slight differences in the fibre density and the percentage of kilocalories from carbohydrate, protein, total fat, and saturated fat observed in the individuals classified in the third tertile of the convenience DP from TT and RRR analyses.

Other studies have applied DP analysis to the ELSA-Brasil dataset or subsets with diverse aims. Bezerra et al.(31) applied latent class analysis and identified DPs labelled as prudent and processed, which shared similar characteristics with the prudent and convenience DPs identified in our study, respectively. Gorgulho et al.(32) applied factor analysis in a subset of ELSA-Brasil (only participants from São Paulo) and also found a convenience DP, as the one that most explained the original data variance, which shared the same food groups in the convenience DP of our study. They also found a DP labelled plant-based and dairy that were highly loaded for similar food groups (fruits, vegetables, and cereals) of our prudent DP identified by factor and treelet transform analyses. The main difference was that we observed that the food group fish was highly loaded in our prudent DP, which could reflect some regional characteristics of the total Brazilian population.

A Brazilian traditional DP sharing the characteristics with the Brazilian traditional (FA) and rice and beans (TT) DP was also observed in four studies conducted with the ELSA-Brasil population(32; 33; 34; 35) that included a range of four different statistical analyses (principal component analysis, factor analysis, cluster analysis, and multiple correspondence analysis). A DP highly loaded with the food groups rice, and beans is commonly identified in studies regarding all ages and sex of the Brazilian population(36; 37; 38).

As expected, the RRR analysis explained a smaller proportion of original dietary data variance (5.8%) than FA and TT, since RRR focuses on identifying DP that most explain the variation of the intermediate variables. Despite this methodological difference, RRR analysis was able to not only identify a DP related to energy density, fibre density and total fat but also a DP that is in fact present in the population, as a similar convenience DP was identified through FA and TT. This result was also observed by Batis et al. and Cunha et al. when comparing RRR with principal component analysis and FA, respectively(39; 40). The food group differences observed between the RRR-convenience DP and convenience DP of other methods may be relevant and bring new insights to understand the associations between this DP and health outcomes in further studies.

FA explained a higher proportion of original dietary data variance when compared with TT (26.9% and 22.2%, respectively). TT only loads the most expressive food groups for a DP, and those that are not relevant receive a loading equal to zero, leading to a lower number of food groups contributing to a DP. This sparsity created by TT is considered as an advantage over FA, which produces a complex factor loading matrix making the interpretation of DP more

susceptible to researcher assumptions. Also, because of the sparsity of TT analysis, a trade-off between the explained variance of the DPs and the interpretability is inevitable(3). Schoenaker et al.(8) introduced a relevant issue for TT analysis: whether it is able to capture the overall diet, as only specific food items are taken into account to predict the DP score at the individual level, some of the synergic aspects of food may be lost in this process and need to be considered when applying this approach. It is important to mention that in both methods, the overall proportion of the variance explained the dietary patterns is not large, which means that only a limited portion of the diet variance is considered when investigating dietary patterns(41).

Several studies have compared DPs identified by RRR with other statistical methods and how they are associated with different health outcomes(42; 43; 44; 45; 46; 47). The findings presented in these studies did not allow us to affirm whether some of these methods are superior in estimating the association with a specific outcome. While some studies suggested that RRR analysis provided better results when investigating the association between DPs and metabolic syndrome in adults(43), obesity in preschool children(45), and bone mass in an elderly population(46), other studies found similar and consistent results independent of the method applied(39; 44; 47). Considering the particularities of the RRR analysis, this may be a promising method when the study goal is exploring the combination of foods that are mediated by specific variables (intermediate variables), also adding the possibility of using metabolome and/or microbiome information(3).

The same controversial results were found when comparing DPs from FA and TT analysis and their association with health outcomes. Schoenaker et al. compared DPs derived both from TT and FA and, even though they have found similar DPs, only those identified by FA were associated with incident diabetes in a middle-aged women's population(8). Whereas, Gorst-Rasmussen et al. obtained similar results in estimating the relative risk of myocardial infraction with DPs identified by TT or FA in a Danish cohort study(9). Since there is not robust evidence of a superior DP method to predict the relationship between diet and health outcomes, the researchers need to keep in mind the research question of their study and then select which method is more appropriate. Also, comparisons between different methods in the same study can bring new insights and complementary results to better understand each statistical method(48).

Our study had some limitations. First, all three statistical techniques applied to identify the dietary patterns require arbitrary decisions and subjective interpretations. In these data-driven

approaches, the researcher defines food grouping and the label of each dietary pattern. Specifically in FA and RRR, the researcher defines the number of factors to retain and the cut-off points that define which food groups are relevant to the dietary patterns. Second, the dietary consumption data were assessed using a FFQ, a self-reported method that has some inherent bias, such as memory or social desirability. Also, in the FFQ the food items are pre-grouped, which made it not possible to have more distinctive food groups (e.g., unhealthy vs. healthy foods groups) and it may have an impact on dietary patterns meaningfulness. The strengths of this study are the use of a validated FFQ, the large sample size, and the application of the TT analysis, a novel method in nutritional epidemiology to identify populations' dietary patterns.

In conclusion, our results showed that three different statistical methods were able to capture the populations' DPs in a similar way while highlighting the importance of the particularities of each method. The different aims and procedures of each method may play a relevant role in identifying associations between DPs and health outcomes, and comparing these results can bring new perspectives to understand this relationship.

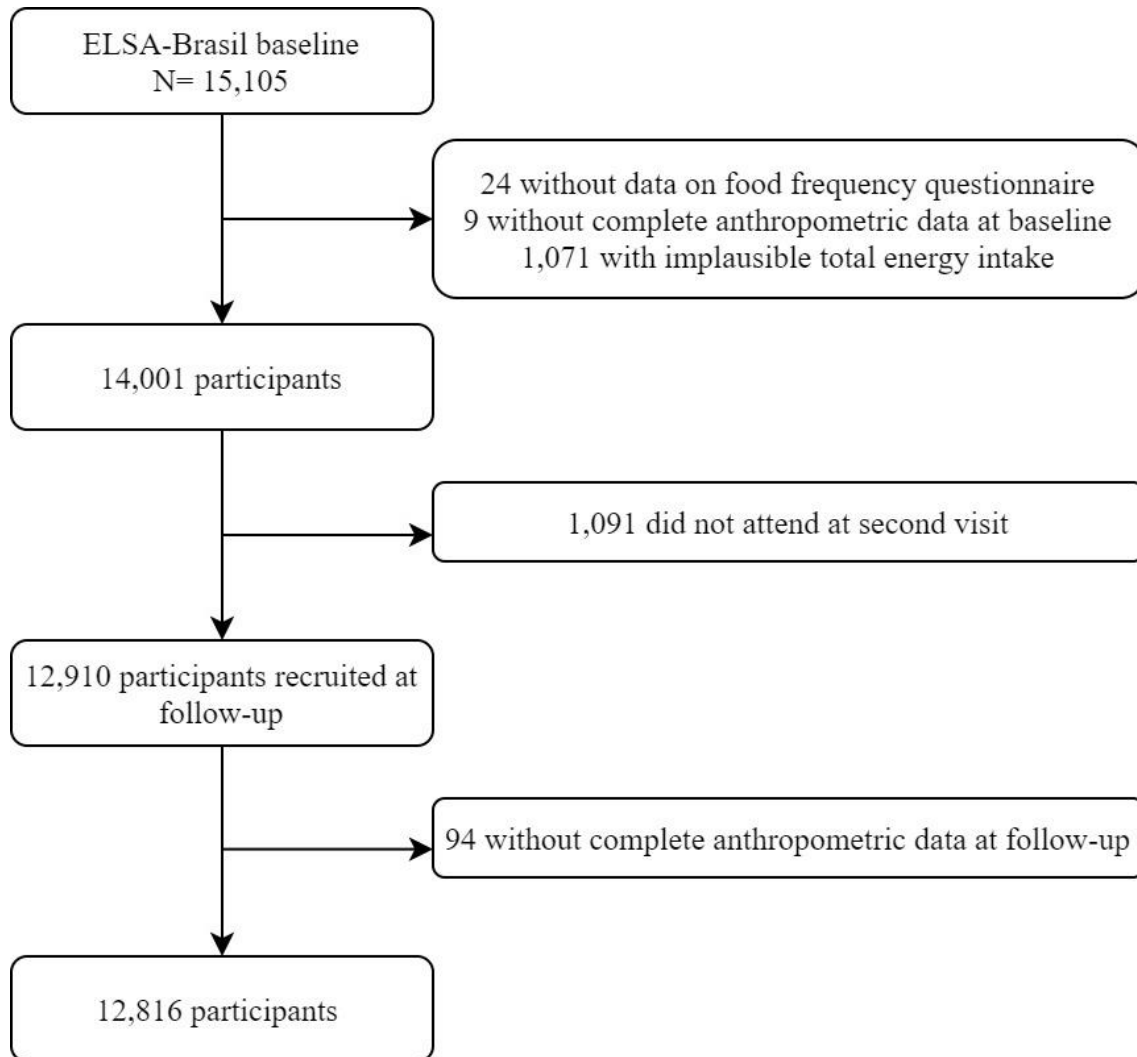
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Figures**Figure 1.** Flowchart of final sample size in analysis. ELSA-Brasil, 2021.

Tables

Table 1. Food groups' factor loadings, eigenvalues and explained variance among dietary patterns (retaining three factors).

Food Group	Convenience	Brazilian Traditional	Prudent
Rice	0.08	0.70	-0.02
Cereals	-0.11	-0.32	0.36
Bread	0.28	0.04	0.03
Fruits	-0.16	-0.03	0.60
Vegetables	-0.05	-0.01	0.69
Beans	0.09	0.66	0.06
Milk	-0.04	-0.11	0.16
Nuts	0.12	-0.41	0.29
Sweets and desserts	0.53	-0.12	-0.02
Tubers	0.20	0.25	0.42
Pasta	0.46	0.09	0.14
Snacks	0.69	0.04	-0.06
Eggs	0.39	0.24	0.16
Cheese	0.33	-0.40	0.20
Butter/margarine	0.32	0.18	-0.07
Red meat	0.31	0.29	0.03
Poultry	0.15	0.44	0.27
Processed meat	0.55	0.22	-0.03
Fish	0.10	0.09	0.50
Soft drinks	0.45	0.08	-0.23
Juice	0.08	-0.12	0.36
Coffee	0.15	-0.04	0.03
Beer	0.23	0.23	0.03
Wine	0.25	-0.33	0.18
Distilled	0.18	0.06	0.05
Eigenvalues	2.68	1.95	1.56
% explained variance	9.66	8.99	8.22
% cumulative explained variance	9.66	18.65	26.87

KMO = 0.73

Table 2. Food groups' loadings, eigenvalues and explained variance among dietary patterns derived by treelet transform.

Food groups	Convenience	Prudent	Rice and beans
Rice	0.28		0.64
Cereals		0.34	
Bread	0.21		
Fruits		0.49	
Vegetables		0.49	
Beans	0.28		0.64
Milk			
Nuts		0.34	
Sweets and desserts	0.37		-0.20
Tubers	0.21		
Pasta	0.27		-0.14
Snacks	0.37		-0.20
Eggs	0.26		-0.14
Cheese		0.29	
Butter/margarine	0.21		
Red meat	0.23		-0.12
Poultry	0.24		
Processed meat	0.32		-0.17
Fish		0.36	
Soft drinks	0.30		-0.16
Juice		0.30	
Coffee			
Beer			
Wine			
Distilled			
Eigenvalue	2.51	1.77	1.27
% explained variance	10.10	7.03	5.07
% cumulative explained variance	10.10	17.10	22.15

Table 3. Food groups' factor loadings, eigenvalues and explained variance among dietary patterns through Reduced Rank Regression.

Food Group	Convenience
Rice	0.01
Cereals	-0.13
Bread	0.05
Fruits	-0.47
Vegetables	-0.30
Beans	-0.24
Milk	-0.10
Nuts	0.12
Sweets and desserts	0.26
Tubers	-0.05
Pasta	0.11
Snacks	0.34
Eggs	0.15
Cheese	0.18
Butter/margarine	0.24
Red meat	0.30
Poultry	0.08
Processed meat	0.27
Fish	0.09
Soft drinks	0.20
Juice	0.03
Coffee	-0.15
Beer	0.14
Wine	0.08
Distilled	0.08
<hr/>	
% explained variance of predictors variables	5.84
% ED ^a explained variance	42.86
% FD ^b explained variance	60.14
% Total fat explained variance	43.22
% explained variance of response variables	48.74

^aED energy density^bFD fiber density

Table 4. Baseline characteristic and nutritional profile of total population and of individuals classified on the third tertile of each dietary pattern (Mean (SD) or N (percentage)).

	Total population	Convenience			Prudent		Rice and beans/Traditional	
		FA ^a	TT ^b	RRR ^c	FA	TT	FA	TT
Age (years)	51.6(8.9)	49.9(8.6)	49.4(8.3)	49.8(8.7)	53.1(8.9)	53.2(9.0)	50.4(8.3)	51.3(8.6)
Female	6,997(54.4)	1,532(35.7)	1,345(31.5)	1,899(44.5)	2,181(51.1)	2,346(54.9)	1,395(32.6)	1,725(40.4)
BMI (Kg/m ²)	27.1(4.7)	27.6(4.6)	27.7(4.8)	27.3(4.7)	27.3(4.7)	27.3(4.7)	27.5(4.6)	27.0(4.7)
Smoker ^d	1,559(12.2)	618(14.7)	631(14.8)	644(15.1)	399(9.3)	373(8.7)	702(16.4)	574(13.4)
Educational level								
Until 8 years	1,428(11.1)	396(9.3)	589(13.8)	294(6.9)	479(11.2)	378(8.8)	912(21.3)	786(18.4)
9 years or more	11,388(88.9)	3,876(90.7)	3,683(86.2)	3,978(93.1)	3,793(88.8)	3,894(91.2)	3,360(78.7)	3,486(81.6)
Self-reported skin colour ^d								
White	6,815(53.8)	2,502(59.3)	2,141(50.6)	2,505(59.4)	2,107(50.1)	2,283(54.3)	1,663(39.3)	1,821(43.1)
Non-white	5,858(46.2)	1,720(40.7)	2,091(49.4)	1,709(40.6)	2,100(49.9)	1,921(45.7)	2,572(60.7)	2,403(56.9)
Energy density (kcal/g)	1.4 (0.3)	1.6 (0.3)	1.6 (0.3)	1.7 (0.3)	1.3 (0.3)	1.3 (0.3)	1.5 (0.3)	1.4 (0.3)
Fibre density (g/1000kcal)	16.0 (5.0)	14.0 (4.6)	15.2 (4.6)	11.9 (2.8)	17.3 (4.8)	17.2 (4.8)	17.1 (5.0)	18.5 (4.9)
%kcal from carbohydrate	56.6 (7.3)	53.5 (6.8)	55.3 (6.5)	51.1 (6.4)	57.7 (7.4)	57.4 (7.6)	57.0 (6.5)	58.6 (6.2)
%kcal from protein	16.8 (2.7)	16.4 (2.6)	16.5 (2.5)	17.1 (3.0)	17.0 (2.6)	17.0 (2.7)	16.8 (2.5)	16.6 (2.4)
%kcal from animal protein	9.1 (3.2)	9.3 (3.1)	8.8 (3.0)	10.4 (3.4)	9.3 (3.1)	9.3 (3.1)	8.4 (3.1)	7.7 (2.7)
%kcal from vegetable protein	7.6 (1.7)	7.1 (1.5)	7.6 (1.7)	6.6 (1.3)	7.6 (1.6)	7.6 (1.6)	8.3 (1.8)	8.9 (1.7)
%kcal from fat	26.4 (5.0)	28.7 (4.7)	27.5 (4.5)	30.1 (4.6)	25.6 (5.0)	26.2 (5.3)	25.2 (4.3)	24.5 (4.2)
%kcal from saturated fat	8.6 (2.4)	9.6 (2.3)	8.9 (2.3)	10.2 (2.3)	8.1 (2.2)	8.4 (2.4)	7.6 (1.9)	7.4 (1.9)

^aFA Factor analysis,^bTT Treelet Transform^cRRR Reduced Rank Regression^dVariable with missing data

Table 5. Cross-classification of dietary patterns, according to each statistical method.

FA ^a and TT ^b -dietary patterns	Total agreement	Opposite tertiles
Convenience	66.1	2.0
Brazilian traditional/rice and beans	58.5	5.3
Prudent	75.9	0.5
FA and RRR ^c -convenience dietary pattern	56.3	6.0
TT and RRR-convenience dietary pattern	44.8	12.3

^aFA Factor analysis,

^bTT Treelet Transform

^cRRR Reduced Rank Regression

4.2 PADRÕES ALIMENTARES E ADIPOSIDADE AO LONGO DO TEMPO

Este subcapítulo apresenta o manuscrito “*Dietary patterns and adiposity over time – Analysis of the ELSA-Brasil*” de autoria de Mariane de Almeida Alves, Paulo Andrade Lotufo, Isabela Martins Benseñor e Dirce Maria Lobo Marchioni. O artigo foi submetido para publicação no periódico *European Journal of Nutrition* (comprovante em anexo).

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Title Page

Title: Dietary patterns and adiposity over time – Analysis of the ELSA-Brasil

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Abstract

Purpose: Data-driven methods applied to identify dietary patterns (DP) are entirely dependent on the study population. Combining different methods may provide new insights on how diet is associated with health outcomes. This study investigated the association of DP derived from three methods and adiposity indicators over time.

Methods: This study used data from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). DP were identified at baseline applying three statistical methods: (1) Factor Analysis (FA), (2) Treelet Transform (TT), and (3) Reduced Rank Regression (RRR). The association between DP and adiposity indicators over the period of 8.2 years of follow-up was assessed by linear mixed-models.

Results: Convenience DP was associated with higher adiposity over the follow-up period, regardless of the method applied. Only the DP identified by TT and marked by high consumption of rice and beans was associated with lower adiposity over time, whereas the similar DP identified by FA, but additionally characterised by consumption of poultry and red meat was associated with higher adiposity over time. Prudent DP identified by FA was associated with lower adiposity status across the median follow-up time.

Conclusion: Applying different methods to identify dietary patterns showed that a convenience DP was associated with higher adiposity level independent of the method applied and also identified the nuances within adherence to a Brazilian traditional dietary pattern characterised by the consumption of rice and beans that only when combined with reduced consumption of red meat and ultra-processed foods was associated with lower adiposity over time.

Keywords: Dietary patterns; adiposity; waist circumference; body fat percentage; fat mass index; longitudinal study

Statements and declarations

Competing interests: The authors declare no competing interests.

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1 **Introduction**

2 Global rates of obesity have increased over the past decades in all age groups and both
3 sexes [1], but with higher prevalence among middle-aged and elderly individuals (50 to 65
4 years) [2]. Epidemiological studies have also shown an increased trend in the prevalence of
5 overweight and obesity in this age group in the Brazilian population from 2006 to 2019 [3,4].
6 As it is well known, obesity is a main risk factor for several chronic diseases, including
7 metabolic syndrome [5,6], type 2 diabetes mellitus, coronary artery disease [7], cerebral
8 vascular disease [8], hypertension, dyslipidaemia, and some types of cancer [6,9,10]. These
9 combined factors are also responsible for reducing quality of life and life expectancy [10]. The
10 excessive fat accumulation characterised in the obesity normally occurs with age progress [11],
11 and combined with this natural process, lifestyle factors play a fundamental role in weight gain
12 and increased adiposity over time.

13 The nutrition transition, marked by switching traditional fresh foods to ready-to-eat and
14 processed meals [12], has set a new challenge for global health in the achievement of breaking
15 the rise of obesity until 2025 [13]. Previous studies exploring the association between specific
16 food groups and longitudinal changes in adiposity have found that ultra-processed food
17 consumption is associated with higher adiposity [14,15], including sugar sweetened beverages
18 [16], processed meat, pre-made meals, snacks, and fast food [17]. In the Brazilian population,
19 the dietary patterns labelled as traditional, prudent and convenience/unhealthy are commonly
20 identified in different studies, but presenting contradictory results with different outcomes [18-
21 20] and it is still not clear what dietary pattern within the Brazilian population may play a risk
22 or protection role for chronic diseases. Data-driven dietary pattern analysis is a comprehensive
23 way to understand how people combine food groups and shape their diet. Different statistical
24 approaches can be applied to identify data-driven dietary patterns and the results are entirely
25 dependent on the study population, culture and ethnicity [21-23].

26 We have previously identified that applying different methods in the same population
27 provides similar dietary patterns [24] that can be compared and may provide new insights on
28 how dietary patterns are associated with adiposity in a specific population. This study aimed to
29 examine how the convenience, Brazilian traditional, rice and beans, and prudent dietary
30 patterns, previously derived from three different statistical methods, are associated with
31 longitudinal changes in adiposity in the ELSA-Brasil population.

32 **Methods**

33 *Study participants*

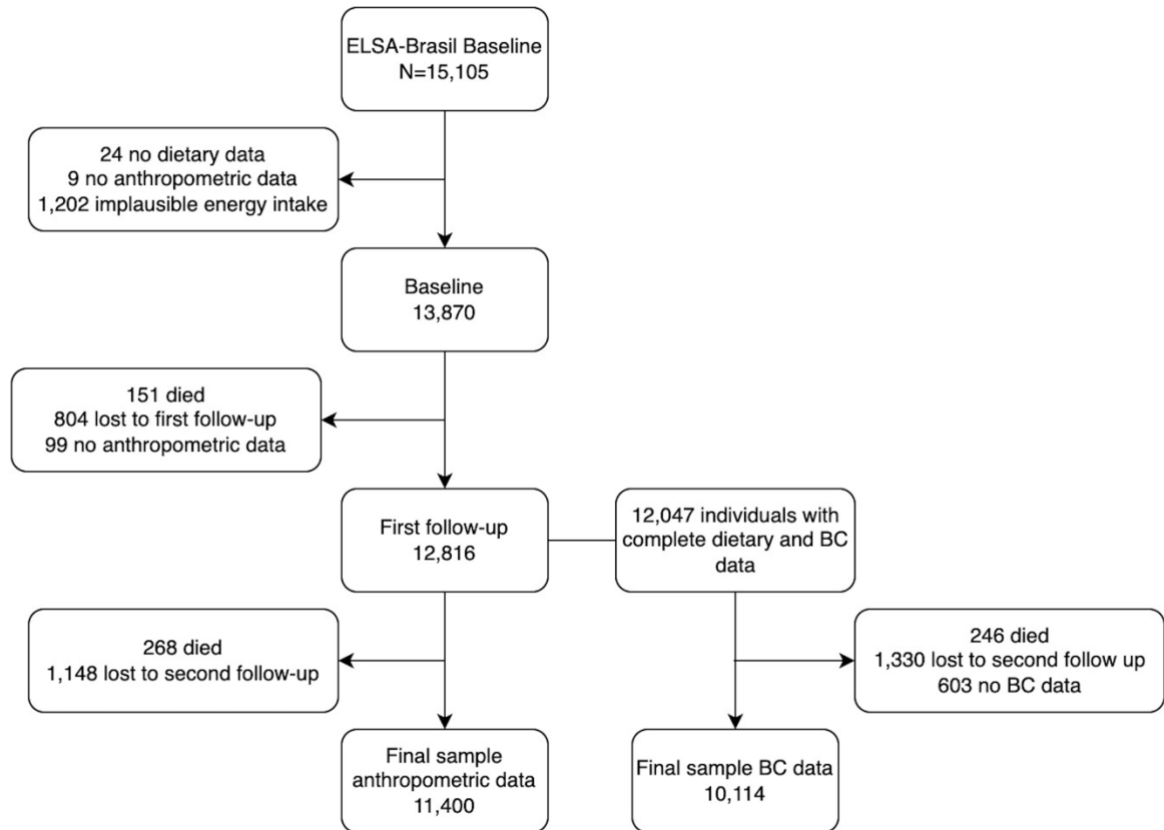
34 Participants were included from the Brazilian Longitudinal Study of Adult Health
35 (ELSA-Brasil), a prospective cohort study whose the main purpose is to investigate factors
36 related to the aetiology of diabetes and cardiovascular diseases. The ELSA-Brasil recruited
37 15,105 active and retired civil servants, aged 35-74 years, from five universities and one
38 research institute covering three Brazilian macro regions (Southeast, Northeast and South).
39 Individuals who related pregnancy, intention to leave work at the institution, severe cognitive
40 damage, and retired individuals who lived outside of the research centre region were not
41 included in the study. All individuals were examined at the baseline between 2008-2010, and
42 two subsequent follow-up visits were conducted, the first one between 2012-2014, and the
43 second between 2017-2019. All data collection was performed by trained personnel with strict
44 quality control. Detailed information regarding the study design, sample and data collection
45 have been previously described [25-27].

46 This study was conducted according to the guidelines laid down in the Declaration of
47 Helsinki and all procedures involving human subjects were approved by all the institutional
48 review boards involved (Universidade Federal da Bahia, Universidade Federal do Espírito
49 Santo, Universidade Federal de Minas Gerais, Fundação Oswaldo Cruz, Universidade de São
50 Paulo and Universidade Federal do Rio Grande do Sul). All participants signed a written
51 informed consent [28].

52 For these current analyses, of the 15,105 participants recruited at the baseline, we
53 excluded 1,226 participants without dietary data or with implausible total energy intake
54 (misreport of energy intake defined by McCroy *et al.*) [29]. Regarding the anthropometric data,
55 we excluded nine participants with incomplete anthropometric data, thus leaving 13,870
56 participants. Of those, 151 died between baseline and first follow-up and 804 did not attend the
57 first follow-up visit. Additionally, 99 did not have completed anthropometric data at that time,
58 remaining therefore 12,816 participants. From those, 268 died between first and second follow-
59 up and 1,148 did not attend the second follow-up visit. For the anthropometric data we had a
60 final sample of 11,400 participants. The bioimpedance body composition data were first
61 measured at the first follow-up. At that time, we had 12,047 participants with completed dietary
62 data and the first bioimpedance exam. Between the first and second bioimpedance examination,
63 268 participants died and 1,416 participants did not attend the second follow-up visit of the

64 study. From those who participated on the second follow-up visit, only 10,637 had completed
 65 data for the bioimpedance examination. **Fig. 1** shows details of the participants selection.

66 **Fig. 1** Flowchart of final sample in this study analyses. ELSA-Brasil, 2022.



67

68 BC: body composition.

69

70

71 *Dietary assessment and dietary patterns*

72 Dietary intake was assessed at baseline by a validate semi-quantitative food frequency
 73 questionnaire (FFQ) comprising 114-food items [30]. Participants were asked to answer the
 74 frequency and portion size of the food items consumed in the last 12 months. Participants
 75 related their consumption using standard household measure and the frequencies of
 76 consumption according to eight categories from “more than 3 times a day” to “1 to 3 times a
 77 month”. The FFQ used the Nutrition Data System for Research (NDSR) software (University
 78 of Minnesota, Minneapolis, USA, 2010) and the *Tabela Brasileira de Composição de Alimentos*
 79 (TACO) (available on: <http://www.unicamp.br/nepa/taco/>) to obtain the nutritional information

80 of the items listed in the FFQ, such as, dietary nutrients, total energy and other food
81 components. More details about the FFQ have been previously published [30,31].

82 The dietary pattern analysis and results have been previously described and published
83 [24]. Briefly, three statistical methods were applied to identify dietary patterns: Factor Analysis
84 (FA), Treelet Transform analysis (TT) and Reduced Rank Regression (RRR). A convenience
85 dietary pattern, commonly loaded for the sweets and desserts, snacks, butter and margarine, red
86 meat, processed meat, and soft drinks food groups was identified by all the three methods. FA
87 and TT also identified a similar prudent dietary pattern, commonly loaded for the cereals, fruits,
88 vegetables, nuts, fish, and juice food groups, as well as a Brazilian traditional (FA) and rice and
89 beans (TT) dietary patterns, which were commonly loaded for the rice and beans food groups.
90 The last two dietary patterns represent the Brazilian traditional food habit of combining rice
91 and beans. **Supplementary Fig. 1, 2 and 3** show the food groups' factor loadings in each dietary
92 pattern identified by Factor Analysis, Treelet Transform and Reduced Rank Regression.

93

94 *Anthropometric measures and body composition assessment*

95 Anthropometric measures were obtained by trained personnel at the research centres
96 following standardized procedures at baseline and the two follow-up visits [32,33]. For the
97 waist circumference and weight measurements, individuals were fasting, with an empty
98 bladder, without glasses or any other personal objects. Waist circumference (cm) was measured
99 using an inelastic measuring tape (Mabis-Gulick®, Waukegan, IL, USA) at the midpoint
100 between the inferior edge of the costal border and the iliac crest in the mid-axillar line. Body
101 weight was measured in an electronic scale with a maximum capacity of 300 kg (Toledo™, São
102 Bernardo do Campo, Brazil). Height (cm) was measured at the nearest 0.1 cm using a
103 stadiometer (Toledo™, São Bernardo do Campo, Brazil) with participants barefoot.

104 At the first and second follow-up visits, body composition was evaluated by segmental
105 bioimpedance with eight electrodes (InBody 230; InBodyCo., Seoul, Korea), capable to
106 differentiate truncal and limb masses. For the exam, the participants were fasting for at least
107 eight hours and they were instructed not to drink caffeine nor engage in physical activity within
108 the previously 24 hours of the exam. Participants were asked to remove all metallic accessories
109 (earring, rings, glasses, etc.) and within minutes prior to the exam participants were asked to
110 empty their bladders. Bioimpedance exam was not performed on those with pacemakers or
111 metal prostheses [34]. Body fat percentage (BF%) and fat mass index (FMI) were analysed as

112 adiposity markers. Fat mass index was calculated dividing total fat mass (kg) by height squared
113 (m^2).

114

115 *Covariates assessment*

116 A comprehensive set of in-person interviews using standardised questionnaires were
117 carried out to assess sociodemographic and lifestyle information. For this analysis we evaluated
118 the sociodemographic and lifestyle data available at the baseline, including sex (male or
119 female), age (completed years), educational level (incomplete primary school, complete
120 primary school, complete high school, and university degree), smoking status (no smoker,
121 former smoker or smoker), level of physical activity during leisure time (inactive, insufficiently
122 active or active) classified according to the data assessed by the International Physical Activity
123 Questionnaire (IPAQ) [35].

124

125 *Statistical analyses*

126 We applied linear mixed models to assess the association between the scores of each
127 dietary pattern at baseline and weight, waist circumference and BMI all measured at three time
128 points, and BF% and FMI measured at two time points. The fixed-effect structure included the
129 dietary pattern scores and possible confounders whereas a random-effect structure accounted
130 for the within individuals' variance of the repeated measures and a random slope for time. To
131 test whether the association between the dietary patterns scores and adiposity differed across
132 the follow-up time, a dietary pattern score \times time interaction term was added. The likelihood
133 ratio test [36] was applied to define fixed-effect and random-effect structure. All models were
134 adjusted for age, sex, time of the repeated measures, total energy intake, education status,
135 physical activity, and smoking status. The effect estimates for the dietary patterns scores in the
136 linear mixed models indicate the association of the dietary pattern scores with adiposity
137 outcomes averaged within the median follow-up of 8.2 years. On additional analyses we tested
138 the association between the dietary patterns scores and fat free mass.

139 All analyses were performed using software R version 4.1.1 (R Foundation for
140 Statistical Computing, Vienna, Austria).

141

142 **Results**

143 Among the dietary patterns from the three methods, the convenience dietary pattern was
144 the most consistent regarding the associations with adiposity outcomes. After multivariable
145 adjustment, an increase in the convenience dietary pattern score from the three methods was
146 associated with higher level of all adiposity indicators evaluated over the median follow-up
147 period of 8.2 years (Table 2). The increase in the Brazilian traditional dietary pattern score
148 derived from FA was associated with a lower mean of 0.51kg for weight (95%CI: -0.83;-0.20),
149 but with higher mean of 0.2 points for body fat percentage (95%CI: 0.02;0.38) and 0.12kg/m²
150 for fat mass index (95%CI: 0.03;0.21) over time. The increase in the rice and beans dietary
151 pattern score derived from TT was associated with lower values of all adiposity indicators
152 evaluated (Table 3). The prudent dietary pattern derived from FA was associated with lower
153 values of waist circumference, BMI, body fat percentage and fat mass index, but was not
154 associated with lower weight over time. The prudent dietary pattern derived from TT presented
155 opposite results, with a higher score on this dietary pattern associated with higher mean of 0.3kg
156 for weight (95%CI: 0.08;0.52) (Table 4).

157 Regarding the interaction between the dietary pattern scores and time, we found a
158 significant interaction term between the convenience dietary pattern score from RRR and time
159 for the outcomes weight, waist circumference, BMI and body fat percentage. The interaction
160 term between convenience dietary pattern from FA was also significant for the outcomes
161 weight, body fat percentage and fat mass index. The interaction term between the convenience
162 dietary pattern from TT analysis was only significant for body fat percentage. All these
163 estimates indicate stronger association between the convenience dietary patterns and the
164 aforementioned adiposity indicators over the follow-up period. The interaction between the rice
165 and beans dietary pattern from TT and time was significant for the outcomes weight, waist
166 circumference and BMI, and the prudent dietary pattern derived from both FA and TT analysis
167 presented a significant interaction with time for the outcomes weight, waist circumference, and
168 BMI. For these estimates we found a weaker association between the dietary patterns and the
169 adiposity outcomes over the follow-up period. All other interaction terms showed a stable
170 association of the dietary pattern with the outcome (non-significant interaction term). The
171 coefficients of the interaction between dietary pattern scores and time are presented on the
172 Supplementary Table 1. Additional analyses showed that convenience dietary pattern derived
173 from the three methods was associated with higher fat free mass over the follow-up and the
174 same was observed for the prudent dietary pattern derived from TT analysis. While the

175 traditional/rice and beans dietary patterns from both FA and TT were associated with lower fat
176 free mass over the median 8.2 years of follow-up (Supplementary Table 2).

177

178 **Discussion**

179 The convenience dietary pattern was consistently associated with higher adiposity over
180 the follow-up period independent of the statistical method applied to identify this dietary
181 pattern. Our results confirmed that a diet marked by high consumption of the snack, processed
182 meat, red meat, sweets and desserts, and soft drinks food groups are associated with higher
183 adiposity. All these food groups, except for red meat, are classified as ultra-processed food and
184 previous studies have shown that higher percentage of energy intake from these foods are
185 associated with higher total body fat percentage, and android and gynoid fat percentage among
186 U.S adults [37]. Similar results were found in a study conducted with Australian adults, where
187 a higher level of energy intake from ultra-processed foods was associated with higher adiposity
188 indicators especially in inactive individuals and those over 40 years old [38]. A longitudinal
189 study conducted with the ELSA-Brasil population identified that individuals classified in the
190 highest level of energy intake of ultra-processed foods had a higher weight and waist
191 circumference gain over a period of 3.8 years of follow-up. These individuals also had greater
192 risk of incident overweight/obesity [15]. All studies mentioned above applied the NOVA [39]
193 food classification to identify the consumption of ultra-processed foods, while our study
194 identified the population's dietary patterns, bringing additional information on how these food
195 groups are consumed in combination. This is clear when we analyse the convenience dietary
196 pattern identified by Treelet Transform method, that was able to identify a diet characterised
197 by the consumption of unhealthy foods combined with the Brazilian traditional foods, that is
198 rice and beans. Additionally, the Treelet Transform method identified a dietary pattern highly
199 and positively loaded for rice and beans while negatively loaded for unhealthy food groups
200 present in the convenience dietary pattern. These results showed us that this method was able
201 to identify two dietary patterns marked by the consumption of Brazilian traditional foods, but
202 only a diet that is not simultaneously marked by the consumption of unhealthy foods was
203 associated with a consistent decrease in the adiposity indicators over time.

204 This hypothesis is strengthened with our results regarding the association between the
205 Brazilian traditional dietary pattern identified by Factor Analysis and adiposity indicators. This
206 dietary pattern, characterised by high and positive factor loadings for rice, beans, and poultry

207 food groups, and also intermediate loadings for red meat, processed meat and beer, was
208 associated with a decrease in weight but an increase in body fat percentage and fat mass index.
209 A study conducted with Brazilian adults also identified a Brazilian traditional dietary pattern
210 (characterised by the consumption of rice, beans and red meat) associated with higher waist
211 circumference, BMI, and higher levels of cytokines and markers of oxidative stress [40]. These
212 results may be explained by the high consumption of red and processed meat by the Brazilian
213 population [41,42]. A study from UK biobank showed that meat intake was associated with
214 higher inflammatory markers, and this association was mostly explained by higher adiposity
215 [43]. Even though legumes are rich in fibre, low in fat and a good source of vegetable protein,
216 characterising a nutrient profile associated with health benefits [44], we should also take into
217 account the possible addition of processed meats, traditionally consumed in Brazil when
218 preparing beans [40].

219 The prudent dietary patterns were positively loaded for cereals, fruits, vegetables, fish
220 and juice for both Factor Analysis and Treelet Transform and showed an inverse association
221 for most adiposity indicators. This may be partly explained by the higher consumption of plant-
222 based foods, considering that these foods contain more fibre, antioxidants, and plant unsaturated
223 fatty acids [45]. Evidence suggests that these components reduce adiposity through certain
224 pathways, such as satiety [46], reducing inflammation and oxidative stress [47-49], as well as
225 gut microbiota composition [50].

226 The strengths of our study include the longitudinal analyses of adiposity, using not only
227 anthropometric measures but also body composition indicators, the quality and the large
228 number of data collected, which allowed for the adjustment of several covariates, the use of a
229 validated food frequency questionnaire to assess dietary data and the application of three
230 methods to identify dietary patterns, which provided new insights on the role of diet in the
231 adiposity process. Still, some limitations need to be addressed. Independent of the method
232 applied, dietary pattern analysis has some level of subjectivity on the interpretation of dietary
233 patterns, food grouping, number of factors to retain and the cut-off points for factor loadings.
234 However, all these decisions were based on previous literature and followed strict and detailed
235 method procedures. Additionally, our results are not representative of all Brazilian population.
236 The ELSA-Brasil study is a cohort with data from civil servants working in universities and
237 research institutes that present higher education, income and socioeconomic level than the
238 general population [51].

239 Conclusion

240 Our findings have significant public health implications. Applying different methods to
241 identify dietary patterns confirmed the consistent unfavourable effect of a diet pattern
242 characterised by ultra-processed foods rich in energy density, saturated fat, and sugar, while
243 low in fibre density. Our results also suggest some nuances within adherence to a Brazilian
244 traditional dietary pattern characterised by high consumption of rice and beans. In this study
245 population we found two different patterns characterised by the consumption of these food
246 groups, showing that a positive effect on adiposity was only present when combined with lower
247 consumption of red and processed meat and other unhealthy food groups.

248 **Tables**249 **Table 1. Participants characteristics at baseline. ELSA-Brasil 2022.**

Characteristics	Baseline
	Mean (SD)
Age (years)	51.2 (8.8)
Weight (kg)	74.0 (15.0)
Waist circumference (cm)	91.1 (12.7)
BMI (kg/m ²)	27.0 (4.7)
Body fat (%) ^a	34.2 (8.7)
Fat mass index (kg/m ²) ^a	9.7 (3.9)
Energy intake (kcal/day)	2,183.6 (689.8)
	N (%)
Sex	
Female	6,316 (55.4)
Educational level	
Incomplete primary school	481 (4.2)
Complete primary school	644 (5.6)
Complete high school	3,821 (33.6)
University degree	6,454 (56.6)
Physical activity	
Inactive	8,585 (76.5)
Insufficiently active	1,592 (14.2)
Active	1,050 (9.4)
Smoking status	
Never	6,711 (58.9)
Former	3,372 (29.6)
Current	1,317 (11.6)

250 Physical activity n= 11,227, body fat and fat mass index n=10,114

251 ^aValues measured in the first follow-up visit. On this study, this is the first-time measurements
252 for these variables

253 Table 2. Associations of the convenience dietary pattern scores derived from factor analysis treelet transform, and reduced rank regression with
 254 longitudinal weight, WC, BMI, BF%, FMI. ELSA-Brasil 2022.

Convenience DP	Weight (kg)	WC (cm)	BMI (kg/m ²)	BF (%)	FMI (kg/m ²)
	β (95%CI)				
Factor analysis	2.44(2.10;2.77)*	2.06(1.78;2.34)*	0.61(0.49;0.72)*	0.64(0.46;0.83)*	0.36(0.27;0.46)*
Treelet transform	1.54(1.26;1.82)*	1.56(1.33;1.80)*	0.49 (0.40;0.59)*	0.63 (0.48;0.78)*	0.35(0.27;0.43)*
Reduced rank regression	0.93(0.68;1.17)*	0.80(0.59;1.01)*	0.26(0.18;0.34)*	0.37(0.22;0.51)*	0.19(0.11;0.26)*

255 Models adjusted for age, sex, total energy intake, time of repeated measurements, education, physical activity, and smoking status.

256 WC: waist circumference, BMI: body mass index, BF: body fat, FMI: fat mass index.

257 *p-value < 0.05.

258 Table 3. Associations of the Brazilian traditional and rice and beans dietary patterns scores derived from factor and treelet transform analyses with
 259 longitudinal weight, WC, BMI, BF%, FMI. ELSA-Brasil 2022.

Brazilian traditional/Rice and beans DP	Weight (kg)	WC (cm)	BMI (kg/m ²)	BF (%)	FMI (kg/m ²)
	β (95%CI)				
Factor analysis	-0.51(-0.83;-0.20)*	0.12(-0.14;0.39)	0.07(-0.04;0.18)	0.2(0.02;0.38)	0.12(0.03;0.21)*
Treelet transform	-1.64(-1.88;-1.40)*	-1.23(-1.43;-1.03)*	-0.42(-0.5;-0.34)*	-0.51(-0.66;-0.37)*	-0.26(-0.33;-0.18)*

260 Models adjusted for age, sex, total energy intake, time of repeated measurements, education, physical activity, and smoking status.

261 WC: waist circumference, BMI: body mass index, BF: body fat, FMI: fat mass index.

262 *p-value < 0.05.

263 Table 4. Associations of the prudent dietary pattern scores derived from factor and treelet transform analyses and longitudinal weight, WC, BMI,
 264 BF%, FMI. ELSA-Brasil 2022.

Prudent DP	Weight (kg)	WC (cm)	BMI (kg/m ²)	BF (%)	FMI (kg/m ²)
	β (95% CI)				
Factor analysis	-0.2(-0.5;0.1)	-0.42(-0.67;-0.17)*	-0.11(-0.21;-0.01)*	-0.37(-0.55;-0.2)*	-0.16(-0.25;-0.07)*
Treelet transform	0.3(0.08;0.52)*	-0.03(-0.22;0.15)	0.04(-0.03;0.12)	-0.08(-0.21;0.05)	-0.02(-0.09;0.04)

265 Models adjusted for age, sex, total energy intake, time of repeated measurements, education, physical activity, and smoking status.

266 WC: waist circumference, BMI: body mass index, BF: body fat, FMI: fat mass index.

267 *p-value < 0.05.

268 **Conflict of interest statement**

269 On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Title: Dietary patterns and adiposity over time – Analysis of the ELSA-Brasil

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Supplementary material

Supplementary Table 1. Interaction terms between dietary patterns scores and time of repeated measures for the associations between the dietary pattern scores and longitudinal measures weight, body mass index, waist circumference, body fat percentage, fat mass index. ELSA-Brasil 2022.

	Weight (kg)	WC (cm)	BMI (kg/m ²)	BF (%)	FMI (kg/m ²)
	β (95%CI)				
Convenience					
FA x time	0.02 (0.01;0.03)*	0.00 (-0.01;0.02)	0.00 (0.00;0.01)	0.03 (0.01;0.05)*	0.01 (0.01;0.02)*
TT x time	0.01 (0.00; 0.02)	0.00 (-0.01;0.01)	0.00 (0.00;0.01)	0.02 (0.01;0.03)*	0.00 (0.00;0.01)
RRR x time	0.03 (0.02;0.04)*	0.02 (0.01;0.03)*	0.01 (0.01;0.02)*	0.02 (0.01;0.03)*	0.01 (0.00;0.01)
Brazilian traditional/Rice and beans					
FA x time	0.00 (-0.02;0.01)	-0.01 (-0.03;0.01)	0.00 (-0.01;0.01)	0.01 (-0.01;0.03)	0.00 (-0.01;0.01)
TT x time	-0.02 (-0.03;-0.01)*	-0.02 (-0.03;-0.01)*	-0.02 (-0.03;-0.01)*	0.00 (-0.01;0.01)	-0.01 (-0.01;0.01)
Prudent					
FA x time	-0.03 (-0.05;-0.02)*	-0.03 (-0.05;-0.02)*	-0.01 (-0.02;-0.01)*	-0.01 (-0.03;0.00)	0.00 (-0.01;0.00)
TT x time	-0.02 (-0.03;-0.01)*	-0.02 (-0.03;-0.01)*	-0.01 (-0.02;-0.01)*	-0.01 (-0.02;0.00)	0.00 (-0.01;0.00)

Beta coefficients from the fully adjusted models. FA: factor analysis, TT: treelet transform, RRR: reduced rank regression, WC: waist circumference, BMI: body mass index, BF: body fat, FMI: fat mass index.

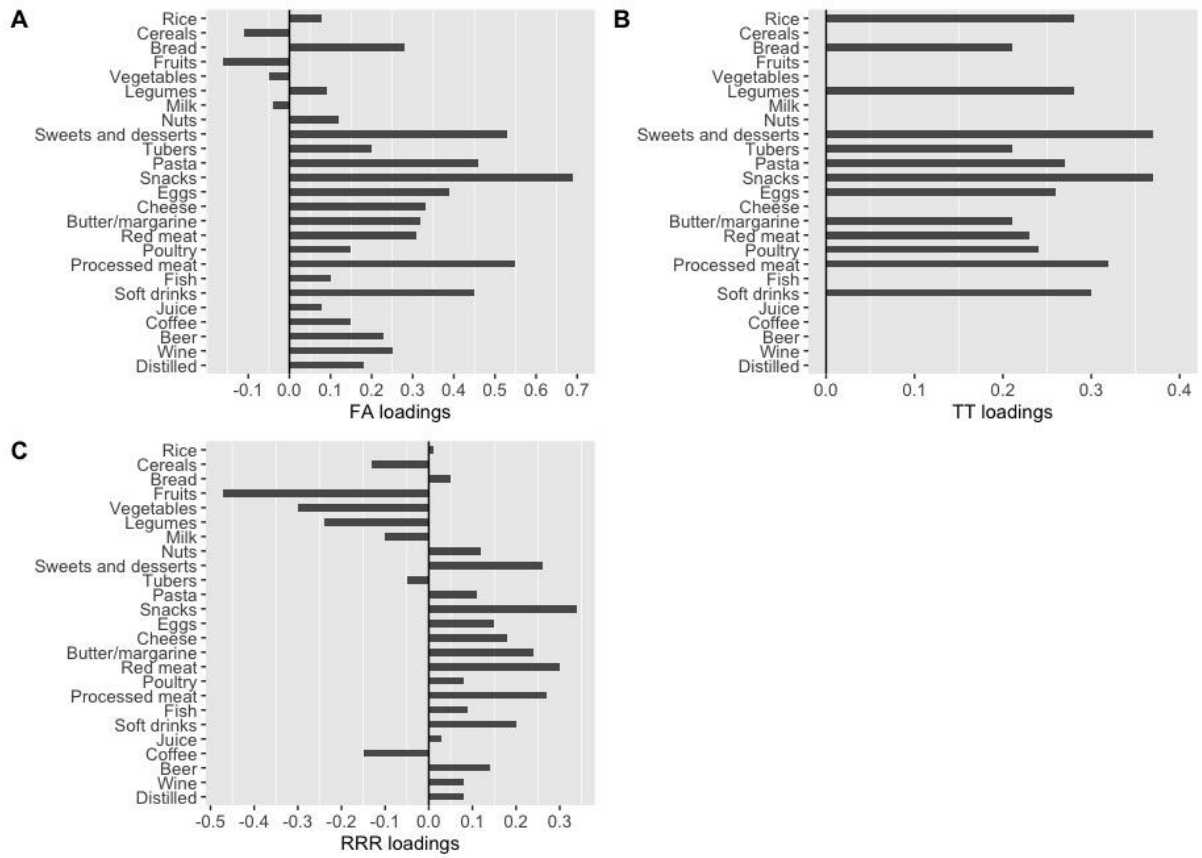
*p-value < 0.05

Supplementary Table 2. Associations between the dietary patterns scores derived from factor, treelet transform, and reduced rank regression analyses at baseline and longitudinal fat free mass assessed by bioimpedance. ELSA-Brasil 2022.

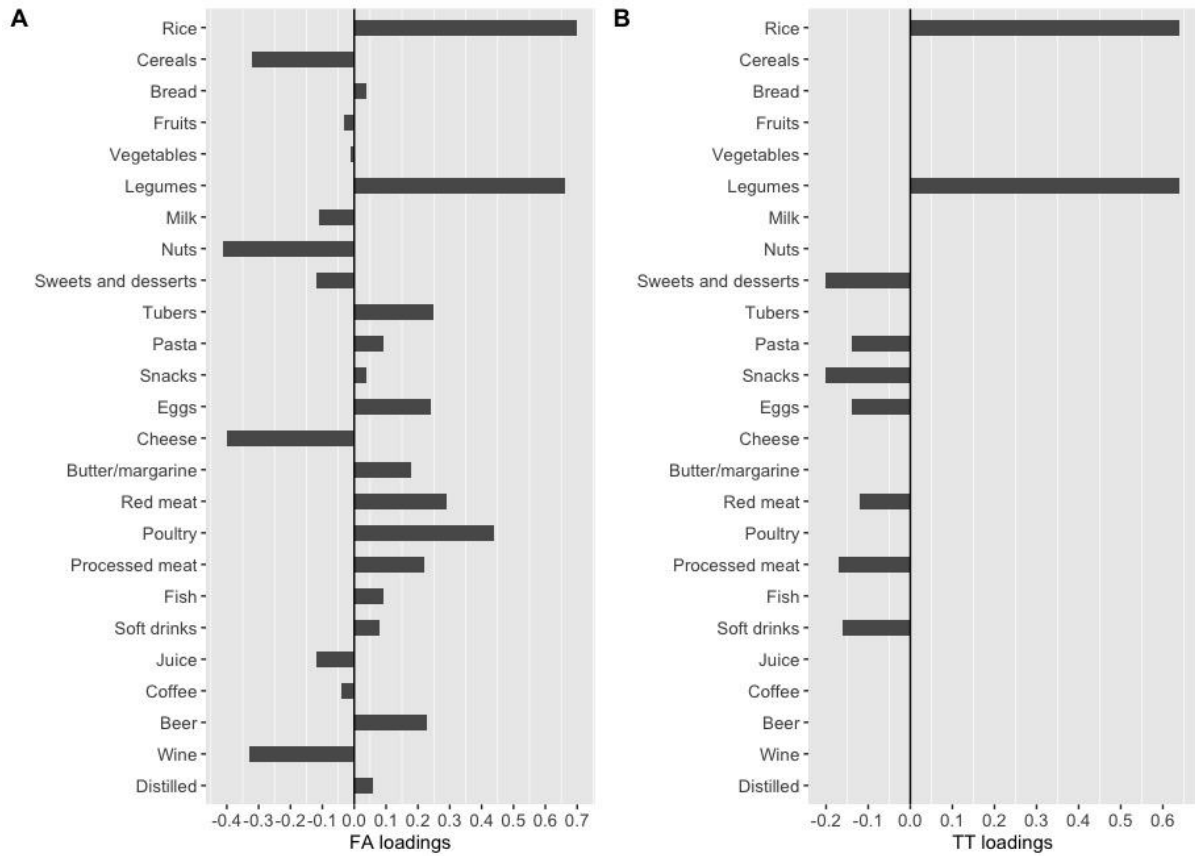
	Fat free mass (kg) β (95%CI)
Convenience	
Factor analysis	0.99 (0.81;1.17)*
Treelet transform	0.47 (0.32;0.62)*
Reduced rank regression	0.30 (0.17;0.44)*
Traditional Brazilian/Rice and beans DP	
Factor analysis	-0.41 (-0.58;-0.23)*
Treelet transform	-0.63 (-0.76;-0.49)*
Prudent	
Factor analysis	0.10 (-0.07;0.26)
Treelet transform	0.22 (0.09;0.34)*

Model adjusted for age, sex, total energy intake, time of repeated measurements, education, physical activity, and smoking status.

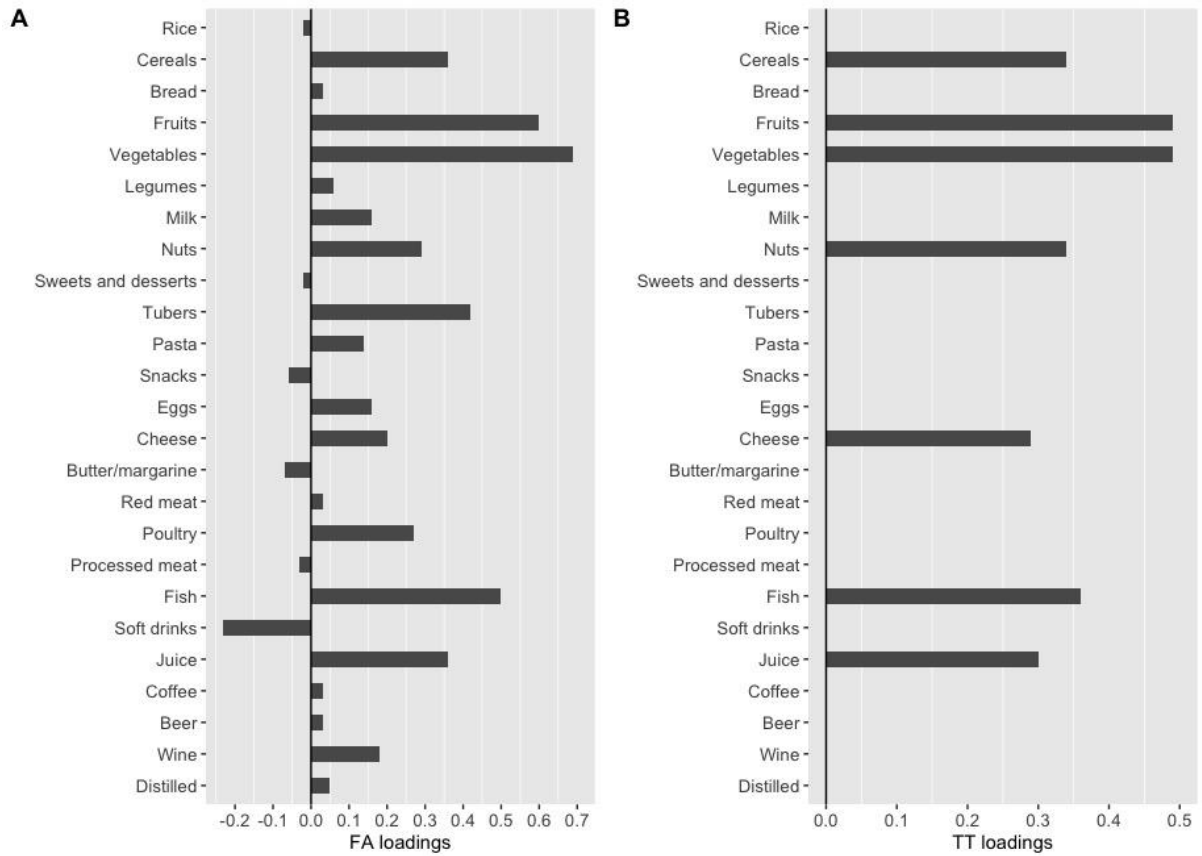
*p-value < 0.05.



Supplementary Fig. 1 Factor loadings for convenience dietary patterns derived from factor analysis (A), treelet transform (B), and reduced rank regression (C) in the ELSA-Brasil study (n=11,400). FA: factor analysis, TT: treelet transform, RRR: reduced rank regression.



Supplementary Fig. 2 Factor loadings for Brazilian traditional and rice and beans dietary patterns derived from factor analysis (A) and treelet transform (B) in the ELSA-Brasil study (n=11,400). FA: factor analysis, TT: treelet transform.



Supplementary Fig. 3 Factor loadings for prudent dietary patterns derived from factor analysis (A) and treelet transform (B) in the ELSA-Brasil study (n=11,400). FA: factor analysis, TT: treelet transform.

4.3 PADRÕES ALIMENTARES E AMINOÁCIDOS DE CADEIA RAMIFICADA ASSOCIADOS COM MARCADORES DE ADIPOSIDADE

Este subcapítulo apresenta o manuscrito “*RRR-dietary pattern explaining circulating branched-chain amino acids is associated with increased levels of adiposity over time in Brazilian adults*” com autoria de Mariane de Almeida Alves, Alexsandro Macedo Silva, Paulo Andrade Lotufo, Isabela Martins Benseñor, e Dirce Maria Lobo Marchioni. O manuscrito será submetido ao periódico *Nutrition* devendo conter de 3000-5000 palavras, incluindo tabelas, figuras e referências.

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ABSTRACT

Objectives

Circulating levels of branched-chain amino acids (BCAA) have been linked with poor health outcome, including overall and abdominal obesity. This study aimed to identify the dietary patterns explaining the circulating levels of BCAA and estimate how it is associated with adiposity outcomes over a follow-up period of 8.3 years.

Methods

This study used data from the Longitudinal Study of Adult Health (ELSA-Brasil). Dietary pattern was identified using reduced rank regression analysis (RRR). The food groups were the predictors variables and the circulating levels of BCAA were defined as the response variable. Linear mixed-models were applied to estimate the association between RRR-dietary pattern and adiposity outcomes over the follow-up period.

Results

A mixed dietary pattern, combining food groups of a typical Brazilian meal and unhealthy food groups, was identified and explained 8.79% of circulating BCAA levels. This RRR-dietary pattern was associated with an increase of all adiposity outcomes averaged over the follow-up period.

Conclusion

Our results suggested that a dietary pattern combining Brazilian traditional and unhealthy food groups explains part of the variability on circulating levels of BCAA and this dietary pattern is associated with higher adiposity measures over time.

Keywords

Dietary patterns; Reduced rank regression; circulating BCAA; adiposity

INTRODUCTION

Branched-chain amino acids (BCAA) comprise the essential amino acids (AA) valine, leucine and isoleucine that are grouped as BCAA due to sharing the same structural features in side-chain and common catabolic pathways(1). Diet is the only source of BCAA and they represent 15-25% of total protein intake(2). BCAA-rich diets have been associated with positive metabolic health, responsible for controlling body weight gain, promoting protein synthesis, and glucose homeostasis(3). Despite these effects of dietary BCAA, studies have indicated that circulating levels of BCAA are highly correlated with obesity and visceral obesity(4), poor cardiometabolic health(5), including insulin resistance(6), type 2 diabetes(7) and cardiovascular disease(8). A previous study has shown a positive association between circulating BCAA and abdominal fat in Asian adults with no obesity and no visceral obesity(4) indicating that high levels of circulating BCAA could be an early marker of adiposity increase. Additionally, Allam-Ndoul et al. identified that individuals with overweight/obesity and metabolic syndrome presented higher levels of these amino acids when compared with their counterparts without metabolic syndrome(9), suggesting that higher levels of BCAA could also be connected with a harmful obesity profile to metabolic health.

This contradictory results between dietary and circulating BCAA make it unclear whether BCAA source foods or dietary patterns determine circulating BCAA or whether other factors affecting individuals metabolism are the drivers of these levels(10). Few studies have investigated the dietary patterns contributing for circulating BCAA levels(11) and the association between these dietary patterns and adiposity is still lacking.

We hypothesized that not only protein intake or BCAA dietary intake are able to explain the circulating BCAA level, but understanding the whole dietary pattern would be a better approach to identify how diet is linked with these metabolites. Thus, the aim of this study was to identify a dietary pattern explaining as much variance on BCAA circulating levels using reduced rank regression analysis and estimate the association of this dietary pattern with longitudinal adiposity measures in a Brazilian cohort over the median follow-up period of 8.3 years.

METHODS

Study population

This study was conducted using data from the Longitudinal Study of Adult Health (ELSA-Brasil), an ongoing prospective cohort study aiming to identify the factor associated with diabetes and cardiovascular diseases among Brazilian adults. A detailed description of the ELSA-Brasil study design, sample, and data collection is described elsewhere(12, 13, 14). Briefly, the cohort started with 15,105 active or retired civil servants aged 35 to 74 years. First examinations took place between 2008 and 2010. Two subsequent follow-up examinations were conducted in 2012-2014 and 2017-2019.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by all the institutional review boards involved (Fundação Oswaldo Cruz, Universidade Federal da Bahia, Universidade Federal do Espírito Santo, Universidade Federal de Minas Gerais, Universidade Federal do Rio Grande do Sul, and Universidade de São Paulo), and all participants signed a written informed consent(15).

For the current analysis, we used data of participants from the São Paulo research center, since only those had available data of circulating BCAA. From 5,022 participants in the ELSA-Brasil from São Paulo, we excluded 1,046 individuals with implausible energy intake leaving 3,976 participants at baseline. From those, a total of 128 participants died and 609 were lost to follow-up between baseline and the third wave examinations leaving a final sample of 3,239 participants for anthropometric data analysis. Regarding bioimpedance analysis, from 3,976 individuals at baseline, a total of 283 individuals died or were lost to follow-up between first and second examination and additionally, 250 individuals did not have bioimpedance data leaving 3,443 participants. From those, 421 died or were lost to follow-up in the third examination leading to a final sample of 3,022 participants for body composition analysis. Details regarding the study sample are presented on Figure 1.

Dietary data

Dietary intake was assessed at baseline using a validated semiquantitative food frequency questionnaire (FFQ) with 114-food items(16). Participants were asked to answer their frequency of food for all food groups considering the last 12 months. To obtain nutrients intake, the FFQ data were linked to Nutrition Data System for Research (NDSR) and Tabela de

Composição de Alimentos (TACO) (available here: <http://www.unicamp.br/nepa/taco/>). More details about the FFQ can be found on previously publications(16, 17). The FFQ food-items were classified into 25 food groups as described on Supplementary Table 1. The food grouping was based on nutritional profile similarities and eating occasion.

Anthropometric and body composition data

Anthropometric data were obtained by trained staff during in person examinations at the research center following standardized protocols(18, 19). For body weight and waist circumference measurements, individuals were dressing standardized clothes, were fasting, with empty bladder, without glasses or any other personal subjects. Body weight was measured in an electronic scale with a maximum capacity of 300 kg (ToledoTM, São Bernardo do Campo, Brazil). Waist circumference (cm) was measures using a inelastic measuring tape (Mabis-Gulick®, Waukegan, IL, USA) at the midpoint between the inferior edge of the costal border and the iliac crest in the mid-axillar line. Height was measured at the nearest 0.1 cm using a stadiometer (ToledoTM, São Bernardo do Campo, Brazil) with participants barefoot. These measures were obtained at baseline, first and second follow-up.

Body composition data was obtained by segmental bioimpedance with eight electrodes (InBody 230; InBodyCo., Seoul, Korea). For the examination, participants were instructed to not drink coffee nor engage on physical activities for the previous 24 hours before the exam and were fasting for at least eight hours. During the exam they used standardized clothes and were asked to remove all metallic accessories (earring, rings, glasses, watches, etc.), additionally, participants were asked to empty their bladders within last minutes before the exam. Bioimpedance exam was not performed on those with pacemakers or metal prostheses(20). Body fat percentage (BF%) and fat mass were obtained and fat mass index (FMI) was calculated dividing total fat mass (kg) by height squared (m²). The first measure of body composition was obtained at the first follow-up and subsequently at the second follow-up.

Circulating BCAA

Circulating branched chain amino acids (BCAA) were measured at baseline by nuclear magnetic resonance (NMR) spectroscopy using a 400 MHz NMR platform, as described for the LP4 NMR MetaboProfileTM Analysis kit(21). Analysis were conducted at the North American LipoScience/LabCorp Global Research Services laboratory. Quantification of valine, leucine,

and isoleucine were computed by mathematical algorithms that allow a precise quantification. Since these three amino acids are highly correlated, we used the sum of them indicating the total BCAA concentration in $\mu\text{mol/L}$.

Covariates

A broad set of in-person interviews were conducted using standardized questionnaires to assess sociodemographic and lifestyle information. Participant answered questions regarding their sex (male and female), age (completed years), educational level (incomplete primary school, complete primary school, complete high school, and university degree), smoking status (no smoker, former smoker, current smoker), and physical activity level assessed and classified according to International Physical Activity Questionnaire (IPAQ)(22).

Dietary pattern analysis

Reduced rank regression (RRR) was applied to identify the dietary patterns that most explain the variation on circulating BCAA concentration. RRR identifies linear functions of predictors of predictors explaining response variation. The food groups were entered in the model as the predictor variables while the circulating BCAA (sum of valine, leucine and isoleucine) was entered as the response variable due to its relationship with adiposity. The number of extracted factors is dependent on the number of response variables. In our analysis we had one response and consequently one dietary pattern as the output. Food groups with factor loadings ≥ 0.20 or ≥ -0.20 were considered on the interpretation of the dietary pattern. Following, a dietary pattern score was calculated for each individual. These values indicate the individual-level weights associated with the dietary pattern and numerically represents the adherence to the dietary pattern. RRR dietary pattern analysis were performed using SAS[®] Studio version 3.8 (SAS Institute Inc.).

Main analyses

To estimate the association between the dietary pattern with adiposity outcomes over time we applied linear mixed-models accounting for the correlation within repeated measures. Two models were computed with a fixed-effect structure accounting for the dietary pattern score, the outcomes, and possible confounders and a random-effect structure including a random intercept for the within individuals' variance of the repeated measures. To test whether the

strength in the association between the dietary pattern and adiposity outcomes differed across the follow-up period, an interaction term between dietary pattern score x time was added to the models. First model was adjusted for age, sex, total energy intake and time of the repeated measures and second models was additionally adjusted for educational level, smoking status and physical activity. The coefficients computed on linear mixed models indicate the association of the dietary pattern scores with adiposity outcomes averaged within the median follow-up of 8.3 years. Main analyses were performed using R software R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

One dietary pattern explaining 8.79% of circulating BCAA variation was identified in our analysis. This dietary pattern was highly and positive loaded for the food groups rice, beans, pasta, snacks, red meat, poultry, processed meat, soft drink, and beer; and was highly and negatively loaded for cereals and milk (Table 1). Regarding the food groups variation, the dietary pattern explained 8.29%.

Baseline characteristics of the study population are shown in Table 2. Comparing participants in the lowest and highest quartile of the dietary pattern score we found that those in the highest quartile were younger, presented higher measures of weight, waist circumference and BMI, but slightly lower measures of body fat percentage and fat mass index, higher total energy intake and circulating BCAA levels. The majority were men, more likely to be inactive and smokers.

After multivariable adjustment, higher adherence to the dietary pattern explaining circulating BCAA was associated with lower adiposity measures averaged across the median follow-up of 8.3 years. The interaction term of dietary pattern score and time showed loss of strength in the association across the follow-up time for the anthropometric outcomes (weight, waist circumference, and BMI). Despite that, our models estimated that for one unit increase in the dietary pattern score the mean weight was 0.64 kg higher (95%CI = 0.09;1.19), waist circumference was 0.96 cm higher (95%CI = 0.52;1.41), BMI was 0.35 kg/m² higher (95%CI = 0.17;0.54), body fat percentage was 0.58 points higher (95%CI = 0.27;0.89), and the fat mass index was 0.33 kg/m² higher (95%CI = 0.17;0.50), all across the median follow-up time of 8.3 years (Table 3). Additionally, the models using the dietary pattern score classified into quartiles showed that individuals in the highest quartile had increase in all adiposity indicators when compared to individuals classified in the lowest quartile (Table 4).

DISCUSSION

In this analysis of ELSA-Brasil sample from the São Paulo research center, we found a dietary pattern explaining 8.7% of circulating BCAA variation and this dietary pattern was characterized with both traditional Brazilian foods, as rice, beans and foods groups that characterize a western diet, as red meat, processed meat, soft drinks, and snacks. Even though, this dietary pattern shows a mixed profile, it consistently represents Brazilian diet. Data from the National Dietary Survey evaluating the most consumed foods between 2008-2009 and 2017-2018 showed that rice, beans and red meat, together with bread, coffee, and vegetables represents the staple Brazilian diet being the most consumed food groups. The food groups poultry, pasta, soft drinks, and processed meat were also ranked within the top 20 food groups most consumed by the Brazilian population(23).

A previous Study conducted with German individuals aged 18 to 80 years also investigated the dietary patterns explaining circulating BCAA using reduced rank regression analysis. They found a dietary pattern positively loaded for sauces, meat, sausage and meat products smoked, beer, soft drinks and desserts(11). This dietary pattern shares similar characteristics with the one found in our study, especially for the presence of meat and processed meat, beer, and soft drinks. These similar results suggest that higher consumption of these food groups may play an important role on BCAA circulating levels and metabolism. Additionally, the dietary pattern identified in our study were derived by dietary data from FFQ, which represents the habitual diet, and as already showed by Floegel et al., the circulating BCAA may be better explained by the long-term diet instead of short-term dietary intake(24).

The dietary pattern explaining 8.7% of circulating BCAA variation identified in our study was consistently associated with higher measures of adiposity over the follow-up period. Previous evidences(25, 26, 27) have shown the role of adipose tissue on regulating BCAA metabolism. Newgard et al. hypothesized that individuals with obesity exposed to a typical American diet, consuming high levels of fat and protein, may present a deficiency on insulin-like growth factor 1 (IGF-1) affecting the already increased circulating BCAA pool (due to overnutrition) that will not be delivered to protein synthesis and catabolic pathways(25), which may explain the high levels of these amino acids on individuals with higher adiposity. Additionally, the dysregulation on BCAA metabolism will lead to changes on several metabolic pathways that can contribute to insulin resistance in individuals with obesity(25, 27). Another

potential pathway explaining the relationship between adiposity and higher circulating BCAA is the metabolization of these amino acids by skeletal muscle, adipose tissue and the brain, rather than by the liver. The adipose tissue plays an important role in BCAA homeostasis, however hypertrophic adipocytes become metabolic inflexible, preventing the utilization of BCAA, in particular when associated with insulin resistance and metabolic syndrome(28). These evidences combined with our results reinforce the importance to identify dietary patterns that may be related with alterations on circulating metabolites that are known as markers of future diseases. Considering the ability of reduced rank regression to combine diet and biomarkers to identify dietary patterns, this approach may be fundamental to identify novel associations between diet and health outcomes.

Our study has several strengths, including the use of metabolomic data available on ELSA-Brasil to identify dietary patterns using reduced rank regression analysis, the longitudinal analyses of adiposity outcomes over a follow-up period of 8.3 years, the dietary data assessed by a validated FFQ able to estimate the habitual diet, and the quality of the data collected that enable the regression models adjustment for several possible confounders. However, some limitations still need to be addressed. Food consumption was assessed by a self-reported method that is not free of biases. Additionally, even though ELSA-Brasil is a multicenter study, we only had circulating BCAA data available for the sample from São Paulo and not from the five research centers, which prevent any extension of our results to the Brazilian population.

CONCLUSION

This prospective cohort study identified a baseline dietary pattern explaining circulating BCAA levels with a mixed profile. This dietary pattern was characterised by high consumption of food groups that compose a typical Brazilian meal (rice, beans, and red meat) and also food groups defined as part of an unhealthy diet (soft drinks, processed meat, snacks, and beer). Our results also suggest that this dietary pattern linked with higher levels of circulating BCAA are associated with higher adiposity over time in this study population.

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FIGURES

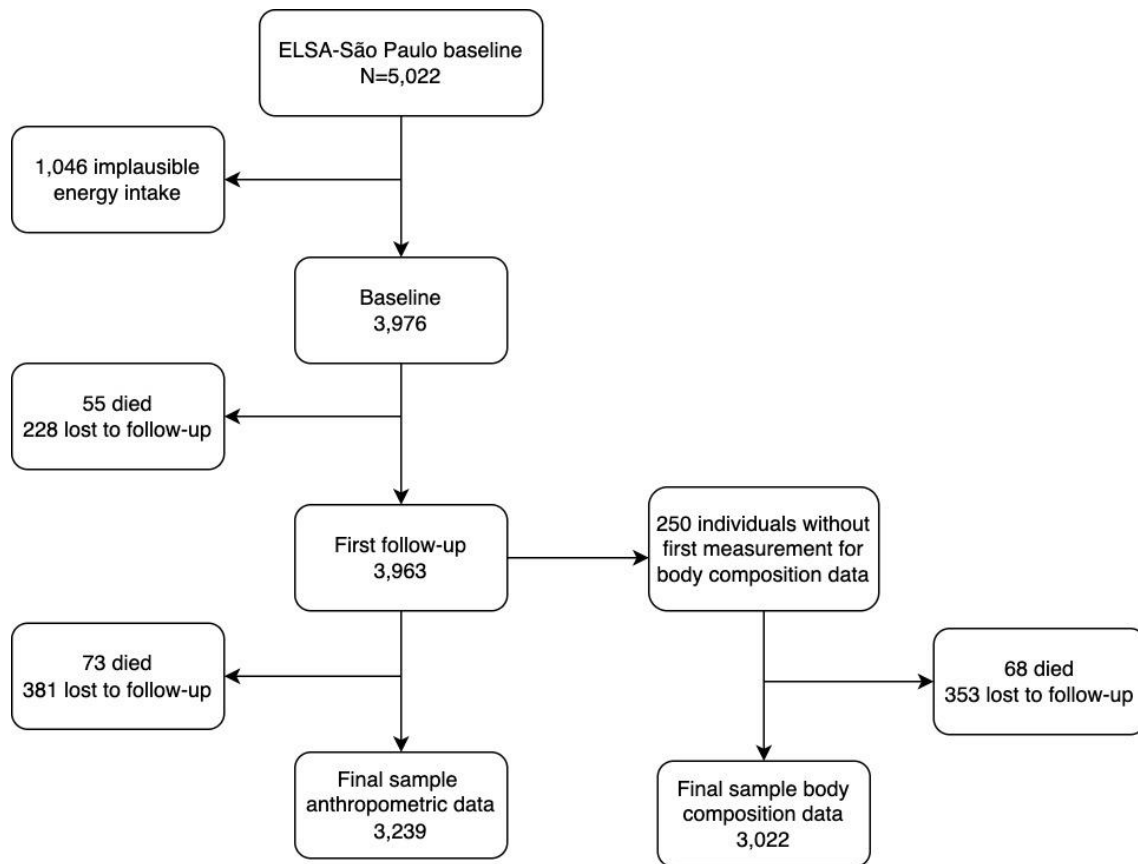


Figure 1. Flowchart of final sample in this study analyses. ELSA-Brasil, 2023.

TABLES

Table 1. Dietary pattern derived by reduced rank regression and food groups factor loadings. ELSA-Brasil, 2023.

Food groups	RRR-DP
Rice	0.42
Soft drink	0.33
Processed meat	0.31
Poultry	0.29
Red meat	0.28
Beans	0.27
Snacks	0.26
Beer	0.24
Pasta	0.22
Milk	-0.21
Cereals	-0.20
Eggs	0.17
Distilled	0.14
Fish	0.13
Bread	0.12
Tubers	0.09
Butter/margarine	0.03
Coffee	0.01
Wine	0.01
Leafy greens and Vegetables	-0.12
Nuts	-0.10
Juice	-0.06
Sweets and desserts	-0.03
Cheese	-0.03
Fruits	-0.02
Explained variance	
Food groups	8.29
Response variable (BCAA)	8.79

BCAA - Branched chain amino acids. Sum of valine, leucine and isoleucine.

Table 2. Participants characteristics at baseline. ELSA-Brasil 2022. (N=3,239)

Characteristics	Overall (n=3,239)	1Q (n=810)	4Q (n=810)
	Mean (SD)		
Age (years)	50.3 (8.6)	51.5 (8.8)	48.5 (7.7)
Weight (kg)	75.9 (15.4)	71.3 (14.2)	81.9 (14.8)
Waist circumference (cm)	91.1 (12.6)	87.8 (12.5)	95.4 (11.8)
BMI (kg/m ²)	27.8 (4.9)	27.3 (4.8)	28.5 (4.9)
Body fat (%)*	35.6 (8.7)	37.8 (8.4)	32.7 (8.6)
Fat mass index (kg/m ²)*	10.4 (4.1)	10.9 (4.1)	9.9 (4.2)
Energy intake (kcal/day)	1989.8 (597.1)	1698.0 (481.4)	2480.3 (560.2)
Circulating BCAA (μmol/L)	432.1 (86.2)	397.7 (75.1)	471.9 (83.4)
RRR-DP score	-0.11 (1.11)	-1.39 (0.42)	1.37 (0.74)
	N (%)		
Sex			
Female	1,741 (53.8)	649 (80.1)	160 (19.8)
Educational level			
Incomplete primary school	198 (6.1)	27 (3.3)	73 (9.0)
Complete primary school	198 (6.1)	28 (3.5)	70 (8.6)
Complete high school	1,265 (39.1)	256 (31.6)	391 (48.3)
University degree	1,578 (48.7)	499 (61.6)	276 (34.1)
Physical activity			
Inactive	2,445 (78.3)	583 (72.0)	651 (80.4)
Insufficiently active	418 (13.4)	118 (14.6)	96 (11.9)
Active	261 (8.4)	81 (10.0)	44 (5.4)
Smoking status			
Never	1,780 (54.9)	482 (59.5)	391 (48.3)
Former	1,000 (30.9)	249 (30.7)	262 (32.2)
Current	459 (14.2)	79 (9.8)	157 (19.4)

Physical activity n= 3,124, body fat percentage and fat mass index n=3,022.

*Values measured in the first follow-up visit. On this study, this is the first-time measurements for these variables.

1Q first quartile, 4Q fourth quartile

BCAA - branched chain amino acids. Sum of valine, leucine and isoleucine.

Table 3. Associations between the RRR-DP scores and longitudinal weight, body mass index and waist circumference. ELSA-Brasil 2022.

Model 1	Weight (kg)	WC (cm)	BMI (kg/m ²)	BF (%)	FMI (kg/m ²)
	β (95%CI)				
RRR-DP	0.60 (0.07;1.13)	1.16 (0.72;1.60)	0.44 (0.26;0.62)	0.73 (0.42;1.03)	0.41 (0.25;0.57)
RRR-DP <i>x</i> time	-0.02 (-0.05; 0.01)	-0.03 (-0.06; 0.01)	-0.01 (-0.02; 0.01)	-0.01 (-0.04; 0.02)	-0.01 (-0.03; 0.01)
Model 2					
RRR-DP	0.64 (0.09;1.19)	0.96 (0.52;1.41)	0.35 (0.17;0.54)	0.58 (0.27;0.89)	0.33 (0.17;0.50)
RRR-DP <i>x</i> time	-0.03 (-0.06; -0.01)	-0.03 (-0.06; -0.002)	-0.01 (-0.02; -0.003)	-0.01 (-0.04;0.02)	-0.01 (-0.02; 0.00)

Model 1 adjusted for age, sex, total energy intake, and time of repeated measurements.

Model 2: model 1 + education, physical activity, and smoking status.

Table 4. Associations of quartile of RRR-DP pattern scores and longitudinal weight, body mass index and waist circumference. ELSA-Brasil 2022.

Model	Weight (kg)	WC (cm)	BMI (kg/m ²)	BF (%)	FMI (kg/m ²)
	β (95%CI)				
Quartile 1	Reference	Reference	Reference	Reference	Reference
Quartile 2	0.53 (-0.86; 1.93)	0.52 (-0.64; 1.69)	0.27 (-0.21; 0.75)	0.14 (-0.68; 0.97)	0.18 (-0.25; 0.62)
Quartile 3	1.90 (0.43; 3.36)	2.03 (0.82; 3.25)	0.69 (0.19; 1.19)	0.75 (-0.11; 1.61)	0.57 (0.11; 1.02)
Quartile 4	1.46 (-0.22; 3.13)	2.51 (1.12; 3.89)	0.95 (0.38; 1.53)	1.57 (0.63; 2.52)	0.97 (0.47; 1.48)
p-value for trend*	0.046	<0.001	0.005	<0.001	<0.001

Model adjusted for age, sex, total energy intake, time of repeated measurements, education, physical activity, and smoking status.

*Trend tests across quartiles of RRR-DP score were performed by assigning median values for these quartiles and treating as a continuous variable in the models.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Food groups description. ELSA-Brasil 2023.

Food groups	Description
Rice	Rice and brown rice
Cereals	Oatmeal, granola, cereals bran and other cereals
Bread	White, wheat, light, and sugar bread
Fruits	All fruits (excluding fruit juice)
Leafy greens/vegetables	All leafy greens, vegetables (excluding potatoes and other tubers), and vegetables soup
Beans	Beans, lentil, chickpea, and peas
Milk	Whole and skim milk, yogurts, and soy milk
Nuts	Walnut, cashew nut, Brazilian nut, peanut, almonds, pistachio
Sweets/desserts	Cakes, cookies, ice cream, chocolate, pudding
Tubers	Potatoes, cassava, yam, cassava flour, and <i>farofa</i>
Pasta	Pasta and polenta
Snacks	Bread cheese, salt biscuits, French fries, pizza, baked and deep-fried snacks
Eggs	Boiled and fried eggs
Cheese	White and yellow cheese
Butter/margarine	Butter and margarine
Red meat	Beef, pork and giblets
Poultry	Fried and cooked poultry
Processed meat	Ham, mortadella, salami, sausage, chorizo,
Fish	Fried, baked, grilled, and stew fish
Soft drink	Regular and diet/light soft drinks
Juice	Fruit juice, industrialized juice, and coconut water
Coffee	Coffee and yerba mate
Beer	Beer
Wine	Red and white wine
Distilled	Distilled beverages

Supplementary Table 2. Food groups intake according to RRR-DP scores classified into quartiles. ELSA-Brasil 2023.

Food groups consumption(grams/day)*	Quartile 1	Quartile 2	Quartile 3	Quartile 4
	Mean score=-1.39 n=810	Mean score=-0.55 n=810	Mean score=0.13 n=809	Mean score=1.37 n=810
Rice	67.5 (32.0-90.0)	90.0 (63.9-180.0)	180.0 (90.0-180.0)	270.0 (180.0-270.0)
Cereals	7.5 (0.0-30.0)	3.2 (0.0-10.7)	0.0 (0.0-4.2)	0.0 (0.0-2.1)
Bread	51.8 (29.0-75.9)	57.0 (36.0-100.0)	57.0 (39.0-100.0)	57.0 (39.5-103.5)
Fruits	338.3 (211.0-520.6)	314.5 (184.6-515.4)	281.0 (157.9-461.2)	258.0 (136.6-466.5)
Leafy greens/vegetables	196.0 (127.0-281.4)	165.2 (108.4-240.2)	144.5 (92.8-213.7)	137.9 (85.2-201.2)
Beans	63.0 (22.4-140.0)	81.2 (40.2-151.2)	140.0 (46.2-280.0)	173.6 (81.2-280.0)
Milk	274.8 (189.6-488.4)	240.0 (104.4-274.8)	178.8 (55.7-255.6)	120.0 (33.6-240.0)
Nuts	2.1 (0.0-6.3)	0.0 (0.0-4.2)	0.0 (0.0-3.2)	0.0 (0.0-2.1)
Sweets/desserts	27.9 (11.2-52.0)	25.5 (11.2-43.5)	23.8 (9.8-41.0)	25.7 (11.2-41.8)
Tubers	20.9 (8.4-37.5)	22.5 (8.7-39.6)	25.2 (10.5-42.8)	28.6 (12.7-49.4)
Pasta	23.8 (11.9-47.6)	35.7 (23.8-49.3)	36.4 (23.8-60.2)	47.6 (23.8-79.8)
Snacks	33.7 (18.2-52.1)	40.4 (23.7-58.9)	47.6 (29.2-72.0)	60.8 (37.5-95.1)
Eggs	6.7 (0.0-10.2)	6.7 (3.5-13.3)	7.0 (3.5-13.3)	10.2 (6.3-17.7)
Cheese	12.7 (5.6-23.2)	11.6 (5.6-23.0)	10.0 (4.2-21.6)	11.2 (4.2-20.0)
Butter/margarine	3.2 (0.2-6.4)	3.2 (0.4-6.4)	3.2 (0.7-6.4)	3.2 (0.9-6.4)
Red meat	29.0 (26.1-58.0)	31.5 (26.1-58.0)	36.0 (29.0-72.2)	62.9 (33.9-100.0)
Poultry	5.6 (0.0-11.2)	11.2 (2.8-16.8)	11.2 (5.6-22.4)	16.8 (8.4-28.8)
Processed meat	4.2 (0.0-8.4)	6.3 (2.1-12.6)	8.4 (4.2-16.8)	13.7 (7.4-23.5)
Fish	28.0 (14.0-44.8)	28.0 (14.0-56.0)	28.0 (14.0-53.2)	44.8 (16.8-67.2)
Soft drink	6.3 (0.0-33.6)	16.8 (0.0-67.2)	33.6 (0.0-69.6)	69.6 (33.6-240.0)
Juice	139.2 (57.4-287.6)	103.2 (40.6-240.0)	93.4 (33.6-227.8)	86.4 (33.6-204.0)
Coffee	100.0 (50.0-149.5)	100.0 (50.0-149.5)	100.0 (50.0-149.5)	100.0 (50.0-149.5)
Beer	0.0 (0.0-33.6)	0.0 (0.0-50.4)	0.0 (0.0-100.8)	67.2 (0.0-268.8)
Wine	0.0 (0.0-10.5)	0.0 (0.0-21.0)	0.0 (0.0-21.0)	0.0 (0.0-15.8)
Distilled	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)

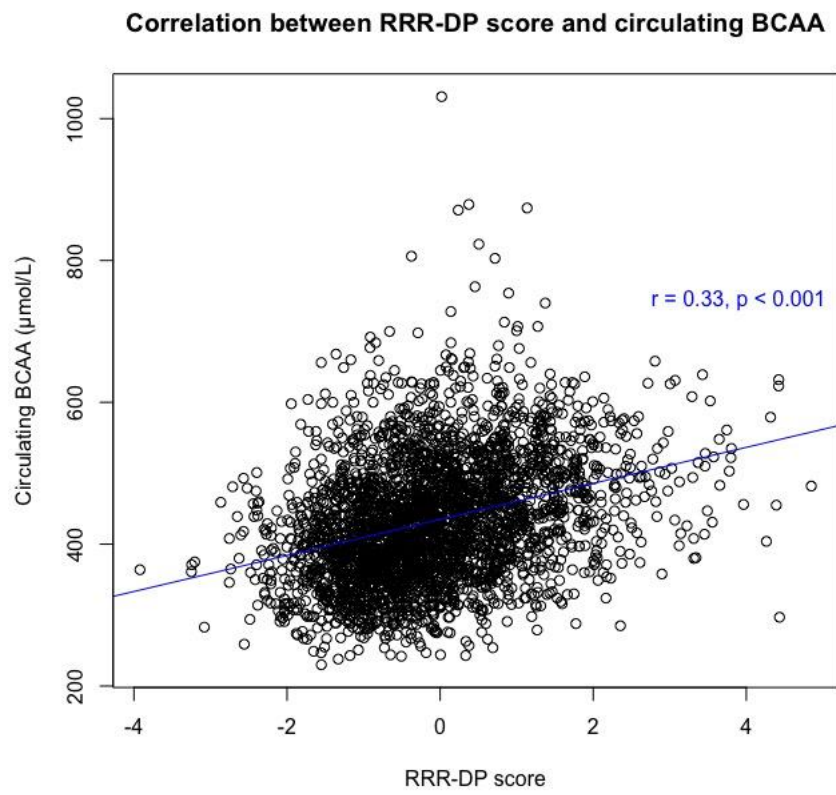
*Values are median (25th percentile-75th percentile) for continuous variables with skewed distribution.

Supplementary Table 3. Nutrient intake according to RRR-DP scores classified into quartiles. ELSA-Brasil 2023.

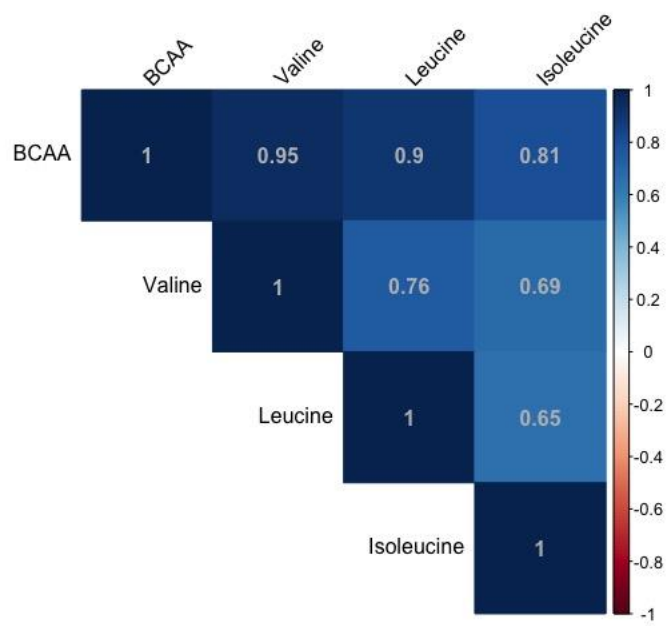
Nutrient*	Quartile 1	Quartile 2	Quartile 3	Quartile 4
	Mean score=-1.39 n=810	Mean score=-0.55 n=810	Mean score=0.13 n=809	Mean score=1.37 n=810
Energy (Kcal/d)	1698.0 (481.4)	1804.4 (492.3)	1976.4 (528.0)	2480.3 (560.2)
Carbohydrate (g/d)	244.8 (77.3)	256.0 (78.5)	275.9 (82.6)	336.9 (89.1)
Protein (g/d)	71.0 (21.3)	76.7 (23.0)	83.1 (24.7)	104.8 (28.0)
Animal protein (g/d)	39.4 (15.6)	42.4 (17.4)	45.0 (18.7)	56.7 (22.9)
Vegetable protein (g/d)	31.6 (12.1)	34.2 (12.6)	37.9 (14.4)	47.8 (16.8)
Total fat (g/d)	51.2 (18.0)	53.9 (18.0)	59.3 (19.5)	74.2 (20.9)
Saturated fat (g/d)	17.7 (7.3)	17.7 (6.7)	18.8 (7.5)	22.5 (7.7)
Monounsaturated fat (g/d)	16.1 (6.0)	16.8 (6.0)	18.4 (6.4)	22.9 (7.2)
Polyunsaturated fat (g/d)	12.9 (4.8)	14.3 (4.9)	16.5 (5.3)	21.7 (6.2)
Cholesterol (mg/d)	152.9 (68.9)	172.4 (73.9)	193.0 (85.5)	246.3 (99.1)
Fiber (g/d)	27.5 (10.9)	28.9 (12.2)	30.5 (13.4)	36.8 (15.4)
Alcohol** (g/d)	0.7 (0.0-3.5)	1.1 (0.0-5.0)	2.2 (0.0-7.3)	4.7 (0.0-13.7)
Total sugars (g/d)	106.6 (39.9)	99.4 (41.0)	94.4 (39.7)	103.6 (45.2)
Valine (g/d)	3.6 (1.1)	3.8 (1.2)	4.2 (1.3)	5.2 (1.4)
Leucine (g/d)	5.4 (1.7)	5.8 (1.8)	6.3 (1.9)	7.9 (2.3)
Isoleucine (g/d)	3.0 (0.9)	3.3 (1.0)	3.6 (1.1)	4.5 (1.2)
Total BCAA (g/d)	12.0 (3.7)	12.9 (4.0)	14.0 (4.3)	17.6 (4.9)

*Values are mean (SD) for continuous variables with normal distribution.

**Values are median (25th percentile-75th percentile) for continuous variables with skewed distribution.



Supplementary Figure 1. Pearson correlation between RRR-DP score and circulating BCAA.



Supplementary Figure 2. Correlation matrix of circulating total BCAA, valine, leucine and isoleucine.

5 CONSIDERAÇÕES FINAIS

5.1 PRINCIPAIS ACHADOS

O objetivo geral desta tese foi explorar como padrões alimentares da população brasileira participante do estudo ELSA-Brasil estão associados com medidas de adiposidade ao longo do tempo de seguimento do estudo e como diferentes métodos estatísticos para a identificação de padrões alimentares podem influenciar estas associações. Nesta seção, os principais objetivos da tese são revisitados, resumindo os principais achados de cada um dos capítulos.

No terceiro capítulo foram descritos os padrões alimentares da população de estudo identificados por cada um dos métodos aplicados. Nós encontramos que a aplicação dos métodos análise fatorial, *treelet transform* e *reduced rank regression* foram capazes de identificar padrões alimentares similares. O padrão alimentar denominado como “conveniência”, além de ser o mais consistente nos três métodos, também foi o que mais explicou a variabilidade nos dados de consumo alimentar da população. Este padrão foi marcado por altas cargas fatoriais para os grupos de alimentos doces e sobremesas, lanches, margarina e manteiga, carne vermelha, carne processada e refrigerantes. Enquanto os outros padrões alimentares (“prudente”, “tradicional brasileiro” e “arroz e feijão”) foram identificados apenas nos métodos análise fatorial e *treelet transform*. O padrão “prudente” foi o segundo com maior similaridade entre os métodos, apresentando altas cargas fatoriais para os grupos cereais, frutas, vegetais, peixes e suco de frutas. Já o padrão caracterizado principalmente pelo consumo de arroz e feijão, combinação tipicamente brasileira, apresentou algumas particularidades de acordo com o método aplicado. O padrão identificado pela análise fatorial e denominado como tradicional brasileiro apresentou as maiores cargas fatoriais para os grupos arroz, feijão, aves, carne vermelha, cerveja e carne processada (ordem decrescente dos valores de carga fatorial), enquanto o método *treelet transform* identificou um padrão com cargas fatoriais positivas para os grupos arroz e feijão e negativas para doces e sobremesas, lanches, carne processada, refrigerantes, ovos e massas.

As similaridades e diferenças entre os padrões alimentares de cada um dos métodos aplicados se refletiram nos resultados de associação com as medidas longitudinais de adiposidade. No capítulo 4 desta tese estão apresentados estes resultados. Maior adesão ao padrão alimentar

“conveniência” foi associado com aumento em todas as medidas de adiposidade ao longo do período de seguimento do estudo. Estes resultados acompanham as evidências já presentes na literatura de que uma dieta marcada por maior consumo de alimentos ricos em gordura, açúcar e sódio estão associados com maior nível de adiposidade em indivíduos adultos em diversos países ^{1;2}, incluindo o Brasil ³. A análise *treelet transform*, onde os alimentos importantes para o padrão alimentar recebem um valor de carga fatorial e os de menor importância recebem carga fatorial zero, nos mostrou que além dos alimentos marcadores de uma alimentação não saudável acima mencionados, o padrão “conveniência” também apresentou carga fatorial positiva para os grupos alimentares arroz e feijão, revelando um padrão alimentar com característica mista. Enquanto o padrão alimentar “arroz e feijão”, identificado por este mesmo método, apresentou carga fatorial positiva apenas para os grupos alimentares arroz e feijão e carga fatorial negativa para grupos alimentares presentes no padrão “conveniência” (doces e sobremesas, lanches, carne vermelha, carne processada, refrigerantes). Estes achados nos mostram a presença de padrões alimentares marcados pelo consumo de alimentos tradicionalmente brasileiros, mas com perfis diferentes. Além disso, apenas o padrão alimentar denominado como “arroz e feijão” foi associado com menores medidas de adiposidade ao longo do tempo.

Ainda em relação aos padrões alimentares marcados pelo consumo de arroz e feijão, na análise fatorial o padrão alimentar “tradicional brasileiro”, além das altas cargas fatoriais para os grupos arroz e feijão, também apresentou cargas fatoriais moderadas para os grupos carne vermelha, carne processada e cerveja. Este padrão alimentar foi associado com aumento nas medidas de percentual de gordura corporal e índice de massa de gordura, novamente sugerindo que o consumo de arroz e feijão combinado com alimentos não saudáveis está associado a desfechos de saúde desfavoráveis como já observado anteriormente em estudo brasileiro ⁴. Destaca-se também o alto consumo de carne vermelha e processada na população brasileira ^{5; 6; 7}. Estes resultados indicam que a redução no consumo de carnes pode trazer benefícios para desfechos relacionados a adiposidade ⁸.

O padrão alimentar “prudente”, identificado pelos métodos análise fatorial e *treelet transform*, caracterizado pelo consumo de alimentos marcadores de uma alimentação saudável, foi associado com redução nos marcadores de adiposidade. Este resultado corrobora com evidências prévias que mostram os benefícios do consumo destes alimentos, principalmente de origem vegetal, devido ao seu perfil nutricional (ricos em fibras, antioxidantes e gordura vegetal

insaturada) e atuação em mecanismos fisiológicos (aumento da saciedade, redução da inflamação e estresse oxidativo, e composição da microbiota intestinal) ^{9; 10; 11; 12; 13}.

Por fim, o capítulo 5 foi dedicado a explorar exclusivamente o método *reduced rank regression*. A característica híbrida deste método possibilitou a utilização de dados de metabolômica disponíveis no ELSA-Brasil para compreender a possível relação entre padrões alimentares, níveis séricos de amino ácidos de cadeia ramificada (BCAA) e os desfechos de adiposidade estudados nesta tese. Nossos resultados mostraram que o padrão alimentar caracterizado pelos grupos alimentares arroz, feijão, carne vermelha, carnes processadas, lanches e cerveja explicou a maior parte da variabilidade nos níveis séricos de BCAA. Novamente um padrão alimentar caracterizado por um perfil misto foi associado com aumento nas medidas de adiposidade ao longo do período de seguimento do estudo, indicando que este padrão de dieta, tipicamente presente na alimentação do brasileiro ⁷, está diretamente relacionado com alterações de um metabólito indicado como possível preditor de obesidade. Este achado nos exige maior atenção ao padrão alimentar que combina alimentos básicos da alimentação brasileira com alimentos ricos em gordura total e saturada, sódio e açúcares, além do álcool.

5.2 IMPLICAÇÕES PARA PESQUISA E POLÍTICAS PÚBLICAS EM ALIMENTAÇÃO E NUTRIÇÃO

A partir da década de 90 a ciência da nutrição passou por uma importante mudança de paradigma. A descoberta das vitaminas e o papel dos nutrientes no desenvolvimento de doenças crônicas não transmissíveis, até então vistos como os principais objetos de estudo, não eram mais suficientes para compreender a relação entre alimentação e saúde. Este cenário exigiu a mudança do ponto de vista da ciência da nutrição, substituindo a visão reducionista dos nutrientes isolados pela busca do entendimento das relações complexas entre alimentos e padrões de dieta na saúde humana ^{14; 15}. Esta tese trouxe uma discussão sobre a análise de padrões alimentares e seus efeitos na saúde, buscando uma visão ampliada da alimentação como alternativa ao reducionismo da análise de nutrientes ou alimentos específicos isolados.

A aplicação de diferentes métodos para a identificação de padrões alimentares é capaz de capturar diferentes aspectos do consumo alimentar ¹⁶, o que contribui para o avanço na compreensão entre dieta e saúde. Além da discussão apresentada sobre a aplicação de diferentes

métodos estatísticos para análise de padrões alimentares, nossos resultados indicam que métodos exploratório e híbrido podem ser aplicados de forma complementar, considerando suas principais vantagens e objetivos.

A produção de evidências científicas geradas no campo da alimentação e nutrição e sua posterior aplicação no desenvolvimento de recomendações nutricionais são o cerne da nutrição em saúde pública ¹⁷. Esta tese buscou gerar evidências da relação entre consumo alimentar e saúde que possam ser utilizadas para a geração de políticas públicas que visem a promoção da saúde e prevenção de doenças crônicas não transmissíveis.

Considerando a complexidade dos fatores envolvidos no comportamento alimentar, é importante destacar que os resultados desta tese não levam em consideração aspectos socioeconômicos, culturais e ambientais que afetam diretamente a dieta de uma população. Os resultados aqui apresentados apontam para a necessidade de investigações futuras que considerem estes diversos aspectos envolvidos na alimentação e desta forma ser capaz de produzir evidências robustas e consistentes com o cenário contemporâneo para nortear decisões políticas que afetam a saúde da população.

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6 ANEXOS

Anexo A – comprovante de publicação do artigo “Different statistical methods identify similar population-specific dietary patterns: an analysis of ELSA-Brasil”



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Different statistical methods identify similar population-specific dietary patterns: an analysis of Longitudinal Study of Adult Health (ELSA-Brasil)

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Abstract

In recent decades, different data-driven approaches have emerged to identify dietary patterns (DP) and little is discussed about how these methods are able to capture diet complexity within the same population. This study aimed to apply three statistical methods to identify the DP of the Longitudinal Study of Adult Health (ELSA-Brasil) population and evaluate the similarities and differences between them. Dietary data were assessed at baseline in the ELSA-Brasil study using a FFQ. DP were identified by applying three statistical methods: (1) factor analysis (FA), (2) treelet transform (TT) and (3) reduced rank regression (RRR). The characteristics of individuals classified in the last tertile of each DP were compared. Cross-classification and Pearson's correlation coefficients were assessed to evaluate the agreement between individuals' adherence to DP of the three methods. A similar convenience DP was identified for all three methods. FA and TT also identified a similar prudent DP and a DP highly loaded for the food groups rice and beans. Individuals classified in the third tertile of similar DP of each method presented similar socio-demographic and nutrient intake characteristics. Regarding the cross-classification, prudent DP from FA and TT presented a higher level of agreement (75%), while convenience DP from TT and RRR presented the lowest agreement (44.8%). The different statistical methods were able to capture the populations' DP in a similar way while highlighting the particularities of each method.

Key words: Nutritional epidemiology; Dietary patterns; Factor analysis; Reduced rank regression; Treelet transform

Anexo B – Comprovante de submissão do manuscrito “Dietary patterns and adiposity over time – Analysis of the ELSA-Brasil”

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