Tatiana Sadalla Collese

**Validação de métodos de avaliação do consumo alimentar em crianças:
SAYCARE study**

Tese de apresentada à Faculdade de Medicina
da Universidade de São Paulo para obtenção do
título de Doutor em Ciências

Programa em Saúde Coletiva

Orientador: Prof. Dr. Heraclito Barbosa de
Carvalho

Co-orientadora: Prof^a Dr^a. Dirce Maria Lobo
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DEDICATÓRIA

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muito sobre o valor da ciência e da curiosidade incessante.

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Abreviaturas dos títulos dos periódicos de acordo com *List of Journals Indexed in Index Medicus*.

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LISTA DE ABREVIATURAS

SAYCARE	<i>South American Youth/Child Cardiovascular and Environmental study</i>
QFA	Questionário de Frequência de Consumo Alimentar
R24h	Recordatório Alimentar de 24 horas
FV	Frutas e Vegetais
Fe	Ferro
MEDLINE	Medical Literature Analysis and Retrieval System Online
IC _{95%}	Intervalo de 95% de Confiança

LISTA DE QUADRO

Quadro 1 Biomarcadores sanguíneos e suas especificações para crianças.

RESUMO

Collese TS. *Validação de métodos de avaliação do consumo alimentar em crianças: SAYCARE study* [tese] São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2020.

Introdução: A avaliação do consumo alimentar em crianças com acurácia, é identificada como importante fator ao se investigar as associações entre a dieta e a saúde. Porém, estudos de validação de métodos para a avaliação do consumo alimentar em crianças são escassos. **Objetivo:** Validar dois questionários [Questionário de Frequência Alimentar (QFA) e Recordatório Alimentar de 24 horas (R24h)] para o consumo habitual de frutas, vegetais, e ferro alimentar em crianças e adolescentes sul-americanos. **Métodos:** Estudo multicêntrico observacional, em amostra de crianças e adolescentes (3-17 anos) participantes do SAYCARE (*South American Youth/Child Cardiovascular and Environmental*) study, e provenientes de cinco cidades: Buenos Aires (Argentina), Lima (Peru), Medellín (Colômbia), São Paulo e Teresina (Brasil). O QFA foi preenchido referente ao consumo nos últimos três meses; O R24h foi aplicado três vezes (dois dias semanais; um dia de fim de semana), e a ingestão média desses três dias (combinada com a frequência da ingestão) foi calculada usando o *Multiple Source Method* (MSM), a fim de validar os questionários para o consumo de frutas e vegetais ($n = 45$) com dosagens plasmáticas de retinol, β -caroteno, α -tocoferol e ácido ascórbico; e para a ingestão de ferro alimentar ($n = 149$) com dosagens séricas de ferro e ferritina. A ingestão alimentar em relação aos biomarcadores sanguíneos foi avaliada por meio de regressão linear múltipla ou de multinível, e análise de curva de características operacionais do receptor (ROC). **Resultados:** A tese é composta por nove artigos, sendo três artigos principais (uma revisão sistemática, um artigo de validação do consumo de frutas e vegetais, e um artigo de validação do consumo de ferro alimentar), além de seis artigos complementares à tese. Os coeficientes de correlação foram de 0,29 e 0,35 para a ingestão de frutas e vegetais estimada pelo QFA e pelo MSM, respectivamente; e os coeficientes de correlação para o consumo de ferro alimentar variaram entre 0,81-0,87 para o QFA, e entre 0,12-0,18 para o MSM. **Conclusão:** O QFA desenvolvido para o SAYCARE study apresentou boa capacidade de classificar a ingestão de frutas e vegetais em crianças, e a ingestão de ferro alimentar em crianças e adolescentes.

Descritores: Pediatria; Questionário de frequência de consumo alimentar; Recordatório alimentar de 24 horas; Frutas; Vegetais; Ferro; Biomarcadores; Estudos de validação.

ABSTRACT

Collese TS. *Validity of dietary assessment methods in children: SAYCARE study* [thesis] São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2020.

Background: Accurate dietary assessment in children is identified as an important fact to investigate the associations between diet and disease. However, there are limited studies about validity of dietary assessment methods in children. **Aim:** To evaluate the validity of two questionnaires [Food Frequency Questionnaire (FFQ) and 24h Dietary Recall (R24h)] to estimate fruit, vegetable, and dietary iron intake in South American children and adolescents. **Methods:** Multicenter observational study, in a sample of children and adolescents (3-17 years) participating in the SAYCARE (South American Youth/Child Cardiovascular and Environmental) study, from five cities: Buenos Aires (Argentina), Lima (Peru), Medellin (Colombia), São Paulo and Teresina (Brazil). The FFQ was filled out according to the consumption over the last three months; the R24h was applied three times (two weekdays, one weekend day) and the mean intake of these three days (combined with the frequency of intake) was calculated using the multiple source method (MSM), to validate the fruit and vegetable consumption (n = 45) against plasma dosages of retinol, β -carotene, α -tocopherol, and ascorbic acid; and for dietary iron intake (n = 149) against serum dosages of iron and ferritin. Dietary intake in relation to blood biomarkers was assessed using multiple linear regression or multilevel regression, and receiver operating characteristics (ROC) curve analysis. **Results:** This thesis consists of nine articles; three principal articles (a systematic review, one article validating fruits and vegetables intake, and one article validating dietary iron intake), in addition to six complementary articles. Correlation coefficients were 0.29 and 0.35 for fruit and vegetable intake estimated by the FFQ and the MSM, respectively; and correlation coefficients for dietary iron intake ranged from 0.81-0.87 for the FFQ, and 0.12-0.18 for the MSM. **Conclusion:** The SAYCARE FFQ exhibited good ability to rank fruit and vegetable intake in children, and dietary iron intake in children and adolescents.

Descriptors: Pediatrics; Food Frequency Questionnaire; 24-hour Dietary Recall; Fruits; Vegetables; Iron; Biomarkers; Validation study.

APRESENTAÇÃO

A alimentação e suas influências sobre a nossa saúde e qualidade de vida é algo que me fascina e intriga, desde a infância. A graduação em Nutrição foi fundamental para aprofundar esses conceitos, e mostrar que esta é uma ciência maravilhosa, da qual sou apaixonada. Mas também, mostrou que é uma ciência complexa, que precisa de muito estudo e constante atualização.

Logo que me formei em Nutrição (2012) pelo *Centro Universitário São Camilo*, fiz o curso de extensão universitária em Nutrição clínica e cardiologia, no *Instituto Dante Pazzanese de Cardiologia*. Após este curso, surgiu o convite do Professor Luis Moreno para trabalhar em seu grupo de pesquisa *GENUD (Growth, Exercise, NUtrition and Development)* na *Universidade de Zaragoza*, Espanha. Trabalhei no GENUD por um ano, em um estudo multicêntrico na Europa, o “*I. Family*”. Ao regressar ao Brasil, em 2014, iniciei o mestrado pela *Faculdade de Medicina da Universidade de São Paulo*, no departamento de *Medicina Preventiva*. Durante o desenvolvimento do mestrado, também colaborei no desenvolvimento do SAYCARE study. Assim que defendi o mestrado, me matriculei no doutorado, na mesma instituição e departamento. Próximo ao término do doutorado, tive o privilégio de fazer parte do doutorado na *Harvard School of Public Health*, departamento de *Nutrição*. Retornei ao Brasil em março de 2020, justo quando a pandemia da Covid-19 se iniciou.

Por conseguinte, esta tese é defendida em meio à crise da pandemia da Covid-19. Uma crise de saúde, socioeconômica, e também uma crise nutricional, principalmente em países de baixa e média renda. Ainda, as crianças são particularmente afetadas, uma vez que as escolas e os programas de refeição escolar estão fechados, os quais constituem importante fonte de alimentação para muitas crianças.

Portanto, nesse momento existe uma necessidade global ainda maior em entender e reconhecer os desafios da nutrição, como parte indispensável à saúde, alimentação, educação e ao desenvolvimento socioeconômico. Atenção particular deve ser dada à nutrição infantil.

Sendo assim, incluímos um capítulo introdutório na tese que aborda sobre a Epidemiologia Nutricional e seus desafios ao avaliar o consumo alimentar em crianças, seja usando questionários ou biomarcadores sanguíneos. A tese é apresentada em forma de compilação de artigos, portanto, é composta por três artigos principais com metodologias distintas: uma revisão sistemática, um artigo de validação do consumo de frutas e vegetais, e um artigo de validação do consumo de ferro alimentar. Para cada um dos três artigos é apresentada uma análise crítica, separadamente. Por fim, é apresentada uma conclusão geral, seguida dos artigos na íntegra (anexos), e as referências. Além disso, seis artigos complementares foram publicados e incluídos à tese, pois estes foram desenvolvidos durante o doutorado e contribuíram para a formação acadêmica.

1 INTRODUÇÃO

1.1 Epidemiologia Nutricional

A epidemiologia nutricional é geralmente criticada pelos vieses existentes ao se avaliar o consumo alimentar ⁽¹⁾. A alimentação humana é complexa e multidimensional, contemplando interações de diferentes compostos alimentares e processos biológicos, sociais e culturais, em proporções variáveis ^(2,3). Além disso, outros fatores como o estilo de vida e nível socioeconômico, também influenciam a alimentação ⁽⁴⁾. Consequentemente, os estudos nutricionais são questionados, pois, muitas vezes, estes resultam em controvérsias ⁽⁵⁾. Por exemplo, concluir que estar sobrepeso diminui o risco de mortalidade geral ⁽⁶⁾, ou que substituir o consumo de gordura saturada por gordura poli-insaturada não influencia na saúde ⁽⁷⁾. Tais conclusões (provenientes de estudos como estes, com baixo detalhamento metodológico) são confusas, e podem gerar a comunicação de mensagens equivocadas, e consequente mudanças de comportamentos que também podem ser prejudiciais à saúde ⁽⁸⁾.

Em 1992, *Faggiano* et al. descreveram que avaliação do consumo alimentar é uma das atividades mais desafiadoras no campo da Epidemiologia ⁽⁹⁾. Apesar de ser um desafio, avaliar o consumo alimentar é importante para se analisar as possíveis associações entre a dieta e o processo saúde/doença/cuidado ⁽⁸⁾. Historicamente, um exemplo claro dessa associação é o papel da vitamina C (ácido ascórbico) no metabolismo do tecido conjuntivo, sobretudo a partir do século XVI, quando a doença de escorbuto, que afetava os marinheiros que permaneciam a bordo dos navios por longos períodos, começou a ser prevenida por meio da ingestão de frutas cítricas, as quais são boas fontes

dessa vitamina ⁽¹⁰⁾. Assim, estudos que abordem metodologias apropriadas e detalhadas, com ferramentas de avaliação dietética validadas para a população em estudo, são necessários a fim de se elucidar as demais associações entre a nutrição e a saúde ⁽⁸⁾. A literatura ressalta que validar os métodos usados na epidemiologia nutricional é essencial frente à crescente incidência de doenças crônicas não-transmissíveis relacionadas à dieta (por exemplo: obesidade, doenças cardiovasculares e diabetes) ⁽¹¹⁾. Além disso, tal validação deve ser realizada considerando o objetivo do estudo em questão, e as características inerentes à população alvo de cada estudo, por exemplo, respeitando seus aspectos socioculturais, econômicos e demográficos ^(12, 13).

1.2 Métodos subjetivos de avaliação do consumo alimentar

Atualmente, se desconhece um único método de avaliação de consumo alimentar ideal ⁽¹⁴⁾. Existem diversos métodos para se realizar essa avaliação, porém, todos estes possuem limitações e erros, tanto aleatórios como sistemáticos ⁽²⁾. Embora os métodos subjetivos de avaliação do consumo alimentar possam parecer semelhantes, a função pretendida de cada um deles é distinta. Abaixo apresentamos dois desses métodos subjetivos, que são usualmente administrados em estudos epidemiológicos de larga escala, principalmente por serem métodos não invasivos, de baixo custo e de aplicação relativamente mais simples, quando comparados a outros métodos, como por exemplo, os biomarcadores sanguíneos ⁽¹⁵⁾. Ressaltamos que a ênfase está nos métodos de avaliação do consumo alimentar entre grupos populacionais, não entre indivíduos, como pode ser o caso em contextos clínicos.

a. Questionário de Frequência de Consumo Alimentar

Entre os métodos subjetivos de avaliação do consumo alimentar, destaca-se o Questionário de Frequência de consumo Alimentar (QFA), o qual é considerado o mais

prático e informativo método de avaliação em estudos de associação entre o consumo alimentar e a ocorrência de desfechos em saúde ⁽¹⁶⁾. Este é desenhado para se estimar o consumo habitual, sendo amplamente aplicado para se avaliar a dieta numa variedade de contextos ⁽¹⁷⁾, incluindo fatores nutricionais que influenciam o desenvolvimento de doenças crônicas não-transmissíveis ^(15, 17).

Trata-se de um questionário estruturado, composto por uma lista de alimentos previamente estabelecida, e uma seção com a frequência de consumo habitual em determinado período (número de vezes que o indivíduo consome um alimento ou grupo de alimentos por dia, semana, mês ou ano) ⁽¹⁶⁾. Alguns QFA, adicionalmente, também podem incluir a porção média do alimento em medida caseira ⁽¹⁶⁾. A seleção dos alimentos que compreendem a lista do QFA deve se basear no objetivo do estudo (alimentos que se pretende investigar) e deve ser validada, o que inclui procedimentos complexos, e portanto, requer esforço e tempo para o seu desenvolvimento ⁽¹⁶⁾. Por ser estruturado, o QFA pode ser auto respondido, ou no caso de crianças, respondido pelos pais ou responsáveis. Logo, outra desvantagem desse método é a necessidade de o relator ser letrado, e ter habilidades cognitivas e memória para estimar o consumo alimentar habitual em longo prazo. Porém, é considerado um questionário de baixo custo, uma vez que pode ser realizado no papel, além de dispensar a presença de profissional treinado para a sua aplicação. Ainda, o QFA estima o padrão de consumo habitual com menos influência do que outros métodos, e classifica os participantes em categorias de consumo, eliminando as variações do dia-a-dia ⁽¹⁶⁾.

b. Recordatório alimentar de 24 horas

Outro método frequentemente empregado é o recordatório alimentar de 24 horas (R24h), pois é relativamente rápido de ser aplicado, culturalmente sensível, e fornece dados quantitativos sobre alimentos e nutrientes ^(18, 19). O R24h é um questionário aberto

retrospectivo, onde se declara os alimentos consumidos nas 24 horas antecedentes ao momento da avaliação, detalhando cada uma das refeições e seus horários, e a quantidade dos alimentos consumidos ⁽¹⁸⁾. No geral, o R24h é conduzido por nutricionista ou profissional treinado. Logo, a qualidade da informação relatada dependerá, não apenas da memória e da cooperação do participante da pesquisa, como também da capacidade do nutricionista ou profissional treinado em estabelecer um canal de comunicação, no qual se obtenha o conhecimento por meio do diálogo, sem que esse diálogo influencie o relato alimentar ⁽¹⁶⁾. Apesar de ser um questionário subjetivo, o nutricionista ou profissional treinado pode usar parâmetros durante a entrevista, a fim de facilitar a recordação do dia anterior, como por exemplo, local da refeição e o horário em que acordou e foi dormir⁽¹⁸⁾.

O R24h é um questionário que mede o consumo alimentar eventual, podendo encontrar alimentos consumidos mais raramente. Essa é uma das vantagens desse questionário, pois como o consumo já aconteceu no passado (24 horas antecedentes), evita as tendências de sub ou superestimar o relato alimentar ⁽¹²⁾. Dois R24h (em dias não consecutivos) foram validados para estimar o consumo de proteínas, frutas e vegetais em estudos europeus multicêntricos com adultos ^(20, 21).

Ambos (QFA e R24h) são métodos subjetivos, que quando associados, apresentam uma estimativa mais próxima do consumo alimentar “verdadeiro” ⁽²²⁾. Entretanto, é importante que estes métodos sejam validados, pois os erros de medida ainda permanecem ⁽²³⁾.

1.3 Método objetivo de avaliação do consumo alimentar: biomarcadores sanguíneos

Considerando a ausência de um método “independente” e “perfeito” para avaliar a real ingestão alimentar, várias técnicas estão sendo desenvolvidas a fim de minimizar e/ou controlar os erros envolvidos nessa avaliação ⁽⁴⁾. Os biomarcadores são medidas objetivas cada vez mais utilizadas para auxiliar, tanto na avaliação nutricional, como na

predição do risco de doenças crônicas, por serem mais consistentes na padronização, validação e precisão quando comparados aos questionários (medidas subjetivas) ^(2, 14). Uma vantagem dos biomarcadores é que estes são menos influenciados pelos erros aleatórios do QFA, e do R24h (por exemplo: memória e dificuldades na estimativa de porção dos alimentos) ^(24, 25).

Existem várias classificações de biomarcadores ⁽²⁾. Dentre os destinados à avaliação dietética, destacam-se os biomarcadores sanguíneos (por exemplo: carotenoides, vitamina C, ferro e ferritina) ^(2, 25). Tais biomarcadores são considerados superiores frente aos questionários de consumo alimentar, e são sugeridos como referência na validação destes questionários ^(2, 26). Contudo, o uso de biomarcadores sanguíneos nos estudos de validade é restrito às associações com a dieta, e devem complementar, mas não substituir o uso de questionários de avaliação do consumo alimentar ⁽²⁷⁾. Os biomarcadores sanguíneos são suscetíveis a complexos processos metabólicos e características individuais (por exemplo: sexo, idade, estado nutricional, variabilidade genética, estilo de vida) ^(2, 25), além de serem medidas invasivas, onerosas, de difícil aplicabilidade e análise laboratorial ⁽²⁾.

Dadas as diferentes vantagens e desvantagens dos métodos subjetivos (QFA e o R24h), e dos métodos objetivos (biomarcadores sanguíneos), caso os resultados entre estes métodos sejam consistentes, isso pode reduzir a probabilidade de viés ao se avaliar o consumo alimentar. Especificamente, isso reduz a probabilidade de viés tanto relacionado a subestimação ou superestimação do consumo alimentar (no QFA e/ou R24h), como os vieses relacionados a absorção e metabolismo de nutrientes (por exemplo, nos biomarcadores sanguíneos) ⁽²⁸⁾.

1.4 População pediátrica

A avaliação da ingestão alimentar em crianças é particularmente ainda mais desafiadora ⁽²⁹⁾. As crianças em idade pré-escolar, todavia não possuem habilidades suficientes para relatar e lembrar dos alimentos consumidos. Já as crianças escolares, muitas vezes, não têm conhecimento de receitas e ingredientes incluídos nas preparações alimentares consumidas ⁽²⁹⁾. Por estes motivos, e pelas dificuldades relacionadas aos métodos de avaliação (subjetivos e objetivos) supramencionadas, vários métodos potenciais de avaliação do consumo alimentar permanecem sem ser validados, principalmente em populações pediátricas ⁽¹¹⁾. Existe uma escassez de estudos que abordem este tema, visto a revisão sistemática publicada em 2018, a qual encontrou apenas dois estudos que validaram questionários para se estimar o consumo de frutas e vegetais em crianças, utilizando biomarcadores sanguíneos como método de referência ⁽³⁰⁾. Visto que a infância é um período de crescimento, durante o qual os hábitos alimentares são formados ⁽³¹⁾, é necessário que estudos de validação de métodos de consumo alimentar sejam realizados desde as primeiras etapas da vida, e considere aqueles alimentos que promovem a saúde, como as frutas e vegetais (FV), e os alimentos fonte de ferro. O quadro 1 resume as principais vantagens de se avaliar o consumo desses alimentos em pediatria.

Biomarcador sanguíneo	Indicador	Vantagens de se avaliar o consumo
Retinol	Consumo de FV	<p>- FV são importantes fontes de nutrientes (ex: fibras, vitaminas, potássio, magnésio e antioxidantes)⁽³²⁾ essenciais para o crescimento e funções dos sistemas (ex: imunológico, neurológico, tegumentar, visual e cardiovascular)^(33, 34)</p> <p>- O consumo de FV é essencial para promover a saúde cardiovascular e prevenir doenças crônicas⁽³⁵⁾;</p> <p>- Revisões sistemáticas⁽²⁵⁾, e metanálises de ensaios clínicos randomizados⁽²⁶⁾, evidenciam de que os biomarcadores sanguíneos de concentração mais utilizados e consistentes para validar o consumo de FV são os carotenoides e a vitamina C.</p>
β-caroteno		
α-tocoferol		
Ácido Ascórbico		
Ferro	Ferro ligado à transferrina no sangue	<p>- A deficiência de ferro é a desordem nutricional mais comum e difundida⁽³⁶⁾;</p> <p>- O ferro desempenha papel-chave na síntese de hemoglobina e mioglobina, bem como na função de ampla gama de enzimas, sendo essencial ao longo do ciclo de vida⁽³⁷⁾;</p> <p>- Crianças e adolescentes deficientes em ferro estão sujeitos a uma série de alterações metabólicas, por exemplo, enfraquecimento do sistema imunológico e déficit no desenvolvimento físico e mental⁽³⁷⁾.</p>
Ferritina	Reserva de ferro	A ferritina sérica é o principal parâmetro utilizado para avaliar as reservas de ferro, pois apresenta forte correlação com o ferro armazenado nos tecidos ⁽³⁸⁾ .

Quadro 1 Biomarcadores sanguíneos e suas especificações para crianças.

Legenda: FV: Frutas e vegetais.

Diante da necessidade de se obter um questionário capaz de avaliar o consumo alimentar de crianças sul-americanas de forma padronizada, por exemplo, como se realizou em estudos multicêntricos com crianças de diferentes países Europeus ⁽²⁹⁾, propusemos esse estudo. Sendo assim, validar os métodos de avaliação do consumo alimentar em crianças sul-americanas (respeitando seus aspectos socioculturais) é relevante, a fim de melhorar a qualidade e precisão dos dados alimentares avaliados nessa região ⁽¹⁹⁾. Logo, essa validação pode contribuir para as discussões sobre o entendimento da epidemiologia nutricional, sua complexidade e desafios, além de permitir a comparação de dados das crianças entre os países sul-americanos.

2 OBJETIVO

Validar dois questionários, QFA e R24h, para o consumo habitual de frutas, vegetais e ferro alimentar em uma amostra de crianças e adolescentes sul-americanos.

3 INCLUSÃO DOS ARTIGOS

A fim de atingir este objetivo, apresentaremos os três resumos dos artigos científicos que compõe a parte central desta tese, seguida de uma análise crítica de cada um, separadamente.

3.1 Revista: *Nutrients* (Fator de Impacto: 4.196), **2018**, Volume 10, Issue 10, 1396. DOI: 10.3390/nu10101396. (Anexo A)

Título: *What is the Validity of Questionnaires Assessing Fruit and Vegetable Consumption in Children when Compared with Blood Biomarkers? A Meta-Analysis*

Autores: **Tatiana S. Collese**; Gabriela Vatauvuk-Serrati; Marcus Vinicius Nascimento-Ferreira; Augusto César Ferreira De Moraes; Heráclito Barbosa Carvalho

Resumo

Fruit and vegetable consumption have been associated with improved health outcomes in children. As an extensive number of questionnaires are currently used to assess fruit and vegetable consumption, we performed a systematic review of criterion validity of questionnaires used to estimate fruit and vegetable consumption in children, considering blood biomarkers as the reference method. Five electronic databases (MEDLINE, CINAHL, Scopus, PsycINFO, Web of Science) were searched from database inception to July 23, 2018. The search strategy used the following sets of descriptors: children; fruits and vegetables; dietary questionnaires; blood biomarkers; validation coefficient. The search terms were adapted for use with other databases in combination with database-specific filters. Potentially eligible articles were selected independently by 2 reviewers, separately, following the PRISMA guideline. 2 articles meeting the inclusion criteria were included. The main reason for study exclusion was the sample age range mixed with adolescents. The pooled correlation coefficient was 0.32 (95% Confidence Interval 0.24, 0.40). This review provides insights into assessment methods for fruit and vegetable consumption in children. Although further studies are required, questionnaires for assessing fruit and vegetable consumption have fair criterion validity in children.

3.2 **Revista:** *Nutrients* (Fator de Impacto: 4.196), 2019, volume 11, issue 1815.
DOI: 10.3390/nu11081815. (Anexo B)

Título: *The Validity of Children's Fruit and Vegetable Intake Using Plasma Vitamins A, C, and E: The SAYCARE Study*

Autores: **Tatiana Sadalla Collese**, Augusto César Ferreira De Moraes, Tara Rendo-Urteaga, Liania Alves Luzia, Patrícia Helen de Carvalho Rondó, Dirce Maria Lobo Marchioni and Heráclito Barbosa Carvalho.

Resumo

Despite that fruits and vegetables are key elements for health promotion, there are limited studies validating their intake in children. We aimed to validate the SAYCARE (South American Youth/Child Cardiovascular and Environmental) Food Frequency Questionnaire (FFQ) and the combination of the FFQ frequency of intake with the 24 h-dietary-recall (24 h-DR) (mean of 3 days), for children's fruit and vegetable intake. The reference methods were plasma dosages of beta-carotene, retinol, ascorbic acid, and alpha-tocopherol, which were collected in the school environment. It is a validity study in a subsample of 45 children aged 6–10 years participating in the SAYCARE study, from São Paulo (Brazil). The FFQ was answered by the parents/guardians over the previous 3 months; the 24 h-DR was answered three times (two weekdays by nutritionists, one weekend day by parents/guardians). The mean fruit and vegetable intake (combined with frequency of intake) was calculated using the multiple source method (MSM). Multiple linear regression showed pooled correlation coefficients of 0.29 to 0.35 for the reported fruit and vegetable intake estimated by the FFQ and the MSM, respectively. The SAYCARE FFQ is an accurate and useful tool for ranking fruit and vegetable intake in children between 6–10 years from the SAYCARE study.

3.3 **Revista:** *Journal of the Academy of Nutrition and Dietetics* (Fator de Impacto: 4.151). Submetido em 22 de maio de 2020*. (Anexo C)

Título: *The validity of a food frequency questionnaire and 24-hour-dietary-recall to assess dietary iron intake in children and adolescents from the SAYCARE study*

Autores: **Tatiana Sadalla Collese**, Augusto César Ferreira De Moraes, Tara Rendo-Urteaga, Laura I Gonzalez-Zapata, Diego Alejandro Gaitán Charry, Carlos A. Delgado, Gabriela Berg, Francisco Leonardo Torres-Leal, and Heráclito B. Carvalho.

* Artigo em revisão em revista indexada de acesso aberto.

Resumo

Background A food frequency questionnaire (FFQ) for South American children and adolescents was developed, but its validity for dietary iron intake has not been evaluated.

Objective To evaluate the validity of the FFQ and 24-hour–dietary-recalls (24h-DR) for dietary iron intake in children and adolescents. **Design** The SAYCARE (South American Youth/Child Cardiovascular and Environmental) is a multicenter observational study, conducted in five South American cities: Buenos Aires (Argentina), Lima (Peru), Medellin (Colombia), São Paulo and Teresina (Brazil). The FFQ was filled out over the previous three months, and the 24h-DR was applied three times (two weekdays and one weekend day). Mean total iron intake was calculated using the Multiple Source Method (MSM). Blood was collected to assess serum iron, ferritin, and hemoglobin.

Participants/Setting Data of 99 children (3-10 years) and 50 adolescents (11-17 years) from public and private schools were collected in 2015-2017. **Main outcome measures**

Dietary iron intake calculated from the FFQ and 24h-DR. **Statistical analyses performed** Dietary iron intake in relation to blood biomarkers were assessed using multilevel linear regression with fixed intercepts and receiver operating characteristics (ROC) curve analysis. **Results** Multilevel regressions showed very good correlation coefficients (range 0.81-0.87) for the FFQ; and weak correlation coefficients (range 0.12-0.18) for the MSM. The accuracy ranged from 49.0-70.6% for the FFQ; and from 38.5-58.9% for the MSM.

Conclusion The SAYCARE FFQ exhibited good ability to rank total dietary iron intake in children and adolescents, and it is recommended instead of the MSM in this study population.

4 ANÁLISE CRÍTICA DOS ARTIGOS

4.1 Artigo 1: Revisão sistemática

O primeiro artigo desta tese é uma revisão sistemática, publicada em primeiro de outubro de 2018 ⁽³⁰⁾, em revista de acesso aberto indexada ao MEDLINE. Essa revisão objetivou responder à seguinte pergunta; "Qual a validade de questionários usados para estimar o consumo de frutas e vegetais em crianças, quando comparados a biomarcadores sanguíneos?"

Os resultados desse estudo indicaram que a validade dos questionários se mostrou aceitável [coeficiente de correlação: 0,32 (IC_{95%}: 0,24 – 0,40)] para classificar o consumo de frutas e vegetais em crianças. Apesar do valor do coeficiente de correlação ser baixo, este foi considerado aceitável, pois deriva dos desafios inerentes à avaliação do consumo alimentar, além do fato de que em crianças menores de dez anos, devemos considerar o relato dos pais/responsáveis (percepção subjetiva) sobre os hábitos alimentares da criança⁽³⁹⁾.

Compreender os principais fatores que influenciam a avaliação do consumo de frutas e vegetais em crianças, pode ser promissor para melhorar o conteúdo e qualidade dos métodos usados nessa investigação. Como os erros de medida são uma dificuldade importante, os estudos de validação de questionários para avaliar o consumo de frutas e vegetais devem ser realizados com cautela, e interpretados à luz do amplo contexto deste consumo. Por exemplo, o desenho do estudo e o desenvolvimento das ferramentas de avaliação da dieta devem ser meticulosos; e tais ferramentas podem ser validadas usando os biomarcadores sanguíneos. Porém, a validação com biomarcadores sanguíneos deve

ser feita desde que todo o processo de coleta e análise de sangue seja adequado, e rigorosamente controlado.

Os dois estudos que foram elegíveis para essa revisão foram realizados nos Estados Unidos da América e na Noruega. Ou seja, países estes considerados desenvolvidos; mas nenhum estudo foi encontrado em países considerados emergentes ou em desenvolvimento (como os países da América do Sul), muito menos com abordagem multicêntrica ⁽³⁰⁾. Os estudos nesses países são mais escassos por diversas razões, dentre elas, está a dificuldade de financiamento e de condições estruturais para a realização de pesquisas ⁽⁴⁰⁾. Por exemplo, apesar de a tecnologia (ex: questionários eletrônicos) ser uma ferramenta cada vez mais empregada nos estudos epidemiológicos, nem sempre é possível aplicá-la na realidade de países menos favorecidos, pois a acessibilidade à tecnologia pode ser um fator complicador em determinados locais. Além disso, as pesquisas de consumo alimentar realizadas na América do Sul são bastante heterogêneas, tanto em relação ao método de avaliação do consumo alimentar aplicado em cada região, como em relação as tabelas de composição de alimentos utilizadas, metodologias de análises, e principalmente, devido à grande diversidade de alimentos e variáveis socioculturais que influenciam o processo alimentar ⁽¹⁹⁾.

Alguns exemplos dessas variáveis foram evidenciados no artigo sobre validação da escala de percepção de imagem corporal em crianças e adolescentes do SAYCARE study (artigo complementar à tese, citado no item 6.3 deste documento) ⁽⁴¹⁾, onde a percepção do peso e da imagem corporal são fatores que potencialmente interferem nas escolhas alimentares. Em adição, outro artigo realizado com adolescentes Brasileiros e de um estudo multicêntrico Europeu (artigo complementar à tese, citado no item 6.4 desta tese) ⁽⁴²⁾, também demonstrou que diferenças entre os sexos, nível socioeconômico, e educação dos pais, influenciaram os comportamentos do balanço-energético (por

exemplo: ingestão de frutas, vegetais, e de bebidas açucaradas; hábitos de sono e de atividade física/sedentarismo) de maneira distinta.

Quando os recursos financeiros são escassos, como frequentemente ocorre em estudos realizados em países da América do Sul, usar a criatividade pode ser uma boa alternativa às tecnologias avançadas. Por exemplo, se não for possível combinar a tecnologia no processo de avaliação alimentar, um simples mascote (desenhado em papel ou um fantoche) pode fazer a diferença na motivação e envolvimento das crianças para responder ao questionário de consumo alimentar, transformando esse processo em um momento mais divertido ⁽⁴³⁾. Sendo assim, tanto o desenvolvimento da tecnologia, como também a criatividade, podem levar a avanços nos métodos de avaliação do consumo alimentar e, portanto, têm um futuro promissor à epidemiologia nutricional.

4.2 Artigo 2: Validação do consumo de frutas e vegetais

O artigo 2 é original desta tese, publicado em 6 de agosto de 2019, em revista de acesso aberto indexada ao MEDLINE. Trata-se da validação do QFA e a combinação da frequência de ingestão do QFA com R24h [média de 3 dias usando o *Multiple Source Method* (MSM) ^(44, 45)], para estimar o consumo de frutas e vegetais em crianças (6 a 10 anos) participantes do SAYCARE study.

Foram desenvolvidos sete QFA semi-quantitativos distintos e adaptados para cada um dos centros participantes do SAYCARE. Mais detalhes sobre o desenvolvimento desse questionário, podem ser encontrados no artigo que publicamos em 2018 ⁽⁴⁶⁾ (artigo complementar à tese, citado no item 6.1 desta tese). Além disso, no estudo que realizamos com a primeira autora Rendo-Urteaga e colaboradores ⁽⁴⁷⁾ (artigo complementar à tese, citado no item 6.2 desta tese) dois QFA foram aplicados a fim de avaliar a sua confiabilidade (coeficientes de correlação de Spearman: 0,47 - 0,73) e validade (coeficientes de correlação de Pearson ajustados para energia: 0,17 - 0,61), para a ingestão de grupos de alimentos (ex: frutas e vegetais), quando comparados ao R24h em crianças e adolescentes do SAYCARE study.

Por esse motivo, nesse artigo foi realizada a validação do QFA e a combinação da frequência de ingestão do QFA com R24h (usando o MSM), considerando as vitaminas plasmáticas (β -caroteno, retinol, ácido ascórbico e α -tocoferol) como um método de referência objetivo. Tais vitaminas são altamente sensíveis à luz, e o ácido ascórbico bastante volátil ⁽²⁵⁾. Dessa forma, estas análises requisitaram metodologia específica e adaptada às condições, principalmente, de luz e temperatura. Sendo assim, realizamos uma coleta de sangue altamente cuidadosa e sofisticada em ambiente escolar (uma escola pública e uma privada), com crianças de 6 a 10 anos (n=45) de São Paulo.

São Paulo foi selecionado por possuir um laboratório especializado neste tipo de análise, o Laboratório de Micronutrientes da Faculdade de Saúde Pública da Universidade de São Paulo (FSP/USP). É importante enfatizar que o exame de sangue foi realizado nas salas de aula, com os tubos de amostra de sangue revestidos em papel alumínio para proteção à luz. Assim que extraídas, as amostras de sangue foram transportadas das escolas ao laboratório por uma empresa especializada em transporte de material biológico (em condições adequadas de luz e temperatura), em um período máximo de 40 minutos, a fim de garantir o rigoroso controle de qualidade dessas amostras e suas análises. Para tal, o trabalho de toda uma equipe treinada foi fundamental, desde o preparo e organização para o exame de sangue, condução da criança ao exame de forma lúdica, extração de sangue por duas auxiliares de enfermagem experientes, transporte das amostras, recebimento das mesmas e entrega ao laboratório, até a realização das análises laboratoriais em duplicata.

Ressalta-se que as crianças desse estudo (n=45) também realizaram as demais avaliações seguindo a mesma estrutura do SAYCARE study, como por exemplo, as análises de ferro e ferritina séricos.

Conforme destacado na revisão sistemática supracitada ⁽³⁰⁾, um possível fator que contribui para a realização do trabalho de campo e o alto controle de qualidade do exame de sangue para as análises de vitaminas plasmáticas, foi o uso de um fantoche de macaco, nomeado "*Ms. Banana's*". O "*Ms. Banana's*" foi criado para brincar com as crianças durante a coleta de dados, visando um processo mais interativo e lúdico durante a participação delas no estudo, especialmente no exame de sangue.

Foram encontrados coeficientes de correlação de 0,29 (IC_{95%}: 0,20 - 0,38) e 0,35 (IC_{95%}: 0,26 - 0,43) para a ingestão de frutas e vegetais estimada pelo QFA e MSM,

respectivamente. Esses resultados sugerem que tanto o QFA como o R24h (combinação de 3 dias com a frequência de ingestão usando o MSM), são métodos válidos e com boa acurácia, para classificar a ingestão de frutas e vegetais das crianças do SAYCARE study. Tais resultados são importantes, para fomentar a possibilidade de se usar apenas o QFA desenvolvido para o SAYCARE, para classificar a ingestão de frutas e vegetais em crianças, como uma ferramenta útil e mais econômica que o R24h. Por exemplo, aplicar apenas um QFA pode otimizar os recursos financeiros e de tempo, uma vez que não há necessidade da presença do nutricionista ou profissional treinado no trabalho de campo para aplicar o R24h (três dias), nem despende tempo e esforços intensivos para analisar os dados do R24h, e calculá-los usando o *Multiple Source Method* (MSM) ⁽¹⁴⁾.

Apesar de ser um objetivo secundário a esse artigo, encontrou-se que quase 80% das crianças participantes desse estudo, cumpriram com as recomendações das vitaminas plasmáticas (retinol, ácido ascórbico e α -tocoferol). Entretanto, a ingestão média de frutas e vegetais ficou abaixo da recomendação (320 a 400 gramas por dia) ⁽⁴⁸⁾, tanto para o QFA [261,2 gramas por dia (IC_{95%}: 195,1 – 327,3)] como para o MSM [301,7 gramas por dia (IC_{95%}: 277,2 – 326,2)]. Sendo assim, sugerimos que os estudos futuros verifiquem se a referência para consumo de frutas e vegetais deve ser a mesma para todas as faixas etárias, uma vez que crianças têm necessidades e comportamentos significativamente diferentes dos adultos ou idosos, por exemplo.

Uma limitação desse estudo foi a impossibilidade de incluir o β -caroteno nas análises de acurácia, pois até o momento, não existia um ponto de corte para identificar se os níveis de β -caroteno estavam adequados no sangue. Os carotenoides e o retinol são precursores da vitamina A, e são importantes para a saúde, crescimento, desenvolvimento infantil ⁽⁴⁹⁾. A deficiência de vitamina A pode estar associada a riscos aumentados de xerofthalmia, baixa densidade mineral óssea, disfunção imune, morbidades infecciosas e

até mortalidade ⁽⁵⁰⁾. Portanto, pesquisadores sugerem que se conduza um estudo explícito, objetivo e transparente, a fim de identificar o ponto de corte adequado de vitamina A no sangue, para determinar a prevalência de deficiência de vitamina A nas populações pediátricas ⁽⁵¹⁾.

Com esse objetivo, uma revisão sistemática foi proposta como trabalho de estágio de pesquisa no exterior, na *Universidade de Harvard*, Boston. Esse estágio foi concluído em fevereiro de 2020, o artigo de revisão sistemática foi finalizado e aceito para publicação na revista *Nutrition Reviews* (*fator de impacto 6.500*), e o resumo deste artigo é apresentado no capítulo de artigos complementares à tese (item 6.6).

4.3 Artigo 3: Validação do consumo de ferro alimentar

O terceiro artigo, também original desta tese, foi submetido em 22 de maio de 2020, em revista indexada ao MEDLINE.

Este artigo se propôs a validar o QFA e o R24h (média dos três dias usando o MSM) para classificar o consumo de ferro alimentar em crianças de 3 a 10 anos (n=99) e adolescentes de 11 a 17 anos (n=50) do SAYCARE study, usando ferro e ferritina séricos como método de referência.

As regressões multiníveis mostraram coeficientes de correlação baixos para o R24h (média dos três dias usando o MSM) em crianças (ferro: 0,18; ferritina 0,16) e também nos adolescentes (ferro: 0,12; ferritina 0,12). No entanto, os coeficientes de correlação encontrados para o QFA foram muito bons (variaram entre 0,81 a 0,87) quando comparado ao ferro e a ferritina séricos, em ambas as faixas etárias. Além disso, o QFA também apresentou boa acurácia (70,6%) para ferritina sérica em crianças, e para o ferro sérico em adolescentes (69,8%). Apesar deste QFA incluir grupos de alimentos fonte de ferro heme e não-heme, este tem capacidade limitada em identificar os constituintes dietéticos do ferro (por exemplo, fatores que podem auxiliar ou prejudicar a biodisponibilidade do ferro).

O QFA desenvolvido para o SAYCARE apresentou boa capacidade de classificar a ingestão alimentar de ferro total em crianças e adolescentes deste estudo. Esse resultado corrobora com o artigo 2, o qual também encontrou que este QFA possui boa capacidade de classificar o consumo de frutas e vegetais em crianças participantes do SAYCARE study.

O QFA não apenas mostrou melhores coeficientes de correlação que o R24h (média dos três dias usando o MSM), como também é uma ferramenta relativamente mais

fácil de se administrar, mais prática e mais econômica que o R24h. Um ponto forte do QFA desenvolvido para o SAYCARE é a adaptação cultural aos diferentes centros sul-americanos participantes desse estudo, e por ser específico à população pediátrica ⁽⁴⁶⁾. Esses fatores podem explicar parte dos coeficientes de correlação muito bons ($> 0,80$) observados entre a ingestão total de ferro (estimada pelo QFA) e os biomarcadores séricos, quando comparados a outros estudos ^(17, 52, 53). Porém, é importante enfatizar que o R24h também é um método de avaliação alimentar útil e amplamente empregado em estudos epidemiológicos ⁽¹⁸⁾. No entanto, neste artigo, a aplicação do R24h por Nutricionista ou profissional treinado foi limitada por apenas um dia (primeiro R24h), e os outros dois dias foram preenchidos em casa pelos pais ou responsáveis das crianças; ou pelo próprio adolescente. Logo, essa foi uma limitação que possivelmente influenciou os resultados do R24h (coeficientes de correlação $\leq 0,18$) encontrados.

Assim, o QFA desenvolvido para o SAYCARE é uma ferramenta epidemiológica útil para classificar a ingestão de total ferro alimentar em crianças e adolescentes deste estudo. Por outro lado, a ingestão média dos três dias de R24h apresentou coeficientes de correlação baixos quando comparada ao ferro e ferritina séricos. Portanto, no caso do SAYCARE study, o uso do QFA é recomendado ao invés do 24h-DR.

5 CONCLUSÕES

Os resultados da revisão sistemática demonstraram que a validade dos questionários se mostrou aceitável para classificar o consumo de frutas e vegetais em crianças, quando comparados a biomarcadores sanguíneos. Porém, ressalta que a avaliação do consumo alimentar em crianças ainda enfrenta muitos desafios, pois a ingestão alimentar é complexa, e repleta de dinamismo, variando entre populações e culturas. Assim, comparar a ingestão alimentar de diferentes centros sul-americanos (com distintos aspectos sociais, culturais e fisiológicos) como foi realizado para o SAYCACRE study, é desafiador. Identificar os principais fatores que podem influenciar essa avaliação, pode ser um caminho promissor para aumentar a validade dos métodos de avaliação dietética. Por exemplo, atenção deve ser dada desde o desenvolvimento dos questionários de avaliação do consumo alimentar (considerando fatores como adaptação a população estudada, sua faixa etária e hábitos culturais), forma de aplicação desses métodos (considerar a incorporação da tecnologia e da criatividade, para tornar o processo mais interativo e lúdico), até o controle rigoroso de todo o processo de coleta e análise de sangue.

Os artigos originais desta tese, sobre validação dos questionários desenvolvidos para o SAYCARE study, sugerem que o QFA desenvolvido possui boa capacidade de classificar o consumo de frutas e vegetais em crianças, e também, de ferro alimentar em crianças e adolescentes da América do Sul. Tais observações se baseiam nos bons coeficientes de correlação, entre o consumo alimentar estimado pelo QFA e as concentrações de biomarcadores sanguíneos (vitaminas plasmáticas e ferro e ferritina séricos). O R24h apresentou coeficientes de correlação similares ou mais baixos que o

QFA. Portanto, apesar do R24h ser uma ferramenta útil e amplamente utilizada em estudos epidemiológicos, no caso do SAYCARE study, o QFA mais é recomendado, por ser relativamente mais prático de ser aplicado que o R24h. Por exemplo, o uso do QFA pode otimizar o tempo e recursos financeiros, visto que não há necessidade da presença de nutricionista ou profissional treinado no trabalho de campo para aplicar o R24h (durante três dias diferentes), nem despende tempo e esforços intensivos para analisar os dados do R24h, e calculá-los usando o software *Multiple Source Method* (MSM).

Porém, por ser uma ferramenta de uso multicêntrico entre países da América do Sul, uma das limitações do QFA desenvolvido para o SAYCARE, é a incapacidade de distinguir entre os diferentes tipos de frutas e vegetais, ou constituintes dietéticos do ferro (por exemplo, fatores que auxiliam ou inibem a biodisponibilidade desse mineral). Mesmo assim, esse QFA mostrou ser uma ferramenta epidemiológica útil para classificar a ingestão de frutas, vegetais, e ferro alimentar em crianças do SAYCARE study.

Por fim, a epidemiologia nutricional pode se beneficiar do desenvolvimento e validação desse QFA, utilizando-o em estudos futuros. O QFA desenvolvido para o SAYCARE pode ser aplicado, principalmente, em estudos realizados em países de baixa e média renda (por exemplo, países da América do Sul), e em diferentes faixas etárias entre a população pediátrica (por exemplo, pré-escolares, escolares e adolescentes).

6 ARTIGOS COMPLEMENTARES

Outros artigos, complementares à tese, que foram publicados ou submetidos, estão citados abaixo:

6.1 **Revista:** *Obesity* (Fator de Impacto: 4.042), **2018**. DOI: 10.1002/oby.22114

Título: *Development of a food frequency questionnaire for assessing dietary intake in children and adolescents in South America*

Autores: Luisa Saravia, Laura I. González-Zapata, Tara Rendo-Urteaga, Jamile Ramos, **Tatiana Sadalla Collese**, Isabel Bove, Carlos Delgado, Florencia Tello, Iris Iglesia, Ederson Dassler Gonçalves Sousa, Augusto César Ferreira De Moraes, Heráclito Barbosa Carvalho, Luis A. Moreno.

Resumo

Objective: This study aimed to describe the development of a food frequency questionnaire (FFQ) to assess dietary intake in South American children and adolescents. **Methods:** A total of 345 children (aged 3-10 years) and 357 adolescents (aged 11-17 years) were included for analysis. The FFQ was designed to be self-administered and to assess dietary intake over the past 3 months. It was developed in Spanish and translated into Portuguese. Multiple approaches were considered to compile the food list, and 11 food groups were included. A food photo booklet was produced as supporting material. **Results:** The FFQ items maintained a common core list among centers (47 items) and country-specific foods. The FFQ for Buenos Aires and Lima had a total of 63 items; there were 55 items for the FFQ in Medellin, 60 items for Montevideo, 58 items for Santiago, 67 items for Sao Paulo, and 68 items for Teresina. Alcohol was also incorporated in the adolescents' FFQ. **Conclusions:** We developed a semiquantitative, culturally adapted FFQ to assess dietary intake in children and adolescents in South America. It has an optimal size allowing its completion in a high proportion of the population; therefore, it can be used in epidemiological studies with South American children and adolescents.

6.2 **Revista:** *Public Health Nutrition* (Factor de Impacto: 2.485), **2019**.
DOI: 10.1017/S1368980019002064

Título: *Reliability and validity of an FFQ for South American children and adolescents from the SAYCARE study*

Autores: Tara Rendo-Urteaga, Luisa Saravia, **Tatiana Sadalla Collese**, Julia María Monsalve-Alvarez, Laura Inés González-Zapata, Florencia Tello, Beatriz Martínez-Oliván, Francisco Leonardo Torres-Leal, Luis A Moreno, Augusto César Ferreira De Moraes and Heráclito Barbosa Carvalho

Resumo

Objective: The purpose of this study was to analyse the reliability and validity of a semi-quantitative FFQ to assess food group consumption in South American children and adolescents. **Design:** The SAYCARE (South American Youth/Child cARdiovascular and Environmental) study is an observational, multicenter, feasibility study performed in a sample of 3- to 18-year-old children and adolescents attending private and public schools from six South American countries. Participants answered the FFQ twice with a two-week interval and three 24-h dietary recalls. Intraclass and Spearman's correlations, weighted Cohen's kappa (κ_w), percentage of agreement and energy-adjusted Pearson's correlation coefficients were calculated. **Setting:** Seven cities in South America (Buenos Aires, Lima, Medellin, Montevideo, Santiago, Sao Paulo and Teresina). **Subjects:** A sample of 200 children and 244 adolescents for reliability analyses and 252 children and 244 adolescents for validity analyses were included. **Results:** Depending on the food group, for children and adolescents, reliability analyses resulted in Spearman's coefficients from 0.47 to 0.73, intraclass correlation coefficients from 0.66 to 0.99, κ_w coefficients from 0.35 to 0.63, and percentage of agreement between 72.75 and 83.52 %. In the same way, validity analyses resulted in Spearman's coefficients from 0.17 to 0.37, energy-adjusted Pearson's coefficients from 0.17 to 0.61, κ_w coefficients from 0.09 to 0.24, and percentages of agreement between 45.79 and 67.06 %. **Conclusion:** The SAYCARE FFQ achieved reasonable reliability and slight-moderate validity for almost all food groups intakes. Accordingly, it can be used for the purpose of ranking the intake of individuals within a population.

6.3 **Revista:** *Public Health Nutrition* (Fator de Impacto: 2.485), **2018**.
DOI: 10.1017/S1368980018004020

Título: *Reliability and validity of body weight and body image perception in children and adolescents from the SAYCARE Study*

Autores: Laura I. González-Zapata, Sandra Restrepo-Mesa, Juan Carlos Aristizabal, Estela Skapino, **Tatiana Sadalla Collese**, Leticia Beatriz Azzaretti, Walter Viana Nascimento-Junior, Luis Alberto Moreno, Augusto César Ferreira De Moraes, Heráclito Barbosa Carvalho, Alejandro Estrada-Restrepo.

Resumo

Objective: To assess the reliability and validity of body weight (BW) and body image (BI) perception reported by parents (in children) and by adolescents in a South American population. Design: Cross-sectional study. BW perception was evaluated by the question "Do you think you/your-child are/is: Severe wasted, Wasted, Normal weight, Overweight, Obese?" BI perception was evaluated using the Gardner scale. To evaluate reliability, BW and BI perceptions were reported twice, two weeks apart. To evaluate validity, the BW and BI perceptions were compared with BMI Z-scores of the World Health Organization. Kappa coefficient and Kendall's Tau coefficient were obtained. Setting: Public and private schools and high-schools from six countries of South America (Argentina, Peru, Colombia, Uruguay, Chile, and Brazil). Subjects: 635 children (3-10 years old) and 400 adolescents (11-17 years old). Results: Reliability of BW perception was fair in children's parents ($k=0.337$) and substantial in adolescents ($k=0.709$). Validity of BW perception was slight in children's parents ($k=0.176$) and fair in adolescents ($k=0.268$). When evaluating BI, most children were perceived by parents as having a lower weight. The reliability of BI perception was slight in children's parents ($k=0.124$) and moderate in adolescents ($k=0.599$). The validity of BI perception was poor in children's parents ($k=-0.018$) and slight in adolescents ($k=0.023$). Conclusions: Reliability of BW and BI perceptions was higher in adolescents than in the parents of the children. Validity of BW perception was good among the parents of the children and adolescents with underweight and normal weight.

6.4 **Revista:** *International Journal of Public Health* (Fator de Impacto: 2.327), **2018**.
DOI: 10.1007/s00038-018-1178-3

Título: *Energy Balance-Related Behaviors: How Do They Cluster in Adolescents?*

Autores: Collese TS, Ferreira de Moraes AC, Fernández-Alvira JM; Michels M; De Henauw S; Manios Y; Androutsos O; Kafatos A; Widhalm K, Galfo M; Beghin L; Sjöström M; Pedrero-Chamizo R; Carvalho HB; Moreno LA on behalf of the HELENA Study Group

Resumo

Objectives To delineate the clustering of energy balance-related behaviors in adolescents and investigate whether these behaviors are associated with the household socioeconomic status and parental education level. **Methods** Two cross-sectional studies assessed information on sedentary behavior, physical activity, sugar-sweetened beverages and fruit and vegetable consumption, and sleep duration by self-reported questionnaires in adolescents (12.5–17.5 years old) from Maringa/Brazil (BRACAH Study; n = 682) and ten European cities (HELENA Study; n = 1252) from nine different countries. Gender-specific cluster analyses were performed separately for each study, applying a combination of hierarchical and non-hierarchical methods. **Results** Girls showed equivalent behaviors: Sedentary; Active; Unhealthy Eating; Healthy Eating; while boys differed (Brazilian: Sedentary; Active; Healthy Eating; European: Sedentary; Healthy; Unhealthy Eating). In Brazil, we found no association between socioeconomic status and parental education. In European girls, the high socioeconomic status and both parents' university degree were associated with Healthy Eating. In European boys, the high socioeconomic status was associated with Unhealthy Eating, and the mothers' university degree was associated with the Healthy cluster. **Conclusions** Adolescents show Sedentary behavior, regardless of their sex, country of origin, or socioeconomic condition.

6.5 **Revista:** *Nutrition Reviews* (Fator de Impacto: 6.500), 2020.
DOI:10.1093/nutrit/nuaa010.

Título: *A posteriori dietary patterns and their association with systemic low-grade inflammation in adults: a systematic review and meta-analysis*

Autores: Marina M. Norde, Tatiana S. Collese, Edward Giovannucci and Marcelo M. Rogero

Resumo

Context: A posteriori dietary patterns are promising ways of uncovering potential public health strategies for the prevention of systemic, low-grade, inflammation-related, chronic noncommunicable diseases. Objective: To investigate and summarize the current evidence on the association between a posteriori dietary patterns and systemic, low-grade inflammation in adults. Data sources: MEDLINE, EMBASE, Web of Science, and LILACS were searched. Data extraction: Data screening, extraction, and quality assessment were performed independently by 2 investigators. Meta-analysis with random effects was conducted. Differences and similarities between reduced rank regression derived dietary patterns were assessed. Results: Healthy dietary patterns are inversely and the Western dietary pattern is positively associated with inflammation ($r = -0.13$, 95% confidence interval: -0.20 to -0.06 ; and $r = 0.11$, 95% confidence interval, 0.09 to 0.12 , respectively). Reduced rank regression–derived anti-inflammatory dietary patterns are consistently characterized by high intake of fresh fruits and inflammatory dietary patterns are consistently characterized by high intake of red and processed meat and low intake of vegetables. Conclusion: Favoring the substitution of a Westernized diet for a healthy diet may lower inflammation, which might improve the prevention of some chronic noncommunicable diseases.

6.6 **Revista:** *Nutrition Reviews* (Fator de Impacto: 6.500), 2020.
DOI: 10.1093/nutrit/nuaa133.

Título: *Which blood cut-off should be used for vitamin A deficiency in children 3-10 years? A systematic review*

Autores: Tatiana S. Collese; Marina M. Norde, Marcus V. Nascimento-Ferreira, Hanseul Kim, Dirce M. Marchioni, Heráclito B. Carvalho and Edward Giovannucci

Resumo

Context: Blood cut-offs for vitamin A deficiency in children aged 3-10 years have not been addressed in the literature. **Objective:** To identify blood retinol concentrations for determining severe vitamin A deficiency in children 3-10 years. **Data Sources:** MEDLINE, Web of Science, EMBASE, and Scopus were searched. **Data Extraction:** Two reviewers independently extracted article data and assessed quality. **Data Analysis:** The hierarchical summary receiver operating characteristic models were applied for the diagnostic accuracy meta-analysis. This review is registered at PROSPERO (CRD42020149367). **Results:** 15 articles met the eligibility criteria, and 9 were included in the diagnostic accuracy meta-analysis. The summary estimates (95% confidence interval) were: sensitivity: 0.39 (0.20–0.62), specificity: 0.79 (0.65–0.88), positive likelihood ratio: 1.85 (1.33-2.57), and negative likelihood ratio: 0.77 (0.60-0.99). The area under the curve of the overall analysis was 0.68 (0.63–0.72). **Conclusions:** Blood retinol concentrations have low diagnostic accuracy for severe vitamin A deficiency in children 3-10 years. Therefore, there is unclear evidence about the preferable cut-off point for determining severe vitamin A deficiency in children 3-10 years.

7 ASPECTOS ÉTICOS

Esta tese sobre validação dos métodos de consumo alimentar em crianças, recebeu aprovação concedida pelo comitê de ética em pesquisa da Faculdade de Medicina da Universidade de São Paulo (FM/USP), sob protocolo de pesquisa **n° 2.022.542** (Anexo D). O termo de consentimento livre e esclarecido foi assinado por todos os pais ou responsáveis dos participantes da pesquisa, e foi obtido assentimento verbal das crianças para participarem do estudo.

O SAYCARE study foi aprovado pelo comitê de ética em pesquisa da Faculdade de Medicina da Universidade de São Paulo (FM/USP), protocolo de pesquisa **n° 232/14** (Anexo E), e também pelos comitês de ética em pesquisa de cada um dos demais centros envolvidos no SAYCARE study (Anexo F).

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Review

What is the Validity of Questionnaires Assessing Fruit and Vegetable Consumption in Children when Compared with Blood Biomarkers? A Meta-Analysis

Tatiana S. Collese * , Gabriela Vatauvuk-Serrati, Marcus Vinicius Nascimento-Ferreira ,
Augusto César Ferreira De Moraes and Heráclito Barbosa Carvalho

YCARE (Youth/Child cArdiovascular Risk and Environmental) Research Group, Department of Preventive Medicine, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP 01246-903, Brazil;

gabrielaverrati@gmail.com (G.V.-S.); marcus1986@usp.br (M.V.N.-F.);

augustocesar.demoraes@usp.br (A.C.F.D.M.); heracc@usp.br (H.B.C.)

* Correspondence: tcollese@usp.br; Tel.: +55-11-3061-7091; Fax: +55-11-3061-8466

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Abstract: Fruit and vegetable consumption has been associated with improved health outcomes in children. As an extensive number of questionnaires are currently used to assess fruit and vegetable consumption, we performed a systematic review of the criterion validity of questionnaires used to estimate fruit and vegetable consumption in children, considering blood biomarkers as the reference method. Five electronic databases (MEDLINE, CINAHL, Scopus, PsycINFO, Web of Science) were searched from database inception to 23 July 2018. The search strategy used the following sets of descriptors: children; fruits and vegetables; dietary questionnaires; blood biomarkers; and validation coefficient. The search terms were adapted for use with other databases in combination with database-specific filters. Potentially eligible articles were selected independently by two reviewers, separately, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Two articles meeting the inclusion criteria were included. The main reason for study exclusion was the sample age range, which included adolescents. The pooled correlation coefficient was 0.32 (95% confidence interval: 0.24–0.40). This review provided insights into assessment methods of fruit and vegetable consumption in children. Although further studies are required, questionnaires for assessing fruit and vegetable consumption have fair criterion validity in children.

Keywords: systematic review; meta-analysis; children; fruits and vegetables; biomarkers; vitamins; validity

1. Introduction

International health associations recommend that children have a diet rich in vegetables, fruits, whole grains, low-fat dairy products, legumes, fish, and lean meat, and low in saturated fat, trans fat, and cholesterol, to help maintain a healthy weight and promote cardiovascular health [1]. Given the role that the diet plays in children's health, it is crucial to accurately determine their dietary intake and to estimate the relationship between the foods consumed and nutrients.

Regular fruit and vegetable consumption is routinely suggested as a key component of promoting health because they are an important source of nutrients, such as water, fiber, potassium, folic acid, vitamins, and phytochemicals [1,2]. Several systematic reviews and meta-analyses have found consistent associations between fruit and vegetable consumption and lower risk of cardiovascular disease, diabetes, cancer, and other chronic diseases [3–5]. Despite the well-known benefits of consuming fruits and vegetables, children are not meeting the recommended level of five servings

per day [6]. Consequently, increasing fruit and vegetable consumption in children is one of the major goals of dietary interventions, worldwide [6]. Thus, valid questionnaires to assess fruit and vegetable consumption are essential to identify eating habit changes in response to interventions, and to analyze the impact of fruit consumption on health [7].

In nutritional epidemiology, the most used methods to estimate fruit and vegetable intake are subjective instruments, such as food frequency questionnaires (FFQs) and dietary recalls, because they are simple, cost-effective methods to assess long-term eating habits for large samples [8,9]. However, these subjective instruments are susceptible to both random and systematic errors [10,11], especially when applied to children, due to their limited skills to report their own dietary intake, depending on parental (or whoever is responsible for their food) literacy and motivation to complete these questionnaires [12].

As all dietary assessment methods (e.g., 24 h recalls, FFQs, smart-phone apps) have their own strengths and limitations, dietary questionnaires are usually validated against an objective measurement, such as blood biomarkers or doubly-labeled water [8,13]. Previous studies proposed that plasma vitamins, such as vitamin A, E, and C, are a reliable concentration biomarker of usual fruit and vegetable intake. Hence they are commonly used in validity studies of dietary assessment methods as independent proxy measures of fruit and vegetable consumption [14], and to evaluate whether sources of random error are independent of errors associated with measurement by questionnaire and/or inaccuracies within nutrient databases [15].

However, these plasma vitamins are still relatively recent, and a significant amount of studies are warranted in nutritional epidemiology [16]. It concerns deciphering whether these biomarkers are markers of dietary consumption or markers of altered metabolism, as a result of the food intake [16], or regarding the individual variability in absorption or availability [17]. Validation studies with blood biomarkers have been carried out in adults [10,11], but relatively few studies were conducted in children, lacking literature on key issues such as the impact of the period between fruit and vegetable consumption and the blood biomarker analysis, or the stability of blood biomarkers in children aged 3 years to less than 10 years.

Therefore, the research question of this review was “What is the criteria validity of questionnaires used to estimate fruit and vegetable consumption in children when compared with blood biomarkers?”

2. Methods

This systematic review was designed and conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (Supplementary Material 1), as described in Reference [18]. At study initiation, no similar systematic reviews were available in PROSPERO (International Prospective Register of Systematic Reviews) or the following databases: Biomed Central, Medical Literature Analysis and Retrieval System Online (MEDLINE), Web of Science, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Scopus, and PsycINFO. Thus, this protocol was registered in PROSPERO (CRD42017072531).

2.1. Study Selection

Five electronic databases—MEDLINE, CINAHL, Scopus, PsycINFO, and Web of Science—were searched from inception to 20 December 2017. The searches were registered in the National Center for Biotechnology Information (US National Library of Medicine, Bethesda, MD, USA), so that continual updates on new publications would be received until 23 July 2018, and the searches were updated until this same date. References of selected articles were analyzed, and the corresponding authors were contacted to identify other relevant studies. The descriptors and medical terms used for the database search were as follows: (*Child OR Children OR school OR boy OR son OR girl OR daughter*) AND (*Fruit OR Vegetable*) AND (*Questionnaire OR 24h Dietary Recall OR 24 h-Dietary-Recall OR 24 h Dietary Recall OR Recall OR Food Frequency Questionnaire OR FFQ*) AND (*Biomarker OR Blood OR concentration*

biomarker OR serum OR serum marker OR carotenoids OR vitamins) AND (Validation OR Validity OR coefficient OR reliability).

Two investigators examined the articles and performed the data screening, the data extraction, and the quality assessment independently. Inter-reviewer discrepancies were resolved by consensus, and a third reviewer was consulted for unresolved discrepancies. Relevant articles were obtained in full and were assessed for eligibility and exclusion criteria.

2.2. Eligibility Criteria

As the goal of this systematic review was to assess evidence, specifically for validation studies of questionnaires used to estimate fruit and vegetable consumption in children when compared with blood biomarkers, this type of review must clearly delineate the population in question. Consequently, the results may apply only to school-aged children.

The literature suggests that children are already able to help their parents to report their food intake with relative accuracy [19]. However, after 10 years of age, the World Health Organization [20] classifies the population as adolescent, with more independence and skills, which strongly influence decision making, peer affiliation, and behavior [21], allowing them to decide on and self-report their own dietary intake. In this sense, we considered only the children between three years of age until nine years and 11 months, as presented in Table 1.

Table 1. Eligibility criteria included in the systematic review.

Eligibility Criteria	
Population	Children (3 years to less than 10 years of age) in a free-living environment, without any specific condition (e.g., eating disorder, obesity, diabetes) or children under medical care.
Exposure	Fruit and/or vegetable consumption estimated by questionnaire.
Comparator	Blood biomarkers for fruit and/or vegetable consumption.
Outcome	Validation coefficients of fruit and/or vegetable consumption.
Study	Original articles.

Exclusion Criteria

Studies where the participants had different diseases (or disturbances) that could interfere with fruit and vegetable consumption were excluded. These criteria were set to increase inter-study comparability. In addition, review articles, conference papers, or books were excluded.

2.3. Data Screening and Extraction

Potentially eligible articles were selected for inclusion according to the following sequence:

1. Articles published in English, Spanish, and Portuguese were identified;
2. Titles of the articles were screened;
3. Abstracts were screened; and
4. The full texts of the articles were reviewed to determine whether they met the inclusion criteria.

Articles were saved in the EndNote Web reference manager software version 3.1 (Thomson Research Software, Carlsbad, CA, USA), and duplicates were removed. The data extracted included the author, title of the study, citation and contact details, and study eligibility (Table 1). When studies did not meet the eligibility criteria, the main reason for exclusion was documented in ink. Thereafter, a table (Supplementary Material 2) was created by each reviewer with additional details about the study, such as the sample size, settings, exposure/outcome definition and unit of measurement, statistical analysis, bias (funding sources, adjustments), key conclusions, authors, miscellaneous comments from the study authors, references to other relevant studies, limitations, correspondence required, and miscellaneous comments by the authors reviewing the included studies.

2.4. Quality Assessment

All retrieved articles were independently assessed and critically appraised using the standardized checklist of the STROBE-nut (adapted to our review) to identify sources of bias, performance, attrition, and detection. Data relevant to this review included the study design, characteristics of the research subjects, dietary assessment methods used, results, discussion, bias assessment, and ethics information.

2.5. Strategy for Data Synthesis

The reporting status of fruit and vegetable consumption in each of the included studies were determined from that listed within the results section of the included articles. The reporting status of each study was determined using three predefined categories. The categories were dependent on the level of accuracy of reported fruit and vegetable consumption, compared to measured blood biomarkers. When available from included studies, agreement estimates were extracted if the reporting status of research subjects correlated to various characteristics of the group. These characteristics included demographic statistics (age and sex), anthropometric characteristics (height, weight, and body mass index), and the study design. Limitations of each study and the evidence level were recorded as well. The agreement estimates from study questionnaires were synthesized.

2.6. Statistics

We used the Stata 14 (Stata Corp., College Station, TX, USA) program for all statistical analyses. Our outcomes were the correlation coefficients between the questionnaire and blood biomarkers, which we calculated with their corresponding 95% confidence intervals (considering $p < 0.05$ as significance level). In addition, we calculated the sample-weighted pooled correlation coefficient for the meta-analysis with the random-effects model, due to the moderate heterogeneity found in each study. We estimated the strength of the agreement of the Spearman rank correlation coefficient cutoff points using the classification for dietary assessment methods: weak < 0.20 , fair = 0.21 to 0.40, moderate = 0.41 to 0.60, good = 0.61 to 0.80, and > 0.80 , very good [22]. Additionally, we verified the heterogeneity of the studies using the *I-squared* test (percentages and p values under 0.05 were considered significant). Heterogeneity levels of 25%, 50%, and 75% were considered low, moderate, and high, respectively [23]. We performed the Egger's regression test to verify potential publication bias (positive or negative results) and small-study effects. We also generated the funnel plot (Supplementary Material 3) to examine the potential bias graphically.

3. Results

The literature search provided 680 potentially eligible records: 4 from PsycINFO, 42 from Embase, 71 from Web of Science, 430 from Scopus, and 133 from Medline (see Appendix 1 in the Supporting Information online). After duplicates ($n = 51$) were excluded, 629 studies remained and were screened by title and, posteriorly, by abstract, according to the established inclusion criteria, resulting in 19 records for which the full-text was assessed for eligibility. Of those, the main reasons for exclusion were the population (studies included participants over 10 years of age) and biomarker (such as skin carotenoids), as shown in Figure 1. More details about these 19 study articles are stated in Supplementary Material 4. Consequently, two studies were eligible and were included in this systematic review.

Figure 1. Flow chart of article identification, retrieval, and inclusion, for the systematic review.

The two final eligible articles included in this systematic review were published in English, but they are from different countries, one from Europe and the other from North America (Table 2). We found one study published in 1993 [24], but Medin et al. [25] was more recent, published in 2016.

Table 2. Description of the included studies.

Reference	Survey Year	Country	Study/Article Title	Sample Age (Years)	Sample Size		Study Type
					Total (n)	Girls (%)	
Byers et al. (1993) [24]	1990	USA	The accuracy of parental reports of their children's intake of fruits and vegetables: Validation of a food frequency questionnaire with serum levels of carotenoids and vitamins C, A, and E.	06 to 10	97	55.7	Validity
Medin et al. (2016) [25]	2013	Norway	Associations between reported intakes of carotenoid-rich foods and concentrations of carotenoids in plasma: A validation study of a web-based food recall for children and adolescents.	08 to 09 12 to 14	121	55.6	Validity

Table 3 shows that both studies asked for the parental report; however, Byers et al. [24] used the FFQ regarding the previous three months and considered fruit juice together with fruit and vegetable consumption. Medin et al. [25] used a web-based food recall, for four consecutive nights, but they assessed only vegetable intake for 4th grade children (8–9 years of age).

Nine different types of biomarkers were used to validate fruit and vegetable consumption. Both studies analyzed carotenoids (α -carotene, β -carotene, and cryptoxanthin), but Medin et al. [25] also included lycopene, lutein, and zeaxanthin in the sum of total carotenoids. On the other hand, Byers et al. [24] analyzed vitamin A (retinol), vitamin E (α -tocopherol), and vitamin C (ascorbic acid). High-performance liquid chromatography (HPLC), which is considered the gold standard analytical technique for characterization and analysis of carotenoids in biological and food samples [26], was used to assess plasma carotenoids in all studies (and for vitamin E in Byers et al. [24]), and spectrophotometry was used to assess plasma vitamin C (Table 3).

The correlation coefficients between fruit and vegetable consumption and blood biomarkers are presented in Table 4. The validity coefficients were fair to moderate, varying from 0.24 (for vitamin E) to 0.47 (carotenoids). Both studies calculated the Spearman rank correlation coefficients and adjusted the analyses for age, sex, ethnicity, and body mass index.

Table 3. Validity studies on questionnaires to assess fruit and vegetable consumption with blood biomarkers in children.

Reference	Exposure Assessment Method	Period of Report	Report	Consumption	Supplements Assessed	Nutritional Database	Blood Biomarkers	Biochemical Method	Fasting Time
Byers et al. (1993) [24]	FFQ (111 food items)	Previous 3 months	Parental	Median fruits, fruit juice, and vegetables Girls: 3.7 Boys: 2.9 (serving/day)	Not declared	Willet	α -Carotene β -Carotene Cryptoxanthin α -tocopherol Ascorbic Acid	HLPC (carotenoids, vitamin A and E), Spectrophotometry (vitamin C)	Not required
Medin et al. (2016) [25]	Web-based food recall	4 consecutive nights	Parental	Median fruits, fruit juice, and vegetables: 225.1 (g/day)	Yes + supplements containing carotenoids	USDA National Nutrient Database	α -Carotene β -Carotene β -Cryptoxanthin Lycopene Lutein Zeaxanthin	HPLC	Non-fasting

Abbreviations: FFQ: food frequency questionnaire; HPLC: high-performance liquid chromatography.

Table 4. Outcome of the included studies.

Reference	Correlations between diet and Blood				Criterion Validity	Covariates in Fully Adjusted Model	Conflict of Interest
	Vitamin A		Vitamin E	Vitamin C			
	Carotenoids	Retinol					
Byers et al. (1993) [24]	0.30	0.25	0.24	0.34	Spearman	Age, sex, ethnicity, family history of coronary artery disease, BMI, TG, serum total cholesterol, total caloric intake	Not declared
Medin et al. (2016) [25]	Vegetable intake: 0.47	-	-	-	Spearman	Age, sex, ethnicity, family structure, BMI, parental education level	None

Abbreviations: BMI: body mass index; TG: serum triglycerides.

The heterogeneity between studies was moderate ($I^2 = 62.9\%$, $p = 0.029$), and the sample-weighted pooled correlation coefficient for biomarkers was fair ($r = 0.32$, 95% CI: 0.24–0.40), as shown in the Figure 2. We were not able to stratify the analysis into subgroup categories due to the small number of eligible studies included in the systematic review.

Figure 2. Forest-plot for correlation coefficients of fruit and vegetable consumption assessed by questionnaires and blood biomarkers.

4. Discussion

At present, there is no strong evidence regarding the validity of questionnaires for assessing fruit and vegetable consumption in children with blood biomarkers. Thus, we conducted a comprehensive systematic review of validity studies of questionnaires to assess fruit and vegetable consumption in children in comparison with blood biomarkers. Our findings indicated that this criteria validity was fair. This validity stems from the challenges inherent to dietary assessment through questionnaires, in addition to the fact that in children, we should consider the parent report, which is the subjective perception of the parents about their children's feeding habits [13,27].

The limited number studies ($n = 2$) meeting the inclusion criteria was likely due to the lack of validated dietary questionnaires, maybe because conducting blood collection in school children is less feasible, for practical and financial reasons [28], and due to the sample age range, since different age group definitions were used for children (e.g., together with infants or adolescents). For example, the study of Biloft-Jensen et al. [29], which fulfilled our eligibility criteria, except for the population age range, which was between 8 and 11 years of age. It is correct that 11-year-old individuals are still children; however, the timing of biological maturation clearly signals entry into adolescence [21], and with more cognitive abilities that enable the researchers to apply the self-report of fruit and vegetable consumption. Therefore, it was excluded from our review.

It is important to emphasize that the credibility of our findings does not rely on the number of studies included in the meta-analysis ($n = 2$), as we conducted an exhaustive and reproducible literature search to produce results with the highest level of quality of evidence available. Murad et al. [30] state that a systematic review or meta-analysis should evaluate the credibility of the methods applied on the systematic review, and that this credibility depends on whether the review addressed a specific and objective question; included an exhaustive literature search; clearly indicated the reproducibility of the selection and assessment of studies; and declared the results in a convenient way that enables future

research development. Furthermore, even though only two studies were eligible in our systematic review, each biomarker was considered separately in the analysis; in other words, we evaluated five different items ($n = 5$).

The sample-weighted pooled correlation coefficient found in our study ($r = 0.32$, 95% CI: 0.24–0.40) was fair, and comparisons with other validity studies carried out in children and adolescents (9–12 years) showed similar Pearson correlation coefficients for vegetable (0.26, $p = 0.13$) and fruit intake (0.49; $p = 0.003$) with total plasma carotenoids [31]. Studies carried out in adults showed a slight increase in the Spearman correlation coefficients for alpha-carotene (0.42; CI: 0.09–0.96), beta-carotene (0.40; CI: 0.09–0.99), and total plasma carotenoids (0.51; CI: 0.12–1.00) [32]. However, these correlations are not so distant from our results, and it is relevant to note that their confidence intervals were broad.

4.1. Dietary Assessment Methods

Measuring fruit and vegetable consumption is complex, with interactions of different food compounds, cultural, and socioeconomic conditions, in variable proportions [10,33]. Until this moment, an ideal questionnaire to assess fruit and vegetable consumption is unknown [28]. There is an extensive number of methods to perform this evaluation; however, all of them have both random and systematic limitations and errors [10].

It is difficult to determine from the studies included in this review who is the most accurate reporter of a child's fruit and vegetable intake, and which method is most accurate and reliable. It is important to note that mere participation in a research study may have biased the data reported for each child, because parents may have selectively reported higher consumption of fruit and vegetable of their child due to their involvement in the study. Reporting methods also depend on the difficulties associated with the method of reporting, and with the parental educational level.

4.2. Food Frequency Questionnaires

Byers et al. [24] used an FFQ to estimate fruit and vegetable consumption in children. Some advantages of the use of the FFQ are as follows: it can be given to the children's parents with limited explanation; it is a good method to obtain data regarding food eaten less than twice a week; questions can be included to allow different portion sizes (adjusted); and the food/nutrient database only needs to cover commonly eaten foods [34].

Disadvantages: it is time consuming; parents may find it difficult to decide how often their child eats fruit or vegetables; the FFQ often underestimates foods eaten regularly or in large quantities if the portion sizes used in the questionnaire are based on average intake; if a portion size is specified in the FFQ, parents may find it difficult to interpret and answer the question meaningfully; and furthermore, it depends on the level of literacy of the parents [34].

4.3. Web-Based Food Recall

Medin et al. [25] showed a higher correlation coefficient (0.47) for vegetable consumption, using a web-based food recall for four consecutive nights. The development of technology-based research tools for the assessment of intake, such as the web-based food recalls, has the potential to reach large populations and reduce language barriers through the use of images rather than verbal descriptions [19]. Technology allows for greater scale and efficiencies for researchers to rely on regular dietary intake data, for surveillance and monitoring [19]. The conversion of paper-based methods into web-based methods may have benefits, including faster completion, greater reach, and the ability to maximize the collection of complete data [19], which might explain part of the higher correlation coefficient found by Medin et al. [25], when compared to other studies that used the FFQ. Furthermore, two small details might have made a great difference in the Medin et al. [25] results: participants were given a personal gift card as an incentive for participating in the study, which may have changed their engagement in it; a voice-assisted cartoon character helped the children and parents to complete the questionnaires, with

pop-up reminders to reduce the problem of food omissions, and in a more friendly way for kids—this was shown to be a promising tool for application in pediatric populations.

In addition, Medin et al. [25] included four nightly 24-h recalls. This period is more than the literature recommendation (three recalls per person) [34], and since parents answered the questionnaire at night (the end of the day), it was easier for them to remember their child's fruit and vegetable consumption on the same day, minimizing the memory bias as well [34].

However, web-based food recalls reduce the limitations in terms of misreporting. Thus, there is a need for continued development of methods, as well as to continue to evolve statistical methods to mitigate error. Training researchers, clinical staff, and research subjects on the use of the technology and the method is still highly warranted and might improve results and compliance with the dietary method [19].

To be confident about the fruit and vegetable data collected, one should combine the FFQ with food recall methods, trying to ascertain both frequent and infrequent fruit and vegetable consumption [35]. Additionally, if possible, combining technology with both dietary assessment methods is a good alternative to reduce the burden of data handling and to make this process more friendly and joyful.

4.4. Creativity

The integration of technology, pictures, drawings, cartoons, and puppets into traditional dietary questionnaires, considering the main factors inherent (Figure 3) to this approach, might provide additional insights to mitigate errors in the fruit and vegetable assessment.

Figure 3. Main factors regarding the validity of dietary assessment methods in children.

When financial resources are scarce, like in studies conducted in low and middle-income countries, using a bit of creativity might be a good solution. For example, if it is not possible to combine the technology in the process of dietary assessment, a simple art-based method like a “mascot” (drawn on

paper, as a cartoon, as a toy or a hand puppet) can make a great difference in the motivation and involvement to answer the questionnaire, turning this process into a fun moment for kids [36,37]. Additionally, pictorial assessment, such as the assessment with the help of food photography albums, food drawings or food replicas (three-dimensional models), might also be a cheaper and smart alternative to enhance motivation and give visual support to estimate fruit and vegetable portion sizes [38,39].

4.5. Administration Mode

A recent systematic review of the validity of dietary assessment methods in children when compared with doubly labeled water suggests that using a parental reporter is the most accurate method for reporting dietary intake in children aged 4–11 years [13]. However, it should be noted that parents were the proxy reporters for the children; thus, some bias can be involved in this report. For example, the parental weight status may influence the accuracy of their report, as observed in a study with obese parents, who may underestimate the food intake of their obese children [40].

4.6. Blood Biomarkers

It is important to bear in mind that there are issues pertaining to bio accessibility and bioavailability of vitamin A, E, and C in fruits and vegetables, depending on several factors, such as genotype, ripening time, cultivation method, and climatic conditions, processing, food source, food particle size and location, cooking method, and the content of fiber of the fruit or vegetable [41,42]. Moreover, different parts of the same plant may also contain different types and amounts of vitamins [41].

In addition, knowing that most vitamins have a half-life of one or 2 months [43], it is difficult to assess their blood availability and to compare it over long previous periods, such as Byers et al. [24] did with their FFQ. Blood vitamins cannot be translated into absolute levels of fruit and vegetable consumption, but these biomarkers do correlate with fruit and vegetable consumption in certain strengths, and they are commonly applied in dietary assessment studies [28].

There are other factors relating to the children that may also affect the measurement and utility of these biomarkers to properly reflect their fruit and vegetable consumption. For example, genetic variability, lifestyle or physiologic factors (e.g., colonic microbiota, body mass index, and metabolic or inflammation disorders) [44], dietary factors (e.g., frequency of intake and nutrient–nutrient interactions), blood sample (e.g., whole blood, plasma, serum, conditions of sample collection, transport, treatment, process, and storage), fasting status before examination (which neither of the studies required), and laboratory analytical methodology (e.g., accuracy, detection of limits, variation from laboratory to laboratory) [28].

Even with all these factors influencing vitamins blood levels, this method is still considered by the scientific community as a reference method for fruit and vegetable consumption in epidemiological and clinical studies.

4.7. Adjustment for Confounders

In addition, appropriate adjustments for confounding variables are critical for understanding how factors of interest are interrelated within a population, as there may be a range of plausible measurement errors that have a slight influence on these associations [45]. The two eligible studies reported adjusting for confounders; however, the variables in the adjusted model differed according to the study. Hence, we suggest that future studies should consider, at least, the sex, age, body mass index, parental education, blood cholesterol, and energy and fiber intake to be included in the fully-adjusted model.

4.8. Methodological Quality

To the best of our knowledge, there is no evidence regarding the validity of questionnaires for assessing fruit and vegetable consumption in children with blood biomarkers. However, other systematic reviews addressing the validity of dietary assessment questionnaires and objective methods, such as double label water [13] or skin biomarkers [19], have consistently reported low methodological quality of the studies as well. Other studies in the field of physical activity in children, validating questionnaires with objective methods (e.g., accelerometers), also reported low methodological quality, especially regarding the absence of information about missing subjects and how the missing data were handled [46].

In this review, only two studies were included. Based on the STROBE checklist, the major bias identified was related to the information bias, particularly due to the heterogeneity between studies in the applied methodologies. The absence of information about how the study size was calculated, or how missing data were handled was a flaw in both studies. Byers et al. [24] did not declare any adjustment for parental education level, nor information on whether fruit and vegetable intake was reported with or without the inclusion of dietary supplement intake, nor about funding sources that could influence their results.

4.9. Bias

The findings of our review indicated that more validity studies of fruit and vegetable consumption need to be carried out in school-aged children. The two eligible studies were published in English and in high-income countries. Therefore, it indicates a potential risk of publication bias, which can practically be explained by the expensive cost of conducting a validity study with blood biomarkers [28].

Moreover, most of the 19 remaining full-text articles assessed for eligibility used the plasma carotenes as a reference method, but only two articles assessed vitamin E [24,47], and one article assessed vitamin C [24]. This bias is also probably related to the costs of vitamin C, to its very sensitive biochemical methodology, and to the many difficulties and bureaucratic barriers for achieving the reactants necessary for this methodology.

As dietary assessment cannot be estimated without error, it is paramount to attempt to understand their effect on the data collected to minimize the confounding effect [17].

Byers et al. studied a sample of parents from a selective group of volunteers who reported their children's food intake once a year. Thus, the validity of their reports was probably higher than other parents, because their motivation, involvement, and knowledge in the next step of the study could be different to other parents who have never been involved in a nutritional research [24].

Another relevant issue of concern is the social approval of fruits and vegetables. As they are widely known as healthy foods [48], parents may have reported their children's fruit and vegetable consumption, either consciously or subconsciously, in ways that make them appear favorable to the researchers [17].

4.10. Heterogeneity between Studies in the Meta-Analysis

A moderate degree of heterogeneity was found in our meta-analyses ($I^2 = 62.9\%$, $p < 0.029$). At first, we found a higher degree of heterogeneity in our meta-analyses ($I^2 = 94.1\%$, $p < 0.001$), and it was more consistent with other systematic reviews regarding dietary assessment [9,19]. However, after critical point-by-point reanalysis in each article, we decided that some characteristics were not very specific to our eligibility criteria. For example, the study of Biloft-Jensen et al. [29] that included the 11-year-old adolescents in the analysis and applied a self-report administration of the questionnaire; or the study of Royo-Bordonada et al. [47] that analyzed fruit and vegetable consumption as part of the dietary variety index (together with sausage and other foods that contributed to the dietary variety). Consequently, we had to exclude these two studies from the meta-analysis, since they had important sources of heterogeneity and did not fulfill our eligibility criteria [49]. After this exclusion, the remaining

heterogeneity could be partially explained by the characteristics inherent to the different types of questionnaires used (e.g., recall period, number of items, recording bias, web-based system) and the biomarker (e.g., type of biomarker analyzed, duration of measure, different cutoff points).

4.11. Strengths and Limitations

The strengths of the current review included the use of a comprehensive search strategy and data collected from children aged 3 years to less than 10 years. To the best of our knowledge, this is the first attempt to provide estimates of the true effect sizes for assessing fruit and vegetable consumption, and to conduct a meta-analysis. Additionally, screening was performed by two independent authors; searches were updated and included papers written not only in English, but also in Spanish and Portuguese. The PRISMA statement was adopted, and a methodological quality rating was performed separately, to assist in interpreting the findings. The strength of this review was that two independent authors conducted the data extraction and the methodological quality assessment.

Despite these strengths, limitations should be noted. Although a comprehensive search was conducted, the agreement analyses were restricted to information from electronically published data, to ensure the reproducibility of the results.

Even though we considered Spanish and Portuguese for the literature search and screening, the two eligible articles were published in English. Both dietary habits and parental awareness can change considerably as children age; therefore, these findings may not be generalized to children of other ages. Moreover, although the dietary methods applied in each study were different, both methods are largely used in nutritional epidemiology [50] and in studies regarding fruit and vegetable consumption by children [51], enabling the meta-analysis, once we defined the correlation coefficients of these methods as our outcome [30].

5. Conclusions

Our findings suggest that the criterion validity of questionnaires and blood biomarkers for assessing fruit and vegetable consumption in children is fair. The assessment of food consumption—especially fruit and vegetable—in children still faces many challenges. However, having a thorough understanding of the main factors that may influence this assessment can be a promising path to enhance the validity of dietary assessment methods. Since measurement error is a key remaining difficulty, validity studies of questionnaires to assess fruit and vegetable consumption should be conducted with caution and interpreted within the broad context of the field. For example, careful study design and meticulous development of the dietary assessment tools can be validated with blood biomarkers, as long as the whole process of blood collection and analysis is adequate and rigorously controlled. Moreover, informed statistical analysis, with appropriate adjustments, can reduce the impact of these errors.

Finally, the incorporation of creativity and the development of technology, despite the challenges, can lead to advancements in the field of dietary assessment methods, and therefore, have an important role in the promising future of nutritional epidemiology.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2072-6643/10/10/1396/s1>, Supplementary Material 1: PRISMA checklist; Supplementary Material 2: Extraction form of full-text articles assessed for eligibility; Supplementary Material 3: Funnel-plot for correlation coefficients of fruit and vegetable consumption assessed by questionnaires and blood biomarkers; Supplementary Material 4: Main reasons for exclusion of the full-text articles assessed for eligibility.

Author Contributions: T.S.C., G.V.-S., M.V.N.-F., A.C.F.D.M. and H.B.C. were responsible for the study concept and study design. T.S.C. and G.V.-S. examined the articles and performed the data screening, the data extraction, and the quality assessment. T.S.C., M.V.N.-F., and A.C.F.D.M. performed statistical analyses and interpreted the data. T.S.C. and G.V.-S. drafted the manuscript. T.S.C., G.V.-S., M.V.N.-F., A.C.F.D.M. and H.B.C. supervised the study. All authors participated in the writing of the paper, provided comments on the drafts, and approved the final version of the manuscript. All authors agree to be personally accountable for the author's own contributions and ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.

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Article

The Validity of Children's Fruit and Vegetable Intake Using Plasma Vitamins A, C, and E: The SAYCARE Study

Tatiana Sadalla Collese ^{1,*} , Augusto César Ferreira De Moraes ^{1,2} , Tara Rendo-Urteaga ^{1,3}, Liania Alves Luzia ⁴, Patrícia Helen de Carvalho Rondó ⁴, Dirce Maria Lobo Marchioni ⁴ , and Heráclito Barbosa Carvalho ¹

¹ YCARE (Youth/Child cArdiovascular Risk and Environmental) Research Group, Department of Preventive Medicine, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo 01246-903, Brazil

² Department of Epidemiology, School of Public Health, University of Sao Paulo, Sao Paulo 01246-904, Brazil

³ Facultad de Ciencias de la Salud, Universidad Isabel I, 09001 Burgos, Spain

⁴ Department of Nutrition, School of Public Health, University of Sao Paulo, Sao Paulo 1246-904, Brazil

* Correspondence: tcollese@usp.br; Tel.: +55-(11)-3061-7091; Fax: +55-(11)-3061-8466

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Abstract: Despite that fruits and vegetables are key elements for health promotion, there are limited studies validating their intake in children. We aimed to validate the SAYCARE (South American Youth/Child Cardiovascular and Environmental) Study Food Frequency Questionnaire (FFQ) and the combination of the FFQ frequency of intake with the 24 h-dietary-recall (24 h-DR) (mean of 3 days), for children's fruit and vegetable intake. The reference methods were plasma dosages of β -carotene, retinol, ascorbic acid, and α -tocopherol, which were collected in the school environment. It is a validity study in a subsample of 45 children aged 6–10 years participating in the SAYCARE Study, from São Paulo (Brazil). The FFQ was answered by the parents/guardians over the previous 3 months; the 24 h-DR was answered three times (two weekdays by nutritionists, one weekend day by parents/guardians). The mean fruit and vegetable intake (combined with frequency of intake) was calculated using the multiple source method (MSM). Multiple linear regression showed pooled correlation coefficients of 0.29 to 0.35 for the reported fruit and vegetable intake estimated by the FFQ and the MSM, respectively. The SAYCARE FFQ is an accurate and useful tool for ranking fruit and vegetable intake in children between 6–10 years from the SAYCARE Study.

Keywords: children; fruits and vegetables; biomarkers; vitamins; validity; food frequency questionnaire; 24-h-Dietary-Recall; Multiple Source Method

1. Introduction

Fruits and vegetables are key elements for the promotion of lifelong health [1]. However, there are limited studies validating questionnaires for children's fruit and vegetable intake [2]. Assessing dietary intake in children is a complex and challenging task [3], because it often relays on the parental report, mainly when children are younger than 10 years of age [4]. As the custom of families eating together is constantly decreasing [5], parental knowledge about their children's dietary intake is difficult to assess with accuracy [6].

Accuracy is the precision of a measurement or the degree to which a tool measures the aspect it was designed to measure, usually assessed to validate a tool [7]. There are several concepts related to the validity of dietary assessment methods, including the validity of their results against a reference method that has a superior level of validity [8]. In the usual fruit and vegetable intake, for example, the validity of a food frequency questionnaire (FFQ) newly developed might be assessed by comparing

it with 24-h dietary recalls (24 h-DR), and with concentration biomarkers. None of these examples is a perfect method, but the validity of the FFQ will be enhanced if there is agreement among the different methods [8].

As all dietary assessment methods have their strengths and limitations, understanding these details may help to apply the dietary assessment methods in the appropriate context [9]. The FFQ and the 24 h-DR are subjective methods widely applied in epidemiological studies, because they are relatively inexpensive and easy to administer, although prone to random and systematic bias [7]. Plasma vitamins can be used as nutritional biomarkers to provide an objective assessment of dietary intake, independently of the errors inherent from the FFQ and the 24 h-DR [10]. Plasma vitamins are a class of concentration biomarkers which are used for comparisons with estimates of fruit and vegetable intake [10]. Concentration biomarkers cannot be translated into absolute levels of fruit and vegetable intake, however, these biomarkers do correlate with fruit and vegetable intake in certain levels [10]. Examples of concentration biomarkers which can be used for ranking fruit and vegetable intake are as follows: Vitamin A (mainly β -carotene, the major source of vegetable pro-vitamin A; and retinol, because most of the β -carotene available is converted into retinol in the blood) [11]; vitamin E [dark-green pigmented leaves and vegetables contain low to moderate levels of α -tocopherol (vitamin E activity)] [12]; and vitamin C (ascorbic acid (vitamin C) contents are found predominantly in citrus fruits, fruit juices, and vegetables) [13,14]. Thus, these plasma vitamins can be applied as a proxy for fruit and vegetable intake, and as a reference method in validity studies of subjective methods ranking fruit and vegetable intake [13].

In spite of that, validity studies ranking children's fruit and vegetable intake are lacking, particularly in low and middle-income countries [3]. For example, in South American countries, where it is important to improve the classification of children's fruit and vegetable intake due to the great variety of fruit and vegetable eating options in these regions [15], and due to changing demographical and epidemiological factors [16].

2. Objective

To validate the FFQ and the combination of the frequency of intake from the FFQ with the 24 h-DR (mean of three days), both designed for the SAYCARE (South American Youth/Child Cardiovascular and Environmental) Study, for children's fruit and vegetable intake, using plasma vitamins A, C, and E.

3. Methods

3.1. Study Design

This is a validity study that is part of the SAYCARE (South American Youth/Child Cardiovascular and Environmental) study. The SAYCARE study is an observational, multicenter, and feasibility study aimed at developing valid and reliable instruments to obtain information about: Social and environmental factors, family environment, food intake and behavior, physical activity and sedentary behaviors, body composition, sleep, oral health and cardiovascular health biomarkers in South American children and adolescents (3–17 years) from seven cities: Buenos Aires (Argentina), Lima (Peru), Medellin (Colombia), Montevideo (Uruguay), Santiago (Chile), and São Paulo and Teresina (Brazil) [17]. Details on the sampling, recruitment, and quality control procedures of the SAYCARE Study were described previously in the SAYCARE Study supplement [17].

3.2. Study Population

The research participants were a convenience sample of children drawn from the SAYCARE study (aged 6–10 years), belonging to the survey center of São Paulo (one public and one private school). Children were selected if they donated blood samples for analyses of complete blood count, total cholesterol and plasma vitamins. These children were selected aiming the highest methodological rigor as possible in the fieldwork logistics and, especially, due to the complexity in conducting the

blood examinations at the school environment and the transport of biological material (more details are given in Section 3.6. Blood processing and analyzes). The calculation for this sample was based on the experience of validity studies of dietary assessment methods in children [3]. This calculation ideally considered the required parameters for validity studies, such as the two-tailed error of 0.05 (type I), error β of 0.20 (error type II), and correlation coefficient of 0.32 (mean of the correlation coefficients of validation studies of dietary assessment methods in children with plasma vitamins) [3], resulting in a total of 30 children.

This sample was randomly extracted from the general sample of the SAYCARE study, with research participants equally distributed by sex and type of school (public and private). Anticipating losses and refusals of 50%, we invited 60 children to participate (30 from each school).

Eligibility Criteria

Inclusion criteria: School children (aged between 6 and 10 years old) enrolled in the selected schools, with signed parental consent form, verbal assent of each child and blood collection performed. Furthermore, children in fasting status (10 h) at the time of collection, and with complete information about sex, age, FFQ, and 3 days of 24 h-DR (with 15 days-interval among each 24 h-DR).

Exclusion criteria: Inability to answer the questionnaires; children with physiologically implausible energy intake for this age group (less than 500 or more than 4000 kcal per day) estimated by the FFQ; children under supplementation with vitamin A, C, and/or E; or children with C-reactive protein concentrations above 10 mg/dL.

3.3. Fieldwork

Fieldwork was done according to the schedule and availability of the schools, between August to November 2017.

Several measurement instruments were used in two main groups: Objective measures (anthropometry and plasma vitamins) and subjective measures (questionnaires). Once the schools had all the information about the study and agreed to participate in it, fieldwork occurred on four school visits:

First visit: Meeting with parents/guardians for the presentation of the study and invitation to participate in it, with the delivery of the parental consent form.

Second visit: Once the parental consent form was signed, it was reviewed and signed by the study researchers as well. An original copy of the consent form was delivered to the parents/guardians, together with the questionnaires, in a colorful bag with the identification of the SAYCARE Study. After each child assented to participate in the study, we performed the anthropometric measures and a nutritionist applied the first 24 h-DR. On that day, the children were instructed about the preparation for the blood test, and this information was also posted on the research participants' agenda. Furthermore, text messages were sent to their parents' cell phones to remind them of this preparation the day before the blood test.

Third visit: Blood extraction and the second 24 h-DR was applied by a nutritionist (with 15 days-interval from the first 24 h-DR). All the children received a snack after the blood exam.

Fourth visit: The results of the blood tests were evaluated individually by a clinical pathologist and then sent to the parents/guardians (direct mail), along with a letter of appreciation for participation in the study and general guidelines for healthy eating. If any variation was found in these results, an accompanying letter to the health service was attached. Thereafter, the researchers went to the schools to acknowledge the participation of the children (and the collaboration of the teachers and school staff) in the study. Plays were done with the children to explain the importance of fruit and vegetable intake and the main sources of vitamins A, C, and E. For that purpose, we used plastic fruits and vegetables; vitamin A, C, and E in balloon shape; and a hand puppet of a monkey named "Ms. Bananas". The "Ms. Bananas" was also used to play with kids in the fieldwork, aiming a more interactive and ludic process during their participation in the study, especially during the blood extraction.

3.4. Anthropometric Measures

Body composition assessments were performed by trained professionals using the standardized SAYCARE Study methodology [17]. Such assessments were conducted in a reserved room within each school, with children wearing lightweight and barefoot clothing (no socks). The variables analyzed, according to the reference manual of anthropometric standardization of the World Health Organization [18], were:

Weight: Measured with an ultra Slim W801 digital scale, with a maximum capacity of 150 kg, and an accuracy of 0.1 kg (Crivitta Diagnostica Ltd., São Paulo, Brazil).

Height: Measured with a wall stadiometer with a millimeter scale (Cardiomed, Paraná, Brazil).

Waist circumference: Measured with a flexible and inextensible tape (in centimeters) positioned between the narrowest point between the last rib and the upper anterior iliac spine.

Measurements were repeated twice, and a third measurement was performed only in case of error higher than 5% between the first and second measurements. Weight and height were used to calculate body mass index (BMI)—weight (kg) divided by height (meters) squared—and it was classified according to the criteria defined by Cole et al., 2007 [19].

3.5. Subjective Dietary Assessment Methods

In order to assist in the estimation of food portions, a food booklet was developed for the SAYCARE Study, with photos of commonly consumed foods (including specific foods from each center participating in the SAYCARE Study) and their standard portion sizes [20]. There are many units of measurement in this food booklet (e.g., spoons, glasses, plates, bowls, bottles, cans), and all have been standardized for the SAYCARE Study. For example, fresh fruit (medium portion 120 g): Orange, apple, banana; fresh fruit juice (with or without sugar and medium portion defined in glasses); raw vegetables (medium portion 50 g): Carrots, beets, tomatoes, cucumbers; Boiled vegetables (medium portion 50 g): Carrot, eggplant, pumpkin, broccoli, cassava, yam; Vegetable soup (average portion of 130 mL). Both FFQ and 24 h-DR (described below) were carried out with the support of this food booklet (delivered with the questionnaires).

3.5.1. Food Frequency Questionnaire (FFQ)

More details on the development and validity of the semi-quantitative FFQ designed for the SAYCARE Study [20] and its validity were described elsewhere [21]. The SAYCARE FFQ refers to usual consumption from the last three months. It was developed considering the national lists of foods commonly consumed by children in each center, and classified according to the food groups from FAO (Food and Agriculture Organization of the United Nations). It consists of a list of 56 basic foods common to SAYCARE participating centers and includes some typical foods, totaling in 67 food groups for São Paulo, for example. Similar to the food booklet, the fruits and vegetables were questioned separately in four different groups such as fresh fruit, fresh fruit juice, raw vegetables, boiled vegetables, and vegetable soup. These groups were defined in nine frequencies of intake, as following: (1) Never/less than once a month; (2) 1–3 times per month; (3) once a week; (4) 2–4 times per week; (5) 5–6 times per week; (6) once a day; (7) 2–3 times per day; (8) 4–5 times per day; and (9) 6 or more times per day [20]. In addition, it was questioned whether the research participant consumed any type of supplement with vitamin A, C, and/or E.

The number of portions of fruits and vegetables consumed was calculated in grams per day, by the sum of the frequency of the fruits and vegetables specified in the FFQ, transformed into frequency of daily consumption, and multiplied by the size of the portion consumed.

For example, if the parent/guardian answered that his/her child usually eats one portion of fruit per day and one portion of vegetables 2–3 times per day, the total fruit and vegetable daily intake was calculated as [fruit: 80 (medium portion) $\times$ 1 (once a day) = 80 g/day] + [vegetable: 50 (medium

portion) $\times$ 2.5 (2–3 times per day) = 125 g/day]. Therefore, the total fruit and vegetable daily intake were (80 + 125) 205 g per day.

3.5.2. 24-h Dietary Recall (24 h-DR)

The 24 h-DR is an open questionnaire on all food consumed in the previous day, at all times and meals, and it was answered three times with 15 days-interval among them (2 non-consecutive weekdays—by trained nutritionists, 1 weekend day—by parents/guardians, with the help of the child, and with the instructions provided).

The intake of foods and beverages reported in the 24 h-DR in home measures were calculated in standard measures (grams or milliliters), with the assistance of the Table for Evaluation of Food Consumption in Domestic Measures [22]. Two research nutritionists (TSC and TRU) with fluent comprehension, speaking and writing in Portuguese and Spanish languages translated the food items from the 24 h-DR in Portuguese to Spanish because the food software used in this study is in the Spanish language. This software was developed to evaluate energy and nutrient intake from Ibero-American food consumption surveys. The food composition databases included in it are as follows: Spanish Food Composition Database (Bedca); National Nutrient Database for Standard Reference Release (USDA); Ibero-American Foods Database. Moreover, some typical Brazilian foods were inserted into this software, considering the Brazilian Food Composition Table (TACO) [23]. Therefore, data from 24 h-DR were entered in the software, and tabulated in an Excel spreadsheet, for further analyzes in multiple source method (MSM). The MSM combines information of frequency of intake (from the FFQ) and the mean intake (from the 3 days of the 24 h-DR) to better predict usual dietary intake [24]. The MSM program is a web-based tool with open access at the website <https://nugo.dife.de/msm/>. At the MSM website, we uploaded an Excel spreadsheet with a structure suitable for MSM analysis, including the habitual frequency of fruit and vegetable daily intake (from the FFQ), and the fruit and vegetable intake (in grams per day) from each of the three days of the 24 h-DR. We defined the MSM Model structure by specifying the response variable as the 24 h-DR, and we defined the MSM regression model using the variable “sex”.

We considered all fruits, fresh fruit juices, vegetables, vegetables soup and tomato sauce in the fruit and vegetable intake, in both dietary assessment methods.

3.6. Blood Processing and Analyzes

Families and children were instructed to keep the research participant fasted (10 h) for blood collection. A snack was offered to the child after the blood examination. Experienced nurses collected 10 mL of blood in the antecubital vein, which were distributed in three tubes (EDTA, heparin, serum).

The blood samples of complete blood count and total cholesterol were sent to the central laboratory of the Clinical Hospital from Medical School/University of São Paulo (USP) for processing and analyze, in the following order: (1) Collection of tube with coagulation accelerator; (2) rest (10 min) at room temperature; (3) centrifugation (15 min at 3000 rpm); (4) serum storage at -20°C [17]. The requirements for pre-analytical and analytical stages are standardized following the guidelines of the World Health Organization [25] and the Clinical Laboratory Standards Institute [26], to diminish errors and variability. Total cholesterol was classified following the Brazilian consensus for the normalization of laboratory determination, 2016 [27], and the c-reactive protein was classified according to Brazil, 2007 [28].

For plasma vitamin analysis, we used the following biomarkers: Retinol, β -carotene, ascorbic acid, and α -tocopherol. Such vitamins are highly sensitive to light, and ascorbic acid is a particularly labile vitamin [29]. Thus, these analyses require a specific methodology and adapted to the conditions, mainly, of light and temperature. Therefore, we performed a careful and sophisticated blood examination only in São Paulo, because it was the only center from the SAYCARE study with a laboratory expert in this type of analysis—the Micronutrient Laboratory of the School of Public Health of the University of São Paulo (FSP/USP). It is important to emphasize that the blood examination was done at schools, with the tubes coated with aluminum paper to protect from light, and the blood samples were immediately

transported by a company specialized in the transport of biological samples (in adequate conditions of light and temperature), in a maximum time period of 40 min from the schools to the laboratory for analysis, in order to minimize the oxidation of vitamins. These samples were stored in its tube, protected from light, at -20°C (for one month), and later at -80°C . All analyses were performed in duplicate, using high-performance liquid chromatography (HPLC). The liquid chromatography equipment used was an LC-20AT HPLC system (Shimadzu, Inc., Chiyoda-Ku, Tokyo, Japan), equipped with an SIL-20AC automatic injector, CBM-20A controller, CTO-20A column oven (40°C), and SPD-M20A diode array detector, using a Luna C18 separation column (250.0×4.6 mm, $5\text{ }\mu\text{m}$ particle size; Phenomenex, Torrance, CA, USA).

Plasma concentrations of retinol, β -carotene, and α -tocopherol were assayed using the methodology proposed by Arnaud et al. (adapted) [30], with $400\text{ }\mu\text{L}$ of plasma collected and stored in the absence of light (coated with aluminum paper). Retinol concentrations were detected at a retention time of 2.5 min; β -carotene in 6.7 min; and α -tocopherol in 4.5 min. Thus, the total time of the chromatographic run was 8 min (approximately). The ascorbic acid was analyzed according to the methodology of Robitaille and Hoffer (adapted) [31], with $200\text{ }\mu\text{L}$ of plasma collected and stored in the absence of light (coated with aluminum paper), and analyzed on the same day of thawing. The following cut-off points were considered acceptable for plasma vitamin level if: Retinol $\geq 0.70\text{ }\mu\text{mol/L}$ [32]; α -tocopherol $\geq 16.2\text{ }\mu\text{mol/L}$ [33]; ascorbic acid $\geq 26.1\text{ }\mu\text{mol/L}$ [31].

3.7. Statistics

We used the Stata 14 (Stata Corp., College Station, TX, USA) program for statistical analyses. We performed sensitivity analysis to detect possible selection biases caused by missing values from the FFQ and maternal education level, and no difference was found. After the calculation of fruit and vegetable intake in grams per day for both dietary assessment methods and the calculation of total energy intake in kcal per day for the FFQ, we excluded children who showed a physiologically implausible energy intake (less than 500 or over 4000 kcal/day) in the FFQ [34]. The Shapiro–Wilk test was used to investigate the distribution of variables and the Wilcoxon to test differences between reported intakes between the FFQ and the mean usual intake estimated by the MSM. Our outcomes were the correlation coefficients between the dietary assessment methods and plasma vitamins, which we calculated using multiple linear regression with their corresponding 95% confidence intervals (considering $p < 0.05$ as significance level). The regressions were adjusted based on the literature and tested in the study data. Consequently, the variables that remained in the regression model were sex, total energy intake, total cholesterol (α -tocopherol), and maternal education for the FFQ; and sex, waist circumference, total cholesterol (α -tocopherol), and maternal education for the MSM.

In addition, we calculated the sample-weighted pooled correlation coefficient using the meta-regression analysis, intending to analyze the combination of all plasma vitamins together and its correlation with the group of fruit and vegetable intake, estimated by each dietary assessment methods. As children were from the same sample (no heterogeneity was found among them), we used the fixed model in the meta-regression analysis. We estimated the strength of the agreement of the correlation coefficients cutoff points using the classification for dietary assessment methods: Weak < 0.20 , fair = 0.21 to 0.40 , moderate = 0.41 to 0.60 , good = 0.61 to 0.80 , and >0.80 , very good [35].

Furthermore, we applied the receiver operating characteristics (ROC) curve analysis to calculate the accuracy of the dietary assessment methods with plasma vitamins (as a binary outcome according to the reference for the age group). ROC curve provides the whole spectrum of accuracy, specificity and sensitivity values for all possible cut-off points. We verified the accuracy of both dietary assessment methods using the ROC curve for fruit and vegetable intake as a continuous variable. Moreover, as an explanatory analyses, we investigated the percentage of accuracy within the recommendation established by the American Heart Association for children and adolescents aged 2–19 years [1], regarding the intake of 4 to 5 cups of fruits and vegetables per day. Since this recommendation is wide and there is no agreed definition on what constitutes the number of grams these cups represent, we

considered that each cup is equivalent (on average) to 80 g. Thus, multiplying the cups into grams, we categorized the American Heart Association recommendation of fruits and vegetables for children in minimum (320 g/day), mean (360 g/day), and maximum (400 g/day). Then, ROC curves were run against plasma vitamins separately, and with these vitamins together (combined in a score of plasma vitamins recommendations), to test whether this combination could improve accuracy, since we are validating the fruit and vegetable intake together, and not individually by type of vitamin. As there is no reference for β -carotene levels in plasma, only ascorbic acid, α -tocopherol and retinol were summed up in this score. Children who accomplished one recommendation in plasma levels of the vitamin received one point, and the ones who did not, received zero points. Then, the children who accomplished two or more recommendations in plasma levels of the vitamins were computed as “good plasma levels of the vitamins score”, and the ones who accomplished only one recommendation or none of the recommendations were computed as “insufficient plasma levels of the vitamins score”. The area under the curve (AUC) was determined from plotting sensitivity versus one—the specificity of a test as the threshold varies over its entire range [36], and it to determine the overall accuracy of each dietary assessment methods (AUC ≥ 0.5 was considered to have diagnostic value, the larger the area, the larger the value) [37].

3.8. Funding

The SAYCARE study was mainly supported by the Brazilian Government through the National Council of Technological and Scientific Development (CNPq; proc. 471266/2013-2) and by the São Paulo Research Foundation (FAPESP; proc. 2014/11468-6). This validity study of children’s fruit and vegetable intake using plasma vitamins A, C, and E was financed by FAPESP through Ph.D. scholarship number: 2016/13922-1.

3.9. Ethics

This study was approved by the Research Ethics Committee of Medical School of the University of São Paulo, under research protocol no. 2,022,542. The parental consent form was signed by all the parents/guardians of the research participants, and all children gave verbal agreement to participate in the study.

4. Results

4.1. Participant Characteristics

Of the 60 children (6 to 10 years) who were identified at schools, 57 of them were interested in participating in the study and signed the parental consent form, 55 completed the blood examination, but four of these children did not have enough plasma samples for vitamin analysis. Thus, 51 children completed all plasma vitamins analyses. Of these children, we excluded two children with physiologically implausible energy intake according to the FFQ, and four children were excluded because they showed blood concentrations of c-reactive protein over 10.0 mg/dL (these levels are suggestive of active inflammation/infection that could affect the bioavailability of the vitamins in plasma) [28].

The selection of participating children ($n = 45$) is presented in the flow chart (Figure 1), and their general characteristics in Table 1. Females represent 53.3% of the sample, the mean age was 8.3 years, 33.3% were with overweight or obesity, and 24.4% were classified with high total cholesterol levels. We questioned about supplementation, but no child was taking supplements with vitamin A, C, and/or E.

Figure 1. Flow chart of children (6–10 years) participating in the validity of questionnaires ranking fruit and vegetable intake with plasma vitamins.

Table 1. Characteristics of the subsample of children (6–10 years) from the SAYCARE (South American Youth/Child Cardiovascular and Environmental) study with plasma vitamins. São Paulo, Brazil. 2017.

	n	% or Mean	(95% CI)
Sex (n = 45)			
Female	24	53.3	(38.3–67.8)
Male	21	46.7	(32.2–61.7)
Age in years (n = 45)		8.3	(7.9–8.6)
Body Mass Index (kg/m ²) * (n = 45)			
Underweight	1	2.2	(0.3–15.1)
Healthy weight	29	64.4	(49.0–77.4)
Overweight	10	22.2	(12.1–37.2)
Obesity	5	11.1	(4.5–24.7)
Waist Circumference (cm) (n = 45)		62.1	(59.0–65.3)
Total Cholesterol (n = 45) **			
Healthy (<170 mg/dL)	34	75.6	(60.4–86.2)
High (>170 mg/dL)	11	24.4	(13.8–39.6)
C-reactive protein (mg/dL) (n = 45)		0.8	(0.4–1.2)
School (n = 45)			
Public	24	46.7	(32.2–61.7)
Private	21	53.3	(38.3–7.8)
Maternal Education Level (n = 32) !			
Incomplete high school	3	9.4	(2.9–26.7)
High school	12	37.5	(22.0–56.1)
Technical	1	3.1	(0.4–20.9)
University degree	16	50.0	(32.5–67.5)

95% CI: 95% Confidence Interval. kg/m²: Kilograms per square meter. mg/dL: Milligrams per deciliters. * Classified according to Cole et al., 2007 [19]. ** Classified by the Brazilian consensus for the normalization of laboratory determination, 2016 [27]. ! Missing data.

4.2. Fruit and Vegetable Intake

The mean usual fruit and vegetable intake estimated by the SAYCARE FFQ (261.2 g/day) was similar to the mean intake (301.7 g/day) calculated by the MSM (using the combination of three days of 24 h-DR with the frequency of intake). The difference is that the MSM showed more precise 95% confidence intervals, and the distribution of the fruit and vegetable intake is parametric (Supplementary Figure S1). No significant difference was found neither between the type of schools nor between fruits and vegetables separately. Therefore, the analyses were conducted with both schools together and with fruits and vegetables together, as well (Table 2). Regarding the 24 h-DR, the children/parent/guardian reported 52 different types of fruits and vegetables (Supplementary List S2). The fruit and vegetable intake is presented as a continuous variable in grams per day, and as explanatory analyses, we investigated the percentage of children who accomplished the fruit and vegetable recommendation by the American Heart Association [1] calculated using the MSM, and the recommendations for plasma ascorbic acid [31], α -tocopherol [33] and retinol [32] (Figure 2).

Figure 2. FFQ: Food frequency questionnaire; MSM: Multiple source method (the combination of frequency of intake with 3 days of 24-h Dietary Recall). These prevalences are based on adherence to the following recommendations: Fruits and vegetables [1]; ascorbic acid [31]; α -tocopherol [33]; retinol [32]. Percentages of accomplished recommendations for children's (6–10 years) fruit and vegetable intake and plasma vitamins.

Table 2. Fruit and vegetable intake and plasma vitamins concentration in a subsample of children (6–10 years) from the SAYCARE Study. São Paulo, Brazil. 2017.

	Total		Public School		Private School	
	n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)
Fruit and Vegetable Intake (grams/day)						
FFQ	45	261.2 (195.1–327.3)	21	225.9 (139.4–312.4)	24	292.1 (189.5–394.7)
Mean intake using MSM	45	301.7 (277.2–326.2)	21	309.0 (278.1–340.0)	24	295.3 (256.2–334.4)
Plasma Vitamins (μmol/L)						
Ascorbic Acid	45	80.9 (66.5–95.4)	21	78.8 (64.9–92.8)	24	82.8 (57.5–108.1)
α-tocopherol	45	21.8 (20.2–23.3)	21	21.1 (18.3–23.9)	24	22.3 (20.5–24.1)
Retinol	45	0.94 (0.84–1.03)	21	0.88 (0.76–1.0)	24	0.98 (0.84–1.12)
β-carotene	45	0.32 (0.26–0.39)	21	0.31 (0.24–0.39)	24	0.33 (0.23–0.44)

95% CI: 95% Confidence interval; FFQ: Food frequency questionnaire; MSM: Multiple source method (the combination of frequency of intake with 3 days of 24-h Dietary Recall).

4.3. Correlation Coefficients

Figure 3 presents the correlation coefficients from multiple linear regression for fruit and vegetable intake and each biomarker separately, and all biomarkers together calculated by the meta-regression analysis [Figure 3a for the FFQ; Figure 3b for the MSM (the combination of frequency of intake with 3 days of 24-h dietary recall)].

Figure 3. 95% CI: 95% Confidence interval; FFQ: Food frequency questionnaire; MSM: Multiple source method (the combination of frequency of intake with 3 days of 24-h Dietary Recall). (a,b) Correlation coefficients from multiple linear regression for children's (6–10 years) fruit and vegetable intake estimated by the FFQ versus plasma vitamins (a); the MSM versus plasma vitamins (b).

4.4. Accuracy of Dietary Assessment Methods

The percentage of accuracy from the ROC curves of fruit and vegetable intake estimated by FFQ and by MSM, according to the type of vitamin is shown in Table 3. The fruit and vegetable intake is presented as a continuous variable, and as an explanatory analyses, we investigated the percentage of accuracy within the minimum (320 g), medium (360 g), and maximum (400 g) fruit and vegetable recommendation by the American Heart Association for children and adolescents (2–19 years) [1]. It indicates that when vitamins are combined in a score, the recommendation of 320 and 360 g of fruits and vegetables showed the highest accuracy (67.9%), for the FFQ; and the recommendation of 360 of fruits and vegetables showed the highest accuracy (61.9%), for the MSM.

Table 3. Percentage of accuracy from receiver operating characteristics (ROC) curve of fruits and vegetables daily intake estimated by FFQ and by MSM according to the type of plasma vitamin. São Paulo, Brazil. 2017.

Fruits and Vegetables Daily Intake	Plasma Vitamins			
	Ascorbic Acid	α -Tocopherol	Retinol	Sum of Vitamins (≥ 2 Points)
FFQ, accuracy (%)				
Continuous	47.7	51.5	64.2	-
Minimum (320 g/day)	67.1	50.0	62.7	67.9
Mean (360 g/day)	67.1	50.0	62.7	67.9
Maximum (400 g/day)	63.6	52.8	58.6	64.3
Mean intake using MSM, accuracy (%)				
Continuous	65.9	71.3	30.1	-
Minimum (320 g/day)	72.7	56.9	31.4	56.0
Mean (360 g/day)	61.4	63.9	33.1	61.9
Maximum (400 g/day)	55.7	56.9	34.0	56.0

FFQ: Food frequency questionnaire; MSM: Multiple source method (the combination of frequency of intake with 3 days of 24-h Dietary Recall).

5. Discussion

This study aimed to validate the FFQ and the combination of the frequency of intake from the FFQ with the 24 h-DR (mean of 3 days), both designed for the SAYCARE Study, for children's fruit and vegetable intake. The reproducibility of the SAYCARE FFQ for this food group was previously tested by comparing it against the 24 h-DR [21], with fair agreement in children. Consequently, now we are conducting the validity the SAYCARE FFQ with a superior and objective method, the plasma vitamins. We found pooled correlation coefficients of 0.29 (95% Confidence Interval: 0.20–0.38) and 0.35 (95% Confidence Interval: 0.26–0.43) for the reported fruit and vegetable daily intake estimated by the FFQ and the MSM, respectively. These findings are comparable to other validity studies assessing fruit and vegetable consumption in children [3,38], and also in adolescents [39]. The MSM showed a slight increase in the correlation coefficients for each plasma biomarker separately, and in the pooled correlation coefficient when compared to the correlations estimated by the FFQ. However, all correlation coefficients from multiple linear regression (separately or together) were in the acceptable range according to the literature [35]. Correlations among plasma vitamins and estimates of dietary intake measured by questionnaires are expected to be considerably lower than 1.0, even when fruit and vegetable intake is perfectly assessed because plasma vitamins are also influenced by numerous variations in absorption and metabolism [13]. Thus, it suggests that both FFQ designed for the SAYCARE Study or the combination of 3 days of 24 h-DR with the frequency of intake using the MSM, are valid dietary methods for ranking children's fruit and vegetable intake.

These results are important to support the possibility of using only the FFQ designed for the SAYCARE Study to rank children's fruit and vegetable intake. Consequently, financial and timing

resources can be optimized, since there is no need to apply the 24 h-DR (three times), the presence of a trained nutritionist in the fieldwork to apply the 24 h-DR, or to dedicate labor-intensive time and efforts for analyzing these data from the 24 h-DR and calculating them with the MSM [40].

5.1. Schoolchildren

We were able to reach more children participating in this validity study ($n = 45$) than our sample size calculation ($n = 30$). The distribution of sex and school was similar within the sample. Furthermore, the population from São Paulo is very diverse in cultural and ethnic aspects, and it might also reflect in the variety of fruits and vegetables consumed [41]. On the other hand, 33.3% of children were with overweight or obesity [42], and 24.4% were classified with high total cholesterol levels [27]. These two facts were considered in the analyses (adjustments for waist circumference and total cholesterol).

Children from public school represent 46.7% of the sample; yet, the majority of mothers had a university degree. Even though maternal education was treated as a confounder in the analysis, it is a limitation of our study, as it might have improved the results found. Besides, 13 mothers did not answer this variable, which might bias the results as well [43]. But after sensitivity analysis, no difference was found between them.

5.2. Subjective Dietary Assessment Methods

FFQs are indicated as the most feasible approach for examining long term intakes on hard endpoints, when they are complemented by nutritional related biomarkers, such as plasma vitamins [44]. The SAYCARE FFQ was designed to be culturally adapted to the South American centers participating in the Study and specific to the pediatric population, to be informative regarding some food groups and dietary patterns, but one of its limitations is the food combination (e.g., different types of fruits and vegetables all together) [44]. However, this may not make a significant difference on validity studies ranking fruit and vegetable intake, since the recommendation of this food group is general, and does not require specification of the fruit/vegetable type [1].

While potentially useful and inexpensive, the FFQ is also prone to other limitations and errors. The FFQ developed for the SAYCARE Study is restricted to the consumption of the previous three months, and omissions can occur possibly due to the fixed food list [20]. Moreover, to answer the FFQ it is necessary that the reporter is literate, and to have cognitive and memory skills to estimate the habitual food consumption [45]. Despite these limitations, a food booklet was developed to assist in the estimation of fruits and vegetables portions. Furthermore, a trained nutritionist applied two of the three 24 h-DR, and we used the MSM to combine the 24 h-DR with the frequency of intake from the FFQ, trying to improve the estimates of fruit and vegetable intake [46].

Another limitation is that we considered fresh fruit juices, vegetables soup and tomato sauce in both dietary assessment methods. This might have improved the fruits and vegetables grams, because of the water content in these preparations. However, as we are validating the consumption in school-age children (6–10 years), these preparations are commonly consumed by this age range, especially in São Paulo, where children are culturally accustomed to drinking fresh fruit juice at lunch, dinner, and sometimes also together with a snack.

5.3. Combination of Biomarkers

As the American Heart Association recommends the intake of 4 to 5 cups of fruits and vegetables per day for children, we aimed to validate the dietary assessment methods considering all fruits and vegetables together (as this recommendation informs). Therefore, we decided to analyze the combination of the plasma biomarkers together as well, for two main reasons. Primarily because the combination of fruits and vegetables is a complex food group, and the bioavailability of phytochemicals vary noticeably between types of fruits or vegetables [47], even in the same fruit or vegetable it may vary according to conditions of storage, transport, cooking method and also to content of the nutrient in the geographic region where it was planted [13]. Secondly, many factors might influence the biomarker

response to fruit and vegetable intake, including inter-individual variation in digestion, absorption, and metabolism [13]. Some of this variation might be related to genetic factors, but environmental factors such as body composition also influence the concentration of the biomarkers [48]. That is the main reason for adjusting the analyses for waist circumference since it correlates better with central adiposity in the body. Thus, other studies also suggested that the combination of biomarkers enhanced the prediction of fruit and vegetable intake [29,47], because, at this moment of validity study, we don't require a complete understanding of the plasma vitamins individually, but whether their overall combination (including the interrelationships cited above) gives a complementary information to validate the questionnaires ranking children's fruit and vegetable intake [44].

5.4. Accuracy of Dietary Assessment Methods

Surprisingly, almost 80% of the sample accomplished the recommendations for plasma vitamins. However, mean fruit and vegetable intake were below the recommendation (320 to 400 g per day) [1] in both dietary assessment methods. The accurate assessment of children's fruit and vegetable intake is challenging, and questions remain regarding the best quantity of fruits and vegetables that children should eat daily [29]. For example, Figure 2 shows that when we considered the minimum recommendation (320 g/day), only 33% and 44% of participants reached this reference when estimated by the FFQ and the MSM, respectively. These percentages decreased for the maximum recommendation (400 g/day), in both dietary assessment methods. It indicates that both methods might be used to rank the children's fruit and vegetable intake, but not to translate it into absolute quantities of intake. Another possible explanation for the difference between lower percentages of fruit and vegetable intake and higher percentages of plasma vitamin levels is that many food products for children are fortified with vitamins [38], and even though children are not eating the recommended amount of fruits and vegetables, their plasma vitamins levels are mostly adequate due to vitamin fortification of commercial food products [13,40]. A study carried out in Finland to assess the relationship between children's fruit and vegetable intake and blood biomarkers also found that the high consumption of fortified foods reflected higher serum carotenoids levels [38]. Furthermore, a good vitamin profile in plasma might also reflect a better dietary variety and quality [49].

Moreover, there is no universally agreed upon recommendation for fruit and vegetable intake specifically for children from 6 to 10 years of age. Even though the American Heart Association targets children and adolescents (2–19 years), its fruit and vegetable recommendation (4–5 cups a day) [1] is different than the recommendation from the USDA for children between 4–8 and 9–13 years of age (2.5 to 5 cups a day) [50]. In addition, the absence of an average portion size for fruit and vegetable that is appropriate for children is an important limitation to translate these recommendations into grams per day.

5.5. Adjustment for Confounders

As previously mentioned, numerous factors may influence the assessment of fruit and vegetable intake with subjective methods (e.g., socially desirable answers and errors in food composition databases) [40] and also the concentration of vitamins in plasma (e.g., genetic, environmental, lifestyle, homeostatic) [29]. Thus, it is extremely important that studies of validity of children's fruit and vegetable intake using plasma vitamins adjust the analyzes for confounders [51]. Dietary assessment questionnaires for children younger than 10 years of age are usually answered by parents [4], therefore maternal education is a paramount adjustment, especially in low and middle-income countries [43]. Waist circumference is also useful, once it is an indicator of central adiposity that may influence the bioavailability of vitamins in plasma [13]. Moreover, α -tocopherol (precursor of vitamin E) should be adjusted for blood cholesterol, because vitamin E is transported in blood lipoproteins and is strongly related to blood cholesterol [13]. Finally, even though in our sample we did not find differences between sex, it is crucial that studies adjust for sex due to different behaviors between girls and boys [3].

5.6. Strengths and Limitations

As far as we know, this is the first study validating usual fruit and vegetable intake in children from middle-income countries, such as Brazil. One limitation of our study is that we were not able to include the β -carotene in our accuracy analyzes, because there is no reference value for β -carotene in blood. The β -carotene and retinol are precursors of vitamin A, and they are important for health, growth, and development, as vitamin A deficiency can be associated with increased risks of xerophthalmia, low bone mineral density, immune dysfunction, infectious morbidities and also mortality in children [52]. Therefore, it is crucial that researchers conduct an explicit, objective, and transparent study to define reliable β -carotene reference values [52]. Also, we suggest that more studies need to verify whether the reference for fruit and vegetable intake should be the same for all populations age, since children have significantly different necessities and behaviors than adults or elders, for example.

6. Conclusions

Our findings suggest that the FFQ designed for the SAYCARE Study is a useful tool for ranking children's fruit and vegetable intake with acceptable accuracy, and with relative precision level as the MSM (the combination of frequency of intake with the 3 days of 24 h-DR) when compared to plasma vitamins. Furthermore, both methods showed to be valid tools for ranking fruit and vegetable intake in children between 6 to 10 years from the SAYCARE Study.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2072-6643/11/8/1815/s1>, Figure S1: Fruits and vegetables intake distribution calculated by the multiple source method (the combination of 3 days of 24-h Dietary Recall with the frequency of intake) in children. List S2: Different types of fruits and vegetables reported at the 24-h Dietary Recall.

Author Contributions: T.S.C., A.C.F.D.M., and H.B.C. were responsible for the study concept and study design. P.H.d.C.R. and L.A.L. performed the vitamins laboratory analyses. T.S.C., A.C.F.D.M., and T.R.-U. executed statistical analyzes and interpreted the data. T.S.C. drafted the manuscript. T.S.C., A.C.F.D.M., D.M.L.M., and H.B.C. supervised the study. All authors participated in the writing of the paper, provided comments on the drafts, and approved the final version of the manuscript. All authors agree to be personally accountable for the author's own contributions and ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.

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The validity of a food frequency questionnaire and 24-hour-dietary-recall to assess dietary iron intake in children and adolescents from the SAYCARE study

Tatiana Sadalla Collese, Augusto César F. De Moraes, Tara Rendo-Urteaga, Laura I Gonzalez-Zapata, Diego Alejandro Gaitán Charry, Carlos A. Delgado, Gabriela Berg, Francisco Leonardo Torres-Leal, and Heráclito B. Carvalho

ABSTRACT

Background A food frequency questionnaire (FFQ) for South American children and adolescents was developed, but its validity for dietary iron intake has not been evaluated.

Objective To evaluate the validity of the FFQ and 24-hour-dietary-recalls (24h-DR) for dietary iron intake in children and adolescents.

Design The SAYCARE (South American Youth/Child Cardiovascular and Environmental) is a multicenter observational study, conducted in five South American cities: Buenos Aires (Argentina), Lima (Peru), Medellin (Colombia), São Paulo and Teresina (Brazil). The FFQ was filled out over the previous three months, and the 24h-DR was applied three times (two weekdays and one weekend day). Mean total iron intake was calculated using the Multiple Source Method (MSM). Blood was collected to assess serum iron, ferritin, and hemoglobin.

Participants/Setting Data of 99 children (3-10 years) and 50 adolescents (11-17 years) from public and private schools were collected in 2015-2017.

Main outcome measures Dietary iron intake calculated from the FFQ and 24h-DR.

Statistical analyses performed Dietary iron intake in relation to blood biomarkers were assessed using multilevel linear regression with fixed intercepts and receiver operating characteristics (ROC) curve analysis.

Results Multilevel regressions showed very good correlation coefficients (range 0.81-0.87) for the FFQ; and weak correlation coefficients (range 0.12-0.18) for the MSM. The accuracy ranged from 49.0-70.6% for the FFQ; and from 38.5-58.9% for the MSM.

Conclusion The SAYCARE FFQ exhibited good ability to rank total dietary iron intake in children and adolescents, and it is recommended instead of the MSM in this study population.

Keywords children; iron; food frequency questionnaire; 24-hour dietary-recall; validity

INTRODUCTION

Iron is a key mineral for hemoglobin function.¹ The primary nutritional role of iron is to support red blood cell production. Childhood growth involves increased red blood cell demand, which enhances iron requirements, thereby increasing the need for extra dietary iron intake.¹ Diet-related iron deficiency occurs when dietary intake is insufficient to cover iron demands (e.g. poor diet or malabsorption), especially during periods when iron requirements are higher, e.g. child development.²

Iron deficiency is indicated by the World Health Organization (WHO) as one of the main diet-related health risks worldwide.² For example, if iron deficiency is untreated, it may progress to anemia (approximately 42% of childhood anemia are related to iron deficiency), and reduced cognitive and motor development outcomes in children². Anemia is a significant public health problem in pediatric populations, mainly (but not exclusively) in low-middle income countries³, including South America. The global anemia prevalence in children (6-59 months) is estimated at 42.6%, which corresponds to 273.2 million affected children.⁴ In Latin America and Caribe, this prevalence is estimated at 29.1%, which represents 15.5 million children with anemia.⁴ In addition, 48% of children and adolescents (5-14 years) from developing countries have anemia.⁵ Adolescents (10-19 years) are also at higher risk of iron deficiency and anemia, due to accelerated increases in iron requirements (for growth and pubertal development) and inadequate dietary iron intake.⁶

This high prevalence of iron deficiency and anemia increases the risk of death, morbidity and susceptibility to avoidable diseases. Thus, reducing iron deficiency is a target of the United Nations' Sustainable Development Goals (SDG) by 2030, especially for low-middle income countries.⁷

Iron intake assessment in childhood is necessary to prevent and control its deficiency. Moreover, iron intake assessment might have a different role in different countries (e.g.

depending on the income of the country, ethnic background, socioeconomic status, dietary patterns, iron supplements).⁸ Food frequency questionnaires (FFQ) can be used to assess dietary iron intake,⁹ and blood concentrations of iron, ferritin and hemoglobin can be used as biochemical indicators of iron status.^{9,10}

Despite the importance of dietary iron intake, limited studies have evaluated the validity of this consumption in pediatric populations from South America. In this context, we sought to evaluate the validity of an FFQ and a 24-hour Dietary Recall (24h-DR) (mean of three days), designed for the SAYCARE (South American Youth/Child Cardiovascular and Environmental) study, to rank dietary iron intake in children and adolescents, using blood biomarkers (serum iron and ferritin) as a reference method.

MATERIALS AND METHODS

Study design

This study was conducted according to guidelines for authors when validating dietary assessment methods (Supplementary Material 1),¹¹ and is part of the SAYCARE, multicenter observational study. The SAYCARE aimed to develop valid and reliable instruments to generate information about social and environmental factors, family environments, food intake and behaviors, physical activities and sedentary behaviors, body composition, sleep, oral health and cardiovascular health biomarkers in children and adolescents from South America.¹² Further details on study sampling, recruitment, and quality control elements were published elsewhere,¹² but the methods regarding this validity study are briefly described below.

Participants

This study was conducted in children and adolescents (aged 3-17 years), from the following SAYCARE centers: Buenos Aires (Argentina), Lima (Peru), Medellin (Colombia),

São Paulo and Teresina (Brazil), where blood analysis was carried out. Study sample calculations were based on studies investigating dietary assessment methods in pediatric populations.^{13, 14} These calculations accounted for a two-tailed error of 0.05 (type I), error β of 0.20 (error type II) and a correlation coefficient of 0.41¹⁴, resulting in 44 children and 44 adolescents, which meant 88 participants. Additionally, a *post-hoc* analysis was conducted, considering the two-tailed error of 0.05 (type I), error β of 0.20 (error type II) and a correlation coefficient of 0.76 (the lowest correlation coefficient found for the FFQ, with biomarkers), resulting in 22 participants (11 children and 11 adolescents).

Eligibility criteria

The selection of children (n = 99) and adolescents (n = 50) is presented (Figure 1). The inclusion criteria were children and adolescents (aged 3-17 years) enrolled in the selected schools, with a consent form signed by parents/guardians. Children/adolescents in a fasting status (10–12 hours) at blood sampling, with information on sex, age, height, weight, FFQ, and a minimum two days of 24h-DR. The exclusion criteria were inability to answer questionnaires, pregnancy, physiologically implausible energy intake for the age (less than 500 or more than 4,000 kcal per day for children¹⁴, and less than 800 or more than 4,500 kcal per day for adolescents^{14, 15}) estimated by the FFQ, and children/adolescents using iron supplements.

Dietary assessment methods

The daily dietary iron intake from the FFQ and 24h-DR was classified using estimated average requirements, from the Institute of Medicine; Panel on Micronutrients, (2001)¹⁶ as described (Table 1).

A food booklet in paper color was created for the SAYCARE study, with photos of commonly consumed foods (including regional foods from participating centers), and standard portion sizes for young children.¹⁷ Measurement units were included in this booklet

(e.g. spoons, glasses, plates, bowls, bottles and cans), and were standardized by an expert consensus.¹⁷ For example, cooked meat (i.e. beef, chicken, pork) and fish (medium portion; 100 g), eggs (average portion; 50 g), cereals (i.e. rice, pasta, bread, crackers - medium portion; 50 g), and legumes (i.e. beans, lentils and chickpeas – medium portion; 80 g). The questionnaire's answers were correlated with a photo of the specific food group in the food booklet, which could be half a portion, one, two, three, or four portions or more. The FFQ and the 24h-DR (described below) were completed using this food booklet, which was distributed with questionnaires.

Food Frequency Questionnaire

The SAYCARE FFQ is a paper questionnaire that refers to children or adolescent habitual food intake from the last three months. It was completed by the children's parents or guardians, or by the adolescents themselves. This FFQ was based on national food lists, commonly consumed by pediatric populations in each center, and was classified according to food groups from the Food and Agriculture Organization (FAO) of the United Nations.¹⁸ This FFQ comprised a core list of 56 basic foods, common to all participating centers. Also, some typical/regional foods were included, totaling 63 food items for the Buenos Aires FFQ, 61 for Lima, 63 for the Medellin FFQ, 67 for Sao Paulo and 69 items for Teresina.¹⁷ Further information on the development of the SAYCARE FFQ¹⁷ and its reliability and validity have been cited.¹⁹ In the study by Rendo-Urteaga *et al.*¹⁹, two FFQs (with 15 days interval) were assessed for reliability (Spearman's coefficients ranged from 0.47–0.73) and validity (energy-adjusted Pearson's coefficients ranged from 0.17–0.61), for food group intake when compared with 24h-DR in children and adolescents. In this study, we investigated the first FFQ because it showed better response rates than the second.

Similar to the food booklet, the FFQ had separate questions in the main eleven food groups, such as cereals; tubers; vegetables; fruits; oils; meats, fish, and eggs; milk and dairy

products; legumes; beverages; sugar products; and miscellaneous. These groups were apportioned to nine intake frequencies, according to Willett¹⁵: 1) never/less than once a month; 2) 1–3 times per month; 3) Once a week; 4) 2–4 times per week; 5) 5–6 times per week; 6) Once a day; 7) 2–3 times per day; 8) 4–5 times per day; 9) 6 or more times per day. Also, the FFQ questioned whether research participants consumed any nutritional supplements (e.g. vitamins and/or minerals).

The number of food portions consumed was calculated as g/day, using the sum of the intake frequency specified in the FFQ, transformed to the daily intake frequency, multiplied by the size of the portion. For example, if the FFQ showed a child/adolescent usually ate one portion of meat per day, and one portion of beans twice a day, the daily intake was calculated as; [meat: 100 g (medium portion) $\times$ 1 (once a day) = 100 g/day] + [legumes: 80 g (medium portion) $\times$ 2 (twice a day) = 160 g/day]. Likewise, this calculation was applied to all eleven food groups in the FFQ. To rank dietary iron intake, we multiplied the weight (g) of each consumed food by the mean iron content of the food/food group, using the Spanish Food Composition Database (BEDCA) (2010),²⁰ the National Nutrient Database for Standard Reference Release 28 (USDA) (2015),²¹ and the Brazilian Food Composition Table (TACO) (2011).²²

24-hour Dietary Recall

The 24h-DR is an open questionnaire on all foods consumed the previous day, at all times and meals. It was a paper questionnaire, including instructions on how to fill in house-measures and food types (e.g. whole bread, skimmed milk and pasta sauces). The 24h-DR was applied on three non-consecutive days (one weekday by a trained nutritionist/dietitian, one weekday and one weekend day by the children's parents or guardians; or by the adolescent themselves).

Food and beverage consumption in the 24h-DR and in-home measures were calculated in g or ml, using the Table for Evaluation of Food Consumption in Domestic Measures²³, and inputted into a food web-based software, version 2015. This software evaluates energy and nutrient intake from Ibero-American food surveys, and includes food composition databases from BEDCA (2010)²⁰ and USDA (2015).²¹ Moreover, some typical/regional foods from each SAYCARE center were added to the software, considering their respective national food composition tables. Thereafter, results from each day of the 24h-DR were tabulated in Excel, to reflect total kilocalorie intake per day, and total iron intake (mg/day). This approach facilitated data organization and enabled analysis using the Multiple Source Method (MSM), which deals with intra-individual variability between consumption days. The program combined information from the three day 24h-DR, and calculated mean daily iron intake for each child or adolescent, to predict dietary iron intake.²⁴ This was a multistep method that estimated habitual iron intake distribution by estimating the probability (step 1) and the amount (step 2) of consumption. Therefore, step 3 was generated by multiplying step 1 (consumption probability) by step 2 (consumption amount) to estimate child/adolescent daily iron intake.²⁵ The MSM program is an open access web-based tool, available at; <https://nugo.dife.de/msm/>.

Blood Analyses

Parents/guardians were instructed to maintain child/adolescent overnight fasting (10–12 hours) prior to blood collecting. In a reserved schoolroom, experienced personnel collected ~10 ml blood from the antecubital vein into Vacutainer tubes (Becton Dickinson, UK) containing ethylenediamine-tri-potassium (EDTA K3, lilac cap) for hematological determination. Tubes with a gel separator without additives were used for clinical chemistry determination (red cap).²⁶

These tubes were placed in racks in a vertical upright position, and placed at 4°C. These boxes were transported to the laboratory for blood processing and analysis, as follows: 1) Collection of tube with coagulation accelerator; 2) Rest (10 min) at room temperature; 3) Centrifugation (15 min at 3000 rpm); 4) Serum storage at -20°C.¹² Pre-analytical and analytical stages were standardized according to WHO guidelines²⁷ and guidelines from the Clinical Laboratory Standards Institute,²⁸ to diminish errors and variability. More details about blood processing can be found elsewhere.²⁶

Statistical Analyses

The Stata program, Ver. 14 (Stata Corp., College Station, TX, USA) was used for statistical analyses. They were conducted separately for children and adolescents. For descriptive analyses, percentages, means, standard deviation, medians and interquartile ranges were used. Sensitivity analyses were performed to detect possible selection bias caused by missing values from the FFQ, and maternal education level in children. The Shapiro-Wilk test was used to investigate the distribution of dietary iron intake estimated by the FFQ and the MSM. The Wilcoxon test assessed for differences between the sexes when reporting dietary iron intake (i.e. using FFQ and MSM).

Correlation coefficients between dietary assessment methods and blood biomarkers were calculated using the hierarchical conceptual model. A potential confounder was retained in the multivariate model, if the significance level in the unadjusted analysis was < 0.2 , or if there was a 10% change in variance estimations. The exception was the study center; this variable was retained in all adjusted models based on its influence on dietary intake. In multivariate analyses, potential confounders were allocated according to the following sequence: Model 0: study center; Model 1: Model 0 plus age and sex; Model 2: Model 1 plus total energy intake.

A multilevel regression model with fixed effects was used after applying the Durbin–Wu–Hausman test. Regressions were adjusted by sex, age, total energy intake (for the FFQ), maternal education (for children only, because their parents/caregivers completed the questionnaires), school (for adolescents), and the study center. The strength of agreements with correlation coefficients cutoff points were estimated using classifications for dietary assessment methods: weak < 0.20, fair = 0.21–0.40, moderate = 0.41–0.60, good = 0.61–0.80, and very good > 0.80.²⁹ Correlation coefficients between dietary assessment methods and blood biomarkers of ≥ 0.5 were considered good evidence that our dietary assessment method adequately ranked participant dietary iron intake.³⁰

Furthermore, receiver operating characteristic (ROC) curve analyses were used to calculate the accuracy of dietary assessment methods with blood biomarkers (binary outcomes according to the reference for the age group). Reference values in this study came from the Institute of Medicine (2001)¹⁶ for estimated average requirements (EAR), and from WHO (2001)⁵ for blood biomarkers (Table 1). ROC curves provided a spectrum of accuracy, specificity and sensitivity values for all possible cut-off points.³¹ The accuracy of both dietary assessment methods were verified using ROC curve analysis, with dietary iron intake as a continuous variable. The area under the curve (AUC) was performed by plotting sensitivity versus 1 - specificity³². To determine the overall accuracy of each dietary assessment method, the AUC > 0.5 was considered to have diagnostic value, ≥ 0.75 was good accuracy, and > 0.9 excellent, assuming that the larger the AUC, the better the diagnostic ability of the test.³³

Ethics

This study was approved by the Medical School Research Ethics Committee of the of the University of São Paulo (Brazil), under research protocol no. 2022542, and also by the other participating centers; Buenos Aires (Argentina), no. CUDAP EXP-UBA: 54555/14,

Lima (Peru) no. 00222-CEI-INSN-2015, and Medellin, (Comité de Bioética para Investigación en Humanos – Universidad de Antioquia, Colombia), no. 14-43-596. Consent forms were signed by all parents/guardians on behalf of participants. The study was performed in accordance with the ethical guidelines of the Declaration of Helsinki, and of the World Medical Association for medical studies in humans.

Table 1. Reference values of iron estimated average requirements and biochemical indicators of iron status for children and adolescents.

Reference values for Children and Adolescents			
Iron Estimated Average Requirements^a (mg/day)	Serum Iron^b (µg/dl)	Serum Ferritin^b (µg/l)	Hemoglobin^b (g/l)
4.1 (≤ 8 years)	45	12 (< 5 years)	110 (< 5 years)
5.7 (girls 9-13 years)		15 (≥ 5 years)	115 (≥ 5-11 years)
7.9 (girls 14–18 years)			120 (12-14 years)
5.9 (boys 9–13 years)			120 (girls ≥ 15 years)
7.7 (boys 14–18 years)			130 (boys ≥ 15 years)

Mg/day: milligrams per day; µg/dl: micrograms per deciliter; µg/l: micrograms per liter; g/l: grams per liter.

^a Institute of Medicine, 2001¹⁶

^b World Health Organization, 2001⁵

RESULTS

Participant characteristics

As shown (Figure 1), of the 1,112 children (3–10 years), and 495 adolescents (11–17 years) with signed consent forms, 691 children and 220 adolescents completed blood analysis, but only 175 children with serum iron and ferritin values, and 151 adolescents with serum iron and ferritin remained. Of these, we excluded 76 children and 101 adolescents due to missing values on sex, age, weight or height, had physiologically implausible energy intakes according to FFQs, and/or had < two 24h-DR days. We questioned participants about supplementation, but none were using iron supplements.

Figure 1. Flow diagram of the selection of children and adolescents from the SAYCARE study to evaluate the validity of a food frequency questionnaire and 24-hour dietary recalls in assessing dietary iron intake in comparison to blood biomarkers, 2015-2017

^aFFQ: Food Frequency Questionnaire; ^b24h-DR: 24 hour Dietary Recall

The general characteristics of the 149 participants (99 children and 50 adolescents) are described (Table 2). Females accounted for 54.5% and 44% of children and adolescents, respectively. Approximately 30% of children and adolescents were overweight or obese. We performed sensitivity analyses for maternal education, because 58.6% of children's mothers, and 34% of adolescent's mothers left this question blank, but no difference was observed between groups. The majority of mothers who filled out this question had a university degree (61% for children and 78.8% for adolescents).

Table 2. Characteristics of the subsample of children (3-10 years) and adolescents (11-17 years) from the SAYCARE Study. 2015-17

	Children (n=99)			Adolescents (n=50)		
	n	Percentage	Mean (SD)	n	Percentage	Mean (SD)
Age in years	99		7.5 (1.9)	50		15.2 (2.0)
Sex (Female)	54	54.5		22	44.0	
Body Mass Index (kg/m ²) ^a						
Underweight	6	6.0		2	4.0	
Healthy weight	65	65.7		34	68.0	
Overweight	20	20.2		13	26.0	
Obesity	8	8.1		1	2.0	
Waist Circumference (cm)	74		58.2 (7.0)	50		73.3 (8.0)
MCV (fL)	58		79.8 (7.5)	46		86.6 (4.8)
C-RP (nmol/L)	67		57.1(0.9)	40		28.6 (0.3)
School						
Public	45	45.5		17	34.0	
Private	54	54.5		33	66.0	
Maternal Education Level						
< High school	5	12.2		3	9.1	
High school	10	24.4		4	12.1	
Technical	1	2.4		--	--	
University degree	25	61.0		26	78.8	

SD: Standard Deviation; kg/m²: kilograms per square meter; cm: centimeters; MCV: Mean Corpuscular Volume; fL: femtolitre; C-RP: C reactive protein; nmol/L: Nano mol per liter.

^aBody Mass Index was classified by Cole et al., 2007¹⁶

Dietary iron intake

The FFQ food group that included meat, fish or eggs was the main contributor of dietary iron intake, representing more than 30% of total dietary iron intake in both age groups (Table 3). Cereals was the second contributor, followed by legumes and then vegetables. Oil groups were practically null contributors for total dietary iron intake.

Table 3. Food groups from the food frequency questionnaire according to their median contribution to dietary iron intake in a subsample of children (3 – 10 years) and adolescents (11-17 years) from the SAYCARE Study. 2015-17

Food Group from the Food Frequency Questionnaire ^a	Children (n=99)	Adolescents (n=50)
	Median (mg/day) (interquartile range)	Median (mg/day) (interquartile range)
Meat, fish and egg	3.0 (2.0 – 4.6)	4.6 (3.0 - 7.2)
Cereals	2.2 (1.4 - 3.1)	3.1 (2.1 - 4.5)
Legumes	0.7 (0.5 - 1.4)	0.9 (0.4 - 2.2)
Vegetables	0.6 (0.3 - 1.1)	0.6 (0.2 - 1.7)
Milk	0.5 (0.3 - 1.2)	0.5 (0.3 – 1.1)
Fruits	0.4 (0.2 - 0.9)	0.4 (0.1 - 0.7)
Sugar products	0.3 (0.2 - 0.6)	0.2 (0.1 - 0.5)
Beverages	0.3 (0.1 - 0.6)	0.2 (0.1 - 0.5)
Tubers	0.1 (0.0 - 0.2)	0.2 (0.1 - 0.5)
Miscellaneous	0.0 (0.0 - 0.1)	0.1 (0.0 - 0.1)
Oils	<0.01	<0.01
Total dietary iron intake	9.8 (7.6 – 13.1)	12.6 (10.6 – 17.5)

Mg/day: milligrams per day.

^a Food groups listed in descending order.

The median dietary iron intake from FFQ was similar to dietary iron intake calculated by MSM (combination of three days of 24h-DR) (Table 4). No differences were observed

between sexes (for both age groups and dietary assessment methods) while reporting dietary iron intake, therefore results are presented for both sexes.

Table 4. Dietary iron intake and concentration of blood biomarkers in a subsample of children (3 – 10 years) and adolescents (11-17 years) from the SAYCARE Study. 2015-17

	Children (n=99)	Adolescents (n=50)
	Median	Median
	(interquartile range)	(interquartile range)
Iron intake (mg/day)		
Food Frequency Questionnaire	9.8 (7.6 – 13.1)	12.6 (10.6 – 17.5)
24-hour Dietary Recall ^a	10.0 (9.0 - 11.7)	12.0 (10.3 – 14.8)
Blood biomarkers		
Iron (µg/dl)	85.0 (72.0 – 108.0)	102.5 (76.8 - 129.3)
Ferritin (µg/l)	55.0 (36.1 – 80.4)	54.9 (36.1 – 87.1)
Hemoglobin (g/l)	133 (127 - 140)	135 (126 – 141)

Dietary Recall (the combination of three days using Multiple Source Method); mg/day: milligrams per day; µg/dl: micrograms per deciliter; µg/l: micrograms per liter; g/l: grams per liter.

For explanatory analyses, Figure 2 shows the proportion of children and adolescents who accomplished the recommendations for dietary iron intake and blood biomarkers, as described in Table 1¹⁶. Most participants (> 80%) achieved iron intake average requirements (independent of the type of dietary assessment method) and blood biomarkers reference levels.

Figure 2. Percentages of children and adolescents accomplishing dietary iron estimated average requirements and blood biomarkers recommendations.

Percentages (95% Confidence Interval); FFQ: Food Frequency Questionnaire; 24h-DR (the combination of three days of 24-hour Dietary Recall).

Dietary iron estimated average requirements were classified according to the Institute of Medicine, 2001.¹⁷

Blood biomarkers were classified according to the World Health Organization, 2001.⁵

Correlation coefficients

Correlation coefficients from multilevel regressions were very good (range: 0.81–0.87) when calculated with the FFQ and serum iron and ferritin, for both age groups (Table 5). When calculated with the 24h-DR, correlation coefficients were weak in both age groups. We performed multilevel regression analysis with hemoglobin levels; correlations coefficients were very good for the FFQ in children (0.86), and good in adolescents (0.76). Unfortunately, data for blood hemoglobin levels were only available in São Paulo and Lima centers. Therefore, these data are not shown in Table 5.

Table 5. Correlation coefficients from multilevel linear regressions and percentage of accuracy from receiver operating characteristics curve of dietary iron intake according to the concentration of blood biomarkers in a subsample of children (3-10 years) and adolescents (11-17 years) from the SAYCARE Study. 2015-17

Dietary iron intake (mg/day)/ Serum biomarkers	Children (n=99)		Adolescents (n=50)	
	Iron (µg/dl)	Ferritin (µg/l)	Iron (µg/dl)	Ferritin (µg/l)
Food Frequency Questionnaire				
Correlation coefficient	0.86	0.87	0.81	0.81
PCV (%)	45.0	37.1	90.6	88.6
Accuracy (%)	61.7	70.6	69.8	49.0
24-hour Dietary Recall (mean intake using MSM)				
Correlation coefficient	0.18	0.16	0.12	0.12
PCV (%)	41.7	36.7	02.0	12.7
Accuracy (%)	58.9	43.3	38.5	42.9

Mg/d: milligrams per day; µg/dl: micrograms per deciliter; MSM: Multiple Source Method (mean of three days of 24-hour Dietary Recall).

PCV: Proportional change attributable to center-level variance estimated by multilevel linear regression using fixed effects.

The influence of contextual factors on the proportional changes attributable to center-level variance (PCV) was significant in all analyses ($p < 0.2$). The PCV for the FFQ in adolescents was very high for serum iron (90.6%) and ferritin (88.6%). The accuracy percentage from ROC curves for the FFQ were good, when compared to serum ferritin in children (70.6%) and serum iron in adolescents (69.8%).

DISCUSSION

The objective of this study was to validate a newly developed FFQ and three 24h-DR for ranking dietary iron intake amongst children and adolescents from South America. These findings suggested that the FFQ has good ability to rank total dietary iron intake amongst children and adolescents. This FFQ includes non-heme and heme iron food sources, but has limited ability in identifying dietary constituents (e.g. enhancers and inhibitors of iron absorption). As a consequence, the FFQ should not be used as a quantitative tool to measure the iron status of pediatric populations.

Serum iron and ferritin are biochemical indicators of iron intake, hence serum iron levels in healthy children are the main determinant of iron absorption efficiency, and serum ferritin is a well-established quantitative measure of iron stores.³⁴ Most iron in the body is found in hemoglobin in circulating erythrocytes,¹⁶ but considerable biological variations exist for hemoglobin, serum iron and ferritin levels, depending on an individual's physiological requirements (e.g. age and sex).¹⁰ Thus, in this context, we used different reference levels (Table 1)^{5, 16} to classify these biomarkers, which were age and sex appropriate.

Equally, generating blood samples from pediatric populations is challenging (e.g. complex ethical reasons for invasive procedures)³⁵, particularly in low-middle income countries, where financial resources and research infrastructure are limited.³⁶ These are some reasons why blood was collected in a subsample from participating study centers. Nevertheless, a *post-hoc* sample size estimation was conducted, and the final sample size was sufficient to assess questionnaire validity with blood biomarkers in children and adolescents. This is an inherent study strength; blood biomarkers objectively assessed dietary iron intake, thus complementing questionnaire validity of dietary assessment, independent of measurement errors in questionnaires.³⁷

When studying relationships between dietary intake and disease development in epidemiology, it is important to account for the ability of the dietary assessment method to correctly categorize participants by dietary intake.¹⁴ The FFQ not only showed better correlation coefficients than the 24h-DR (mean intake using MSM), but it was relatively easier to administer, was more practical and cheaper. A strength of this FFQ was its cultural adaptability to the different South American centers, and its specificity for pediatric populations.¹⁷ These factors may partly explain the good correlation coefficients (> 0.80) observed between total iron intake (estimated by the FFQ) and serum iron and ferritin, when compared to previous studies with children^{14, 38} and pregnant women.³⁹ It is important to emphasize that the 24h-DR is a useful and widely applied dietary assessment method,⁴⁰ that can also be used to improve the food list and the portion size options in the FFQ.¹⁴ However, in this study, its application was limited by only the first 24h-DR interviewed by a trained Nutritionist, and the two others were filled at home by the children's parents or guardians; or by the adolescent themselves. This was a significant limitation that possibly affected 24h-DR data (correlation coefficients ≤ 0.18) in this study.

To our knowledge, no prior study has validated an FFQ for total iron intake using biochemical iron status biomarkers, in a South American pediatric population. The FFQ was created to be a standardized questionnaire that could be applied to all SAYCARE centers, and therefore it should be more generalist with respect to food items. In this sense, the FFQ cannot assess the type of iron bioavailability. This FFQ limitation is relevant because heme iron (present in foods such as meat, poultry, fish and eggs) and non-heme iron (present in food groups such as legumes and vegetables) have different absorption mechanisms.¹⁶ Heme iron is highly absorbed and influenced by dietary factors.¹⁶ Non-heme iron is influenced by solubility, and its interaction with other dietary components in the intestine, such as fiber¹⁶ and phytates which inhibit dietary iron bioavailability.^{41, 42} Also, we did not assess the

potential influence of cooking processes on iron, e.g. boiling rice.⁴³ Additionally, we did not assess nutrient interactions, e.g. vitamin C or other organic acids that may modify iron absorption.⁴⁴

Another study limitation was that iron intake was probably underestimated, since iron fortification in some foods were not considered. These fortifications were not assessed because SAYCARE is a multicenter study, and different centers' governments have distinct fortification policies, with diverse food products and different iron quantity and quality levels.⁴⁵ These would be difficult to analyze, both singularly and together. For example, Columbian wheat flour is fortified with reduced iron compounds (44–65 mg/kg), and in Peru, with ferrous sulfate (30 mg/kg).⁴⁵ In Brazil, wheat and maize flours are fortified with reduced iron (30 mg/kg).⁴⁶ In this context, not including iron fortification measures showed that the correlation coefficients could have been better than correlation coefficients without fortifications, but even so, the majority of participants (> 94%) achieved average iron requirements¹⁶ (regardless of the dietary assessment method).

Assessing dietary intake is complex and challenging; dietary intake is replete with multidimensionality and dynamism, and varies amongst populations and cultures.¹¹ Thus, comparing dietary intake for different South American centers, with distinct social, cultural and physiological aspects is problematic.¹¹ This fact is evident in this multicenter study, from the high influence of contextual factors in the proportional change attributable to center-level variance for the FFQ, especially in adolescents (90.6% for serum iron 88.6% for ferritin).

Questionnaires were completed by parents/guardians for children younger than 10 years old. Thus, maternal education was a primary adjustment for such children, especially in South American countries.⁴⁷ Nonetheless, 58.6% of children's mothers left the maternal education status blank, and 61% of mothers who filled it out, stated they had a university degree. Despite this limitation, no differences between groups were observed after sensitivity analyses, and the maternal education variable was treated as a confounder.

Lastly, nutritional epidemiology benefits from FFQ development and validity, particularly amongst low-middle income countries (e.g. South American countries), and different age groups (e.g. preschoolers, children and adolescents).³⁶

CONCLUSION

Our findings suggest that the SAYCARE FFQ has a good ability to rank total dietary iron intake in children and adolescents. This observation was based on good correlation coefficients with serum iron and ferritin concentrations. Thus, this FFQ is a useful epidemiological tool for ranking dietary iron intake in children and adolescents from the SAYCARE study. On the other hand, the mean intake of three days of 24h-DR showed weak correlation coefficients with blood biomarkers in this study population. Therefore, the SAYCARE FFQ is recommended instead of the 24h-DR in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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47. Inhulsen, MM, Merelle, SY, Renders, CM. Parental feeding styles, young children's fruit, vegetable, water and sugar-sweetened beverage consumption, and the moderating role of maternal education and ethnic background. *Public Health Nutr* 2017;20(12):2124-2133.

Supplementary Material 1

Topic	Description	Page
Title and abstract	Indicate the study's design and purpose, including the construct of interest (ie, dietary components of interest and over what time frame) and dietary assessment measure undergoing validation, in the title and/or abstract. Provide an informative and balanced summary of what was done and what was found. Avoid summary statements that do not reflect the totality of the findings and that treat validity as dichotomous rather than properties operating on a continuum of low to high.	1
Introduction		
Background and rationale	Explain the scientific background and rationale for the validation study. Place the study in the context of existing research on dietary assessment methods, and build justification for the specific focus of the validation study.	2
Objectives	State specific objectives, including any prespecified hypotheses. Objectives should explicitly identify the aim of the study in terms of the properties assessed to examine suitability of the measure to address a given research purpose in a specified population and setting.	3
Methods		
Study design	Present key elements of the study design early.	3
Measure	Describe the measure undergoing validation in detail, including how it was developed/adapted, its format, the method and location of administration, and salient characteristics such as the associated food composition database(s) and whether supplement intake is assessed. Describe the intended use of the measure (ie, to capture occurrence of consumption (or not), to rank intake among a group of persons, or to estimate absolute intake), as well as the dietary components (foods, food groups, nutrients, patterns, or other food components) and time frame of interest.	5-7
Settings	Describe the setting, locations, and relevant dates, including periods of recruitment and data collection. Describe any characteristics of the study setting that might influence participants' dietary intake.	3-4
Participants	Provide the eligibility criteria and sources and methods of selection of participants. Justify the sample size. Describe the representativeness of the sample to the target population and discuss response rates. Report characteristics related to nutrition, dietary intake, and physiology considered in defining the eligibility criteria.	3-4

Supplementary Material 1. Checklist for authors and reviewers when describing studies to validate dietary assessment measures. Italicized text is adapted from: Lachat C, Hawwash D, Ocké MC, et al. Strengthening the reporting of observational studies in epidemiology— Nutritional epidemiology (STROBE-nut): An extension of the STROBE statement. PLOS Med. 2016;13(6). 2016:e1002036.86

Procedures	<p>Is the measure well-constructed and grounded in an understanding of the underlying phenomenon of interest? (face and content validity)• Provide a detailed presentation of the procedures employed to gather feedback to assess whether items reflect the construct of interest (eg, reviews of the relevant literature(s), surveys of lay persons and/or relevant experts). Address approaches employed to ensure the items included, for example, in a frequency-based questionnaire, are comprehensive and reflect the most commonly-consumed sources of the dietary components of interest, as well as relevant portion sizes (if applicable). Does the measure perform in a manner consistent with the theory underlying its construction? (construct validity)• Describe the methods used to examine whether or not the measure assesses the intended construct (eg, contrasted groups) and the theory that underlies this particular approach to assessing this aspect of validity. Include a detailed description of statistical procedures used. Is the measure accurate within specified performance standards? (criterion or relative validity)• Describe criterion or comparison reference measure(s) (eg, biomarkers, observation, feeding studies, other self-report tools) used to validate the measure of interest in detail, including potential biases. Avoid language such as gold standard to describe reference measures. Justify the use of particular reference measure(s) in terms of the dietary components and time frame of interest. Address whether the reference measure(s) and the measure undergoing validation assess intake over the same period of time. Describe considerations regarding number and timing of administration(s) of both the measure to be evaluated and the reference measure(s). Explicitly describe and justify the statistical procedures used, including multiple tests as appropriate. Does the measure produce data that are precise and dependable? (reliability)• Describe the particular aspects of reliability of interest; for example, precision (test–retest reliability) or interrater reliability. If test–retest reliability, describe the relevant period of time over which reliability is of interest. Discuss whether dietary intake could be expected to change over the relevant time period due to confounding factors and how this was addressed. Explicitly describe the statistical tests used. Use multiple tests as appropriate. Is the measure responsive to change? Describe procedures used to assess responsiveness to meaningful change over time and to assess the smallest detectable change for given dietary components. Comment on issues related to statistical power related to responsiveness. Does the measure produce data that are equivalent or comparable across populations? Discuss approach to comparability in terms of adapting measures for different contexts or identifying particular variables captured using different measures but that can be theoretically harmonized.</p>	3-9
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Supplementary Material 1. (*Continued*) Checklist for authors and reviewers when describing studies to validate dietary assessment measures. Italicized text is adapted from: Lachat C, Hawwash D, Ocké MC, et al. Strengthening the reporting of observational studies in epidemiology Nutritional epidemiology (STROBE-nut): An extension of the STROBE statement. PLOS Med. 2016;13(6). 2016:e1002036.86

Results		
Participants	Report the numbers of individuals at each stage of the study and give reasons for nonparticipation at each stage. Consider the use of a flow diagram to illustrate. Report the results of each procedure implemented for each dietary construct of interest.	11
Descriptive data	Give information on the study participants (eg, demographic characteristics). Provide salient information regarding dietary intake data (eg, low or high values, avoidance of certain foods)	12-13
Discussion		
Key results	Make use of all statistical tests and/or procedures to objectively summarize the key results with reference to the study objectives. Discuss the degree of validity, reliability, sensitivity to change, and/or equivalence of the evaluated measure as appropriate to the study, rather than referring to these properties as present or absent. When comparing error-prone measures to one another, consider the contribution of correlated error to measures of association. Avoid overstating the level of validity or reliability based on the available data.	17-19
Limitations	Describe study limitations that may affect conclusions. This may include the reference measures in studies of validity or recruitment methods (eg, paid volunteers) in any study.	18-19
Interpretation	Limit interpretations about validity, reliability, responsiveness, and/or equivalence of the evaluated measure to the specific populations and contexts evaluated, as well as the particular objectives (eg, interpretations of an evaluation of validity should be limited to validity and not reliability). Base interpretations on the totality of the evidence, including all tests and comparisons conducted, as well as results from similar studies. Place the findings in the context of other literature.	18-19
Generalizability	Describe potential appropriate and inappropriate uses of the measure given the study design and findings. Describe features of the measure that may influence the design of studies proposing to make use of it; for example, sample size calculations to account for loss of power due to biased measurement of dietary intake.	17
Other information		
Funding	Give the source of funding and the role of the funders in the validation study and, when applicable, in prior studies on which the present validation study is based.	20-21
Ethics	Describe the procedures for consent and study approval from ethics committee(s).	9-10

Supplementary Material 1. (*Continued*) Checklist for authors and reviewers when describing studies to validate dietary assessment measures. Italicized text is adapted from: Lachat C, Hawwash D, Ocké MC, et al. Strengthening the reporting of observational studies in epidemiology— Nutritional epidemiology (STROBE-nut): An extension of the STROBE statement. PLOS Med. 2016;13(6). 2016;e1002036.86.

Frequência de Consumo Alimentar

Caro(a) papai ou mamãe, o senhor(a) está participando de um estudo que tem por objetivo conhecer a alimentação do seu filho(a).

Para isso, é necessário que o senhor(a) assinale com um X a resposta mais apropriada sobre a frequência, a forma e o local; que seu filho(a) normalmente se alimenta.

Lembrando que:

1. O questionário deve ser preenchido pela pessoa que passa mais tempo em casa com a criança;
2. As perguntas fazem referência aos alimentos e preparações consumidas nos últimos três meses;
3. Deve-se responder sobre todas as comidas e bebidas consumidas durante este período (últimos três meses), incluindo os alimentos que seu filho(a) consumiu fora de casa, na escola, restaurante e com amigos ou familiares.

Algumas instruções para completar o questionário:

1. Leia atentamente todas as instruções do questionário;
2. Assinale com um X somente a resposta correta (uma por pergunta).

Para completar este questionário, você poderá olhar o álbum de todos os alimentos para saber qual foi a quantidade consumida.

Exemplo:

1 CEREAIS E PANIFICADOS

	ALIMENTOS	NOS ÚLTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
1.3	Pães (Pão francês, pão de hamburger, pão de hot dog, bisnaguinha, etc) (Ver Foto 2-A, 2-B)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☒ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☒ 1 porção ☐ 2 porções ☐ 3 porções	☐ Branco ☒ Integral

1. ONDE SEU FILHO(A) REALIZA ESTAS REFEIÇÕES?

	CASA			ESCOLA			OUTRO LUGAR		
	Todos os dias	Segunda a Sexta	Finais de semana	Todos os dias	Segunda a Sexta	Finais de semana	Todos os dias	Segunda a Sexta	Finais de semana
Café da manhã ☐ não toma	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lanche da manhã ☐ não toma	☐	☐	☐	☐	☐	☐	☐	☐	☐
Almoço ☐ não toma	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lanche da tarde ☐ não toma	☐	☐	☐	☐	☐	☐	☐	☐	☐
Jantar ☐ não toma	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lanche antes de dormir ☐ não toma	☐	☐	☐	☐	☐	☐	☐	☐	☐

1 CEREAIS E PANIFICADOS

	ALIMENTOS	NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
1.1	Arroz (Ver Foto 1-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/4 porção ☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
1.2	Massas (Macarrão ao sugo ou alho e óleo; lasanha; ravióli; capeleti) (Ver Foto 1-B; 1-C; 1-D)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/4 porção ☐ 1/2 porção ☐ 1 porção ☐ 2 porções	
1.3	Pães (Pão francês, pão de hamburguer, pão de hot dog, bisnaguinha, etc) (Ver Foto 2-A, 2-B)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	☐ Branco ☐ Integral
1.4	Pão de forma (Ver Foto 2-C, 2-D)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	☐ Branco ☐ Integral

	ALIMENTOS	NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
1.5	Bolachas salgadas tipo água e sal (Ver Foto 3-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	
1.6	Bolachas doces sem recheio tipo maisena e maria (Ver Foto 3-B)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
1.7	Bolachas doces com recheio (Ver Foto 3-C)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	
1.8	Produtos de padaria (bolos, pães doces, sonho, etc) (Ver Foto 4)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	

	ALIMENTOS	NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
1.9	Cereais matinais tipo Sucrilhos (Ver Foto 5)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	☐ Açucarados ☐ Sem açúcar
1.10	Pizza (Ver Foto 6-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
1.11	Salgados assados e fritos (coxinha, pastel, pão de queijo, esfirra, enroladinho, croissant) (Ver Foto 28)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	
1.12	Mingau (de aveia, maizena ou milho) (Ver Foto 29-B)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	

1.13

ALIMENTOS	NOS ÚLTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?		TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
Farofa pronta ou caseira (Ver Foto 30)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção
	☐	1 a 3x por mês	☐	1 porção
	☐	1x por semana	☐	2 porções
	☐	2 a 4x por semana	☐	3 porções
	☐	5 a 6x por semana		
	☐	1x por dia		
	☐	2 a 3x por dia		
	☐	4 a 5x por dia		
	☐	6 ou mais vezes por dia		

02

TUBÉRCULOS

2.1

ALIMENTOS	NOS ÚLTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?		TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
Batatas fritas (Ver Foto 7-A)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção
	☐	1 a 3x por mês	☐	1 porção
	☐	1x por semana	☐	2 porções
	☐	2 a 4x por semana		
	☐	5 a 6x por semana		
	☐	1x por dia		
	☐	2 a 3x por dia		
	☐	4 a 5x por dia		
	☐	6 ou mais vezes por dia		

2.2

Batata cozida ou purê (Ver Foto 7-B; 7-C)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção
	☐	1 a 3x por mês	☐	1 porção
	☐	1x por semana	☐	2 porções
	☐	2 a 4x por semana	☐	3 porções
	☐	5 a 6x por semana	☐	4 porções
	☐	1x por dia		
	☐	2 a 3x por dia		
	☐	4 a 5x por dia		
	☐	6 ou mais vezes por dia		

	ALIMENTOS	NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
2.3	Mandioca cozida (Ver Foto 8-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	
2.4	Mandioca frita (Ver Foto 8-B)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	
2.5	Inhame ou Cará (Ver Foto 8-C)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	

03 VERDURAS E HORTALIÇAS

	ALIMENTOS	NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
3.1	Verduras e Hortaliças cruas (Ver Foto 9)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/4 porção ☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
3.2	Verduras e Hortaliças cozidas (Ver Foto 10)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/4 porção ☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
3.3	Sopa de legumes (Ver Foto 11)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	

04 FRUTAS

	ALIMENTOS	NOS ÚLTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
4.1	Frutas frescas (Ver Foto 12)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	
4.2	Açaí (Ver Foto 29-C)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	☐ Com granola ☐ Sem granola
4.3	Banana frita (Ver Foto 8-D)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/4 porção ☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
		☐ 6 ou mais vezes por dia

05 ÓLEOS E AZEITES

	ALIMENTOS	NOS ÚLTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
5.1	Manteiga (Ver Foto 13-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/4 porção ☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ Porção individual	
5.2	Margarina (Ver Foto 13-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/4 porção ☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ Porção individual	
5.3	Azeite (adicionado na comida depois de pronta, na salada, etc) (Ver Foto 13-B)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	

06 CARNES E DERIVADOS, PEIXES E OVOS

	ALIMENTOS	NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
6.1	Carne de vaca (Ver Foto 14-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	☐ Bife ☐ Moída
6.2	Carne de frango (Ver Foto 14-B; 14-C)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
6.3	Carne de porco (Ver Foto 14-D)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
6.4	Filé à milanesa (carne de frango, carne de vaca) (Ver Foto 14-E)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções

ALIMENTOS		NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?		TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?		ESCOLHA A OPÇÃO MAIS FREQUENTE
6.5	Hamburguer (Ver Foto 6-B)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção	
		☐	1 a 3x por mês	☐	1 porção	
		☐	1x por semana	☐	2 porções	
		☐	2 a 4x por semana	☐	3 porções	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia			
6.6	Embutidos (linguiça, salsicha, etc) (Ver Foto 15-A; 15-B)	☐	Nunca ou menos de 1x por mês	☐	1/3 porção	
		☐	1 a 3x por mês	☐	1/2 porção	
		☐	1x por semana	☐	1 porção	
		☐	2 a 4x por semana	☐	2 porções	
		☐	5 a 6x por semana	☐	3 porções	
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
6.7	Frios (presunto, salame, mortadela, etc) (Ver Foto 15-C; 15-D; 15-E)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção	
		☐	1 a 3x por mês	☐	1 porção	
		☐	1x por semana	☐	2 porções	
		☐	2 a 4x por semana	☐	3 porções	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia

ALIMENTOS		NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?		TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?		ESCOLHA A OPÇÃO MAIS FREQUENTE
6.8	Peixes (Ver Foto 16-A)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção	
		☐	1 a 3x por mês	☐	1 porção	
		☐	1x por semana	☐	2 porções	
		☐	2 a 4x por semana	☐	3 porções	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia			
6.9	Peixe enlatado (Ver Foto 16-B)	☐	Nunca ou menos de 1x por mês	☐	1/3 porção	
		☐	1 a 3x por mês	☐	1/2 porção	
		☐	1x por semana	☐	1 porção	
		☐	2 a 4x por semana	☐	2 porções	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia			
6.10	Ovos (ovo frito, ovo cozido, ovo mexido, omelete) (Ver Foto 16-C; 16-D)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção	
		☐	1 a 3x por mês	☐	1 porção	
		☐	1x por semana	☐	2 porções	
		☐	2 a 4x por semana	☐	3 porções	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia			

07 LEITES E DERIVADOS

	ALIMENTOS	NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
7.1	Leite líquido ou em pó (Ver Foto 17)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ Xícara 1 ☐ Xícara 2 ☐ Xícara 3	☐ Integral ☐ Semidesnatado ☐ Desnatado
7.2	Iogurte (natural, de frutas, grego, etc) (Ver Foto 18)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ Embalagem 1 ☐ Embalagem 2 ☐ Embalagem 3 ☐ Embalagem 4 ☐ Embalagem 5 ☐ Embalagem 6	☐ Integral ☐ Semidesnatado ☐ Desnatado ☐ Com açúcar ☐ Sem açúcar
7.3	Leite com achocolatado, vitamina, milk shake (Ver Foto 17 e 19)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ Xícara 1 ☐ Xícara 2 ☐ Xícara 3 ☐ Copo 1 ☐ Copo 2 ☐ Copo 3 ☐ Copo 4

ALIMENTOS		NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?		TODA VEZ QUE SEU FILHO(A) CONSUME, QUAL A QUANTIDADE?		ESCOLHA A OPÇÃO MAIS FREQUENTE
7.4	Sobremesas lácteas (flan, danette, iogurte com confetes) (Ver Foto 20)	☐	Nunca ou menos de 1x por mês	☐	Embalagem 1	
		☐	1 a 3x por mês	☐	Embalagem 2	
		☐	1x por semana	☐	Embalagem 3	
		☐	2 a 4x por semana	☐	Embalagem 4	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia			
7.5	Queijo tipo ricota, cottage (Ver Foto 21-A; 21-B)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção	
		☐	1 a 3x por mês	☐	1 porção	
		☐	1x por semana	☐	2 porções	
		☐	2 a 4x por semana	☐	3 porções	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia			
7.6	Queijo branco tipo minas, Requeijão, Mussarela (Ver Foto 21-C; 21-D; 21-E)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção	
		☐	1 a 3x por mês	☐	1 porção	
		☐	1x por semana	☐	2 porções	
		☐	2 a 4x por semana	☐	3 porções	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia			
7.7	Queijo Prato, Queijo ralado (Ver Foto 21-F; 21-G)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção	
		☐	1 a 3x por mês	☐	1 porção	
		☐	1x por semana	☐	2 porções	
		☐	2 a 4x por semana	☐	3 porções	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia			

08 LEGUMINOSAS

ALIMENTOS		NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
8.1	Feijão (Ver Foto 22-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/4 porção ☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
8.2	Lentilhas (Ver Foto 22-B)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/4 porção ☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
8.3	Grão-de-bico (Ver Foto 22-C)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/4 porção ☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	

09 BEBIDAS

	ALIMENTOS	NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
9.1	Água (Ver Foto 19)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ Copo 1 ☐ Copo 2 ☐ Copo 3 ☐ Copo 4	
9.2	Suco de fruta natural (Ver Foto 19)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ Copo 1 ☐ Copo 2 ☐ Copo 3 ☐ Copo 4	☐ Com açúcar ☐ Sem açúcar
9.3	Suco de fruta em caixa (incluindo de soja) (Ver Foto 19)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ Copo 1 ☐ Copo 2 ☐ Copo 3 ☐ Copo 4 ☐ Caixa individual	☐ Com açúcar ☐ Sem açúcar
9.4	Refrigerante (incluindo bebidas energéticas) (Ver Foto 19)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ Copo 1 ☐ Copo 2 ☐ Copo 3 ☐ Copo 4	☐ Com açúcar ☐ Sem açúcar

	ALIMENTOS	NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
9.5	Sucos artificiais em pó (Ver Foto 19)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ Copo 1 ☐ Copo 2 ☐ Copo 3 ☐ Copo 4	☐ Com açúcar ☐ Sem açúcar
9.6	Café, chá, mate (não quantificar o leite) (Ver Foto 17)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ Xícara 1 ☐ Xícara 2 ☐ Xícara 3	☐ Com açúcar ☐ Sem açúcar
9.7	Caldo de cana (Ver Foto 19)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ Copo 1 ☐ Copo 2 ☐ Copo 3 ☐ Copo 4	

10 PRODUTOS AÇÚCARADOS E SALGADINHOS

	ALIMENTOS	NOS ÚLTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
10.1	Mel (Ver Foto 23-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 colher rasa ☐ 1 colher rasa ☐ 2 colheres rasas ☐ 3 colheres rasas	
10.2	Geléia (Ver Foto 23-B)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 colher rasa ☐ 1 colher rasa ☐ 2 colheres rasas ☐ 3 colheres rasas	
10.3	Doce de leite (Ver Foto 23-C)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 colher rasa ☐ 1 colher rasa ☐ 2 colheres rasas ☐ 3 colheres rasas	
10.4	Doces de corte (Goiabada, Marmelada, Bananada) (Ver Foto 23-D)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	
	ALIMENTOS	NOS ÚLTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A)	TODA VEZ QUE SEU FILHO(A) CONSOME,	ESCOLHA A OPÇÃO MAIS

		COMEU ESTES ALIMENTOS?	QUAL A QUANTIDADE?	FREQUENTE
10.5	Sorvetes (picolé de fruta, picolé de leite, bola) (Ver Foto 24-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	☐ N° 1 ☐ N° 2 ☐ N° 3
10.6	Gelatina (Ver Foto 24-B)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções	
10.7	Guloseimas (bala, chiclete, pirulito, bala de goma, etc) (Ver Foto 25)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	☐ N° 1 ☐ N° 2 ☐ N° 3 ☐ N° 4
10.8	Chocolate (barra, bombom, brigadeiro, etc) (Ver Foto 25)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 porção ☐ 1 porção ☐ 2 porções ☐ 3 porções ☐ 4 porções	☐ N° 5 ☐ N° 6 ☐ N° 7

ALIMENTOS		NOS ULTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?		TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?		ESCOLHA A OPÇÃO MAIS FREQUENTE
10.9	Salgadinhos tipo chips (Ver Foto 26)	☐	Nunca ou menos de 1x por mês	☐	1/2 porção	
		☐	1 a 3x por mês	☐	1 porção	
		☐	1x por semana	☐	2 porções	
		☐	2 a 4x por semana	☐	3 porções	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia			
		10.10	Barra de cereal	☐	Nunca ou menos de 1x por mês	☐
☐	1 a 3x por mês			☐	1 unidade	
☐	1x por semana			☐	2 unidades	
☐	2 a 4x por semana			☐	3 unidades	
☐	5 a 6x por semana					
☐	1x por dia					
☐	2 a 3x por dia					
☐	4 a 5x por dia					
☐	6 ou mais vezes por dia					
10.11	Pipoca doce (Ver Foto 29-A)			☐	Nunca ou menos de 1x por mês	☐
		☐	1 a 3x por mês	☐	1 unidade	
		☐	1x por semana	☐	2 unidades	
		☐	2 a 4x por semana	☐	3 unidades	
		☐	5 a 6x por semana			
		☐	1x por dia			
		☐	2 a 3x por dia			
		☐	4 a 5x por dia			
		☐	6 ou mais vezes por dia			

11 DIVERSOS

	ALIMENTOS	NOS ÚLTIMOS TRÊS (3) MESES, QUANTAS VEZES SEU FILHO(A) COMEU ESTES ALIMENTOS?	TODA VEZ QUE SEU FILHO(A) CONSOME, QUAL A QUANTIDADE?	ESCOLHA A OPÇÃO MAIS FREQUENTE
11.1	Maionese (Ver Foto 27-A)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 colher rasa ☐ 1 colher rasa ☐ 2 colheres rasas ☐ 3 colheres rasas ☐ 4 colheres rasas	
11.2	Outros molhos (Ketchup, mostarda, barbecue, molho rosé) (Ver Foto 27-B; 27-C; 27-D; 27-E)	☐ Nunca ou menos de 1x por mês ☐ 1 a 3x por mês ☐ 1x por semana ☐ 2 a 4x por semana ☐ 5 a 6x por semana ☐ 1x por dia ☐ 2 a 3x por dia ☐ 4 a 5x por dia ☐ 6 ou mais vezes por dia	☐ 1/2 colher rasa ☐ 1 colher rasa ☐ 2 colheres rasas ☐ 3 colheres rasas ☐ 4 colheres rasas	

Muito obrigado por responder este questionário. Suas respostas são muito importantes para nós!

Recordatório Alimentar de 24h

Por favor, preencher este recordatório no dia _____, referente ao que seu filho(a) consumiu no dia _____.

MANUAL DE APOIO PARA O PREENCHIMENTO DO RECORDATÓRIO ALIMENTAR DE 24 HORAS

Mediante este questionário gostaríamos de recordar todos os alimentos que seu filho (a) consumiu nas últimas 24 horas. Suas respostas são muito importantes para nós!

Trata-se de um exercício de memória, no qual você terá que fazer um esforço para lembrar tudo que seu filho (a) comeu e bebeu ontem, desde a hora que ele (a) acordou, até a hora que foi dormir.

O questionário tem divisões de acordo com o período do dia: café da manhã, lanche da manhã, almoço, lanche da tarde, jantar e lanche da noite. Você deverá descrever detalhadamente todos os alimentos e bebidas consumidos ontem pelo seu filho (a) em cada um desses momentos.

Vamos começar o preenchimento

1) A pergunta inicial de cada período do dia é se seu filho(a) comeu algum alimento ou bebida nesse momento do dia. Exemplo: “*Carlos, ontem você tomou café da manhã?*”. Se SIM, você irá preencher os alimentos e bebidas referentes a este mesmo período do dia, se a resposta for NÃO, o questionário irá informar que você passe para o período seguinte.

3) Lembre-se de sempre preencher o horário e o local que os alimentos e bebidas foram consumidos pelo seu filho(a), haverá um espaço no questionário para isso.

4) No espaço reservado para você registrar os alimentos e bebidas, utilize uma linha por alimento. Lembre-se que há preparações que devem ser detalhadas utilizando-se mais de uma linha. Exemplo: *salada de alface e tomate*. Nesse caso, é necessário detalhar a quantidade de alface consumida e a quantidade de tomate consumida, além de qualquer molho ou tempero adicionado.

Você deverá detalhar os alimentos e bebidas incluindo os ingredientes, forma de preparo, e marca. Exemplos:

- **Carne vermelha:** carne assada, carne ensopada, bife acebolado, bife à rolê, bife à *parmegiana*, carne seca, bife à milanesa. Se souber, informar o tipo: file *mignon*, patinho, alcatra, picanha, músculo, fígado.
- **Frango:** frango à milanesa, frango assado, frango ensopado, frango frito, frango grelhado. Coxa, sobrecoxa, peito, asa. Frango com pele, sem pele.
- **Peixe:** peixe frito, peixe à milanesa, peixe cozido, peixe ensopado, peixe grelhado. Atum, sardinha, bacalhau, cação, salmão, tainha?
- **Arroz:** branco, integral, de forno, à grega, carreteiro, arroz doce.
- **Feijão:** marrom, preto, branco, feijoada.
- **Suco:** Preparado com a fruta? Qual a fruta? Era de que sabor? Com ou sem açúcar? Era em pó? Concentrado (Maguary®)? Era polpa congelada? Era de caixinha? Qual era a marca?
- **Refrigerante:** Qual a marca? Qual o sabor? Era *light*? Era *diet*? Era normal (que não é *diet* e nem *light*)?
- **Bolacha:** Doce ou salgada? Qual a marca? Com ou sem recheio? Qual o sabor?
- **Pão:** Francês (pão comum), integral de forma, de leite de forma, de queijo, de centeio de forma, pão doce, de cachorro quente, de hambúrguer, de batata, pão sírio, *croissant*? Com ou sem recheio?
- **Bolo:** Com recheio, sem recheio? Com cobertura, sem cobertura? Qual sabor?

- **Salgadinho:** Qual o sabor? (presunto, queijo). Qual o nome? (*Cheetos, Fandangos, Ruffles*)
- **Sanduíche:** Qual o nome (misto-quente, X-burguer)? Quais os ingredientes? Tinha presunto? Queijo? Maionese?
- **Salgado:** Coxinha, esfirra, pastel? Qual o recheio? De carne, de palmito, de frango, de camarão?
- **Macarrão:** Com molho? Qual o tipo de molho? À bolonhesa, ao alho e óleo, ao sugo, ao molho branco? Com ou sem recheio?
- **Nhoque:** Com molho? Qual o tipo de molho?
- **Massas:** Com recheio ou sem recheio? Caneloni, rondeli, panqueca? Com molho? Qual o tipo de molho?
- **Leite:** Integral, semidesnatado, desnatado, de soja, em pó, leite condensado? Com ou sem açúcar?
- **Vitamina:** Qual o sabor da vitamina (de banana, de mamão, de maçã)? Quais os ingredientes? Tinha leite? Banana? Açúcar?
- **Iogurte:** Integral, desnatado? Qual sabor? Morango, chocolate, ameixa? Era *diet, light* ou normal?
- **Queijo:** Prato, mozzarella, ralado, minas, polenguinho?
- **Requeijão:** Era *light*, normal?
- **Frutas:** Banana prata, banana nanica, uva Itália, uva rubi?
- **Verduras:** alface lisa, tomate cereja, repolho roxo, cebola, pepino enlatado? Cru, cozido?
- **Molhos:** *Catchup*, mostarda, maionese, molho de salada, azeite, vinagre.
- **Chocolate:** Preto, branco, ao leite, meio amargo? Era *light, diet*? Qual a marca?
- **Embutidos:** presunto magro, presunto de peru, mortadela de frango, linguiça, calabresa, salsicha de porco.
- **Salada:** de alface, de cenoura, de pepino, de rúcula?
- **Ervilha, milho:** natural, em lata ou congelada.

5) Na próxima coluna, você precisará detalhar a quantidade de cada alimento ou bebida que você descreveu. Para isso, é importante você descrever o utensílio que foi utilizado, como por exemplo, o tipo de colher (de café, de chá, de sobremesa, de sopa); de copo (xícara, copo de requeijão); de prato (fundo, raso, de sobremesa, pires), e se colocou uma medida cheia ou rasa. Nesse momento, você poderá utilizar as fotos de utensílios que foram entregues junto com o questionário.

- **Colher:** de servir, de sopa, de sobremesa, de chá, de café. A colher estava cheia (bastante quantidade) ou rasa (pouca quantidade)?
- **Escumadeira ou concha:** pequena, média, grande. A escumadeira ou concha estavam cheias (bastante quantidade) ou rasas (pouca quantidade)?
- **Copo:** pequeno (café), médio (requeijão), grande ou extra grande.
- **Pacote:** em gramas (g), por exemplo: pacote de salgadinho com 100g.
- **Pegador de macarrão:** Estava cheio (bastante quantidade) ou raso (pouca quantidade)?
- **Xícara:** de café, de chá.
- **Fatia:** pequena, média, grande.
- **Rodela:** de tomate, de pepino.
- **Ponta de faca:** com grande quantidade ou com pequena quantidade

Tamanho

- **Frutas:** pequena, média, grande.
- **Bife de carne:** pequeno, médio, grande.
- **Filé de frango ou peixe:** pequeno, médio ou grande.
- **Folhas de alface:** pequena, média, grande.

Quantidade

- Número de colheres (2 colheres de servir de arroz branco; 3 conchas pequenas de feijão preto).

No quadro abaixo, lembraremos você de alguns itens e como detalhá-los:

CAFÉ DA MANHÃ

Lembre-se! Seu filho (a):

- Tomou leite: anote se era integral, desnatado, semidesnatado, fortificado, “de soja”, e se foi acrescentado alguma coisa (achocolatado, café, açúcar, adoçante, etc);
- Tomou suco: anote se era suco natural ou artificial, se foi adicionado açúcar ou adoçante;
- Comeu bolo ou pão: anote o tipo (pão francês, pão de forma branco ou integral, broa, pão caseiro, pão doce, torrada, bolo com ou sem recheio, etc) e se ele (a) passou alguma coisa (manteiga, margarina, requeijão, geleia);

PERÍODO DA MANHÃ/TARDE/NOITE

Lembre-se! Seu filho (a):

- Comeu algum chocolate, chiclete, bala, pirulito, salgado, salgadinho de pacote, bolacha recheada ou sem recheio, ou qualquer outro alimento, anote todos os detalhes que lembrar e a quantidade de cada um deles;
- Bebeu algum suco, iogurte, refrigerante ou qualquer outro líquido, anote qual foi e a quantidade de cada um.

ALMOÇO/ JANTAR

Lembre-se! Seu filho (a):

- Comeu carne, anote o tipo (ave, porco, boi, peixe), como foi preparada (grelhada, frita, assada, empanada, ensopada, cozida) e se retirou a pele do frango, o couro do peixe ou a gordura visível da carne;
- Comeu salada: diga todos os ingredientes e suas quantidades, se foi temperada ou não e o que foi utilizado para isso (óleo, azeite, vinagre, limão, sal, molho industrializado, etc);
- Tomou algum líquido junto com a refeição, não deixe de anotar qual e a quantidade, além de descrever se foi adoçado (açúcar, adoçante), se já vinha adoçado, etc.
- Comeu alguma sobremesa: não deixe de anotar qual e a quantidade.

Para completar este questionário, você poderá olhar o álbum de fotos de alimentos para saber qual foi a quantidade consumida.

		Dia	Mês	Ano
1	Data de realização do recordatório			
2	Nome do entrevistador (se aplicável)			
3	Nome de quem responde			

		Segunda	Terça	Quarta	Quinta	Sexta	Sábado	Domingo
4	Que dia da semana foi ontem?							

- Que horas seu filho(a) acordou ontem?: |__||__|:|__||__|

		Não <i>(Passe para a pergunta N° 9)</i>	Sim
5	Ontem seu filho(a) tomou café da manhã?		

6	A que horas seu filho(a) tomou café da manhã?	
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		Em casa	Na escola	Em outro lugar (qual?)
7	Onde seu filho(a) tomou seu café da manhã?			

8	Descreva todos os alimentos e bebidas que seu filho(a) consumiu no café da manhã:
---	---

Alimentos, bebidas e preparações consumidas	Quantidade (em medida caseira)	

9	Ontem, entre o café da manhã e o almoço, seu filho(a) comeu ou bebeu alguma coisa?	Não (Passe para a pergunta N° 12)	Sim

10	Onde seu filho(a) consumiu esses alimentos e bebidas?	Em casa	Na escola	Em outro lugar (qual?)	A que hora?

11	Descreva todos os alimentos e bebidas que seu filho(a) consumiu entre o café da manhã e o almoço:
----	---

Alimentos, bebidas e preparações consumidas	Quantidade (em medida caseira)	Código (não completar)

12	Ontem seu filho(a) almoçou?	Não (Passe para a pergunta N° 16)	Sim

13	A que horas seu filho(a) almoçou?	
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14	Onde seu filho(a) almoçou?	Em casa	Na escola	Em outro lugar (qual?)

15	Descreva todos os alimentos e bebidas que seu filho(a) consumiu no almoço:
----	--

Alimentos, bebidas e preparações consumidas	Quantidade (em medida caseira)	

		Não (Passe para a pergunta N° 19)	Sim
16	Ontem entre o almoço e o jantar, seu filho(a) comeu ou bebeu alguma coisa?		

		Em casa	Na escola	Em outro lugar (qual?)	A que horas?
17	Onde seu filho(a) consumiu esses alimentos e bebidas?				

18	Descreva todos os alimentos e bebidas que seu filho(a) consumiu entre o almoço e o jantar:
----	--

Alimentos, bebidas e preparações consumidas	Quantidade (em medida caseira)	

		Não (Passe para a pergunta N° 23)	Sim
19	Ontem seu filho(a) jantou?		

20	A que horas seu filho(a) jantou?	
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		Em casa	Na escola	Em outro lugar (qual?)
21	Onde seu filho(a) fez seu jantar?			

22	Descreva todos os alimentos e bebidas que seu filho(a) consumiu no jantar:
----	--

Alimentos, bebidas e preparações consumidas	Quantidade (em medida caseira)	

23	Ontem depois do jantar ou antes de dormir, seu filho(a) comeu ou bebeu alguma coisa?	Não	Sim
		(Questionário Finalizado)	

24	Onde seu filho(a) consumiu esses alimentos e bebidas?	Em casa	Na escola	Em outro lugar (qual?)	A que horas?

25	Descreva todos os alimentos e bebidas que seu filho(a) consumiu depois do jantar ou antes de dormir:
----	--

Alimentos, bebidas e preparações consumidas	Quantidade (em medida caseira)	

- Que horas seu filho(a) foi dormir ontem?: |__||__|:|__||__|

Muito obrigado por responder este questionário. Suas respostas são muito importantes para nós!

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Validação de Métodos de Avaliação do Consumo Alimentar em Crianças: SAYCARE Study

Pesquisador: HERACLITO BARBOSA DE CARVALHO

Área Temática:

Versão: 1

CAAE: 66002917.6.0000.0065

Instituição Proponente: Faculdade de Medicina da Universidade de São Paulo

Patrocinador Principal: FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E INOVAÇÃO

DADOS DO PARECER

Número do Parecer: 2.022.542

Apresentação do Projeto:

Trata-se de um sub-projeto de doutorado dentro de um protocolo já aprovado previamente nesta CEP (projeto 232/14).

A avaliação do consumo alimentar de crianças com acurácia é essencial para se investigar as associações entre dieta e doença. Diante da crescente prevalência de obesidade, fatores de risco cardiovascular, diabetes e demais doenças com consequências em longo prazo, torna-se fundamental prevenir o surgimento das mesmas, desde a infância. Portanto, validar métodos de consumo alimentar em crianças é primordial para a identificação e prevenção primária destas doenças.

Objetivo da Pesquisa:

Validar em crianças de 6 a 10 anos de idade dois questionários [Recordatório Alimentar de 24 horas (R24h) e Questionário de Frequência Alimentar (QFA)], para estimar o consumo habitual de ferro, e de Frutas, Legumes e Verduras (FLV), em crianças sul-americanas.

Avaliação dos Riscos e Benefícios:

Riscos mínimos

Endereço: DOUTOR ARNALDO 251 21º andar sala 36

Bairro: PACAEMBU

CEP: 01.246-903

UF: SP

Município: SÃO PAULO

Telefone: (11)3893-4401

E-mail: cep.fm@usp.br

**USP - FACULDADE DE
MEDICINA DA UNIVERSIDADE
DE SÃO PAULO - FMUSP**

Continuação do Parecer: 2.022.542

Comentários e Considerações sobre a Pesquisa:

Pesquisa bem delineada, pertencente a um estudo multicêntrico em diferentes pontos da América do Sul. Pergunta de pesquisa atual, clara, sem ambiguidades e testável.

Considerações sobre os Termos de apresentação obrigatória:

Não apresentado, mas já aprovado anteriormente (projeto 232/14)

Recomendações:

Aprovação

Conclusões ou Pendências e Lista de Inadequações:

Aprovado

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_824610.pdf	20/03/2017 11:40:03		Aceito
Outros	DocCepFmusp.pdf	20/03/2017 11:39:23	HERACLITO BARBOSA DE CARVALHO	Aceito
Outros	parecer.pdf	20/03/2017 11:39:02	HERACLITO BARBOSA DE CARVALHO	Aceito
Outros	Anexo6CEPColaboradores.pdf	20/03/2017 11:36:00	HERACLITO BARBOSA DE CARVALHO	Aceito
Outros	Anexo5CEPSAYCARE.pdf	20/03/2017 11:35:36	HERACLITO BARBOSA DE CARVALHO	Aceito
Outros	Anexo4QFA.pdf	20/03/2017 11:35:08	HERACLITO BARBOSA DE CARVALHO	Aceito
Outros	Anexo3.pdf	20/03/2017 11:33:58	HERACLITO BARBOSA DE CARVALHO	Aceito
Declaração de Instituição e Infraestrutura	Anexo2.pdf	20/03/2017 11:27:37	HERACLITO BARBOSA DE CARVALHO	Aceito
Recurso Anexado pelo Pesquisador	Anexo1SAYCAREStudy.pdf	20/03/2017 11:25:27	HERACLITO BARBOSA DE CARVALHO	Aceito

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Continuação do Parecer: 2.022.542

Projeto Detalhado / Brochura Investigador	ProjetoDoutoradoTSC.pdf	20/03/2017 11:21:23	HERACLITO BARBOSA DE CARVALHO	Aceito
Folha de Rosto	folhaderosto_ap.pdf	20/03/2017 11:15:04	HERACLITO BARBOSA DE CARVALHO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	10/11/2016 16:01:11	HERACLITO BARBOSA DE CARVALHO	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

SAO PAULO, 19 de Abril de 2017

Assinado por:

**Maria Aparecida Azevedo Koike Folgueira
(Coordenador)**

Endereço: DOUTOR ARNALDO 251 21º andar sala 36

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APROVAÇÃO

O Comitê de Ética em Pesquisa da Faculdade de Medicina da Universidade de São Paulo, em sessão de **17/09/2014**, **APROVOU** o Protocolo de Pesquisa nº **232/14** intitulado: **“DESENHO E IMPLEMENTAÇÃO DO ESTUDO SAYCARE: SOUTH AMERIC YOUTH/CHILD CARDIOVASCULAR AND ENVIRONMENT STUDY”** apresentado pelo Departamento de MEDICINA PREVENTIVA

Cabe ao pesquisador elaborar e apresentar ao CEP-FMUSP, os relatórios parciais e final sobre a pesquisa (Resolução do Conselho Nacional de Saúde nº 466/12).

Pesquisador (a) Responsável: Heráclito Barbosa de Carvalho

Pesquisador (a) Executante: Augusto César Ferreira de Moraes

CEP-FMUSP, 17 de Setembro de 2014.

Prof. Dr. Roger Chammas
Coordenador
Comitê de Ética em Pesquisa

Buenos Aires, Jueves 09 de Octubre de 2014

Dictamen del Comité de Ética en Investigación Clínica

Visto el proyecto epidemiológico "Diseño e implementación del estudio SAYCARE: South American Youth/Child cARdiovascular and Environmental Study" de diseño multicéntrico, observacional y transversal, co-dirigido y presentado por la investigadora responsable en la Argentina, la Prof Dr. Gabriela Berg del Departamento de Bioquímica Clínica- Laboratorio de Lípidos y Aterosclerosis- de la Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, este Comité considera:

Que en el proyecto, se constata que la sede correspondiente a la institución en Argentina se desarrollará en los Laboratorios de Lípidos y Aterosclerosis, ubicado en el ámbito físico del Departamento de Bioquímica Clínica de la Facultad de Farmacia y Bioquímica, se propone investigar los estilos de vida, la salud cardiovascular y el estado nutricional de niños y adolescentes de 7 ciudades de Sudamérica, con el objetivo de desarrollar metodologías estandarizadas fiables y validadas para que pueden ser aplicadas en estudios epidemiológicos multicéntricos de mayor alcance en América del Sur.

Que participarán individuos voluntarios de entre 3 y 17 años sin condición patológica existente, que serán sometidos a diferentes protocolos experimentales que se detallan en el proyecto.

Que el proyecto cuenta con un profesional médico a cargo (Dr. Máximo Soto, MN 98978).

Que se incluyen los aspectos éticos de confidencialidad y de reclutamiento de los participantes.

Que se incluyen los criterios de inclusión y exclusión de los pacientes.

Que se adjunta el formulario para el consentimiento informado y de información al paciente (y el contenido de una encuesta sobre datos personales, hábitos de vida y antecedentes patológicos de los mismos).

Por lo tanto este Comité de Ética en Investigación Clínica considera que se encuentran cumplidos los requisitos y no encuentra objeciones para la realización del Proyecto de Investigación Clínica arriba mencionado, por lo que recomienda su aprobación.

Firma y aclaración de los integrantes del comité:

 Dr. Marcelo R.

 Wilson

Universidad de Buenos Aires
Facultad de Farmacia y Bioquímica

CUDAP: EXP-UBA: 54555/14

BUENOS AIRES, 09 de Octubre de 2014

Visto las presentes actuaciones, mediante las cuales la Doctora Gabriela A. Berg del Laboratorio de Lípido y Lipoproteínas del Departamento de Bioquímica Clínica, solicita la aprobación del Proyecto denominado: "Diseño e implementación del estudio SYCARE: South American Youth/Child Cardiovascular and Environmental Study"; y

CONSIDERANDO:

Que el citado proyecto cuenta con la conformidad de los expertos que han intervenido en el tema;

Que en consecuencia, cabe promover la aprobación pertinente;

Por ello y en uso de las atribuciones que le son propias

**LA DECANA DE LA FACULTAD DE FARMACIA Y BIOQUIMICA
RESUELVE:**

ARTICULO 1º - APROBAR el Proyecto denominado "Diseño e implementación del estudio SYCARE: South American Youth/Child Cardiovascular and Environmental Study", co-dirigido y presentado por la Doctora Gabriela Berg, del Departamento de Bioquímica Clínica.

ARTICULO 2º - Regístrese, Pase para su conocimiento a la Secretaría de Ciencia y Técnica, por su intermedio notifíquese a la interesada. Cumplido, archívese.

RESOLUCION No 3776

Laura Schreier
Secretaria Académica

Cristina Arranz
Decana

Sede de Investigación Universitaria - SIU

Medellín, 25 de agosto de 2014

Doctor

Diego Alejandro Gaitán

Grupo de Investigación en Alimentación y Nutrición Humana
Universidad de Antioquia

Referencia: *"Diseño e implementación del estudio SAYCARE: Estudio sobre los determinantes de la salud cardiovascular en niños y adolescentes de Medellín-Colombia"*

Cordial saludo,

Por medio de la presente comunicación, los miembros del CBE-SIU hacen constar que en su reunión N° 12 del año 2014, realizada el día 20 de agosto, revisaron las respuestas a las observaciones realizadas al acta de aprobación 14-43-596. De esto:

Se consideran satisfactorias, en su totalidad, las respuestas a las observaciones realizadas por el Comité de Bioética al proyecto de la referencia.

Recordamos nuevamente parte del contenido de nuestra anterior comunicación:

1. El Comité considera que las medidas que están siendo tomadas para proteger a los sujetos humanos son adecuadas.
2. El Comité considera adecuada la forma de obtener el consentimiento informado de los participantes en el estudio.
3. El Comité informará inmediatamente a los entes reguladores, correspondientes según el caso
 - a. Todo desacato de los investigadores a las solicitudes del Comité.
 - b. Cualquier suspensión o terminación de la aprobación por parte del Comité.
4. El Comité informará inmediatamente a las directivas de la SIU, toda información que reciba acerca de:
 - a. Lesiones o daños a sujetos humanos con motivo de su participación en la investigación
 - b. Problemas imprevistos que involucren riesgos para los sujetos u otras personas.
 - c. Cualquier cambio o modificación a este proyecto que haya sido revisado y aprobado por este comité.
8. El presente proyecto queda aprobado por un periodo de un (1) año más a partir de la fecha de aprobación.

Comité de Bioética de Investigación en Humanos de la Sede de Investigación Universitaria CBE-SIU
Edificio SIU: Calle 62 52-59 Telefax (574) 2196402
Medellín - Colombia

Sede de Investigación Universitaria - SIU

Los proyectos de duración mayor a un año, deberán ser sometidos nuevamente a este Comité, con todos los documentos que permitan su revisión actualizada: estos incluyen, informe de actividades, fecha de iniciación, modificaciones solicitadas y previamente aprobadas por este Comité

9. El Investigador principal deberá:

- a. Informar cualquier cambio que se proponga introducir en el proyecto. Estos cambios no podrán ejecutarse sin la aprobación previa del CBE-SIU excepto cuando sean necesarios para minimizar o suprimir un peligro inminente o un riesgo grave para los sujetos que participan en la investigación.
- b. Avisar cualquier situación imprevista que se considere implica riesgos para los sujetos o la comunidad o el medio en el cual se lleva a cabo el estudio.
- c. Informar cualquier evento adverso serio de algún paciente, comunicando la situación a la secretaría y al presidencia del CBE-SIU, dentro de las primeras 24 horas de ocurrido el incidente, tal como lo dispone la resolución 2378 de 2008 y la resolución 2011020764 de 2011, ambas del Ministerio de la Protección Social.
- d. Poner en conocimiento del comité toda información nueva importante respecto al estudio, que pueda afectar la relación riesgo/beneficio de los sujetos participantes.
- e. Comunicar cualquier decisión tomada por otros comités con respecto a la investigación que se lleva a cabo.
- f. Informar de la terminación prematura o suspensión del proyecto explicando las causas o razones y las implicaciones que esto tiene para el cumplimiento de los objetivos del proyecto.
- g. Avisar oportunamente sobre la fecha de inicio de esta investigación.
- h.

El Comité de Bioética cuenta con siete (9) integrantes, para que haya quórum es necesario la presencia de al menos cuatro (5) de ellos, en la reunión del 20 de agosto participaron:

ROSALBA DURÁN FORERO- PhD Universidad de Barcelona
DANIEL FLÓREZ MEDINA- especialista en pedagogía virtual TIC's
JUAN CAMILO MEJÍA WALKER- MSc Derecho
IVÁN DARÍO VÉLEZ BERNAL-PhD en enfermedades Infecciosas
MARÍA C. CORREA TABARES- Administradora en Salud

Atentamente,

Nombre: ROSALBA DURÁN FORERO

Título: PhD. Universidad de Barcelona, España

Cargo CBE SIU: Presidenta

Nombre: MARÍA C. CORREA

Título: Administradora en Salud, Universidad de Antioquia

Cargo CBE SIU: Secretaria

PERÚ

Ministerio
de Salud

Instituto Nacional
de Salud del Niño

"Año de la Promoción de Diversificación Productiva
y del Fortalecimiento de la Educación"

Lima, 25 de febrero del 2015

OFICIO N° 00222-CEI-INSN-2015

Doctor

CARLOS DELGADO BOCANEGRA

Investigador Principal

De mi consideración:

Es grato dirigirnos a usted para saludarle cordialmente y con relación al proyecto **E-56/14** titulado: "South American Youth/Child cARdiovascular and Environmental Study" SAYCARE-nodo Perú.

El Comité de Ética en Investigación, aprueba el proyecto de investigación.

La vigencia de esta aprobación es desde el 25 de febrero del 2015 al 24 de febrero del 2016.

Los trámites deberán iniciarse 30 días antes de su vencimiento.

Sin otro particular, quedo de Ud.,

Atentamente,

DR. GAMANIEL GUEVARA CHACABANA

Presidente

Comité de Ética en Investigación

Instituto Nacional de Salud del Niño

RESOLUCIÓN APROBADA

Dr./Lic María Isabel Bove
Tel. Correo E. Isabelbove.uruguay@gmail.com
Dirección: Pagola 3290 apto. 1002

Distinguida Dra:

El Comité de Etica en Investigación con Seres Humanos de la Universidad Católica del Uruguay, después de estudiar toda la documentación relativa al Protocolo de Investigación, presentado por Ud. (**expediente A141014**) en su calidad de Investigador Principal a evaluación de este Comité, bajo el título: “***Desarrollo de métodos para evaluar el comportamiento alimentario, la composición corporal y la actividad física en niños y adolescentes de Montevideo***”, considera que dicho Protocolo:

- cumple los principales requisitos necesarios para la aprobación y no se registran objeciones insuperables que impidan su realización.
- Sin embargo, el CE-UCU estima que deben realizarse cambios de ciertos aspectos previos a un nuevo escrutinio por parte del Comité y a una aprobación definitiva. Dichas enmiendas se detallan a continuación:

1) Con respecto a los **sujetos participantes**:

- a) Se deberá decir con exactitud en qué institución se realizará la investigación (nombre del colegio o escuela), cuántos y que edades concretas tendrán los menores participantes;
- b) Deberá informarse la cantidad de horas que les implicará a los menores participar de esta investigación, dando detalle de las diversas actividades o visitas;
- c) Si el tiempo dedicado será durante o fuera del horario escolar;
- d) Se deberá aclarar también si se informará a cada familia de los resultados de las mediciones hechas a cada uno de sus hijos.

2) **Consentimientos**:

- a) Las informaciones antes detalladas deberán ser incorporadas a cada uno de los tres consentimientos previstos (consentimiento de la familia, del menor y de la institución);
- b) En cada consentimiento debe indicarse que el Protocolo ha sido aprobado por nuestro Comité y el nexo electrónico para comprobar dicha información;
- c) Deberá adjuntarse nota con el consentimiento firmado por la institución donde se va hacer este estudio piloto;
- d) Deberá informarse a los padres y a la institución que características precisas tiene el instrumento llamado “acelerómetro” y que tipo de molestias específicas puede causar a los menores.

3) **Instrumentos de medida**:

- a) Debe informarse al Comité que características precisas tiene el aparato llamado “acelerómetro”. En particular nos interesa saber si emite ondas electromagnéticas o similares y qué tipo de molestias específicas puede causar.

b) Deben adjuntarse al Comité todos los cuestionarios que se van a utilizar en la investigación, con sus textos o consignas completos.

4) Confidencialidad.

El Comité necesita saber las medidas adecuadas que se aplicarán para asegurar el respeto a la intimidad cuando se interroga o se recaben los datos de a cada menor o a sus padres.

5) Compromiso de los investigadores.

Se deberá presentar al Comité nota con el compromiso de los investigadores que deberán suscribir todos los investigadores.

- En suma: que si se cumplen las modificaciones referidas el citado **Protocolo desde el punto de vista científico y ético podrá ser oportunamente aprobado, una vez que nos remita las enmiendas y se dicte la resolución definitiva.**

Dr. Omar França
Presidente

Dr. Raúl Gamarra
Secretario

Montevideo, 16 de octubre de 2014

COMITÉ DE ETICA

Acta de Aprobación Nº 4 Miércoles 18 de Marzo de 2015

Asisten: Ana María Pino presidenta (Bioquímico, Prof Asociado), Cecilia Algarín (Médico Cirujano, Prof. Asociado), Marcela Castillo (Sicóloga, Prof. Asistente), Gerardo Weisstaub (Médico Cirujano, Prof. Asistente)

Preside: Prof Ana María Pino Z

Proyecto: "Diseño e implementación del estudio saycare: south american youth/child cardiovascular and environmental study"

Investigadora: Juliana Kain

Documentos revisados: Proyecto y documentos de consentimiento informado.

Se analiza el Proyecto a la luz de los postulados de la Declaración de Helsinki, del Código de Nüremberg y del Reglamento para comités de Ética de la Universidad de Chile.

Sobre la base de la información proporcionada en el texto (Copia en archivo) y de los antecedentes aportados personalmente por la investigadora, el Comité de Ética, estima que el estudio no significa para los sujetos involucrados, riesgos físicos, psíquicos, social, legal o de otra naturaleza, propios de este tipo de investigación.

En la evaluación del proyecto el comité de ética del INTA consideró los siguientes fundamentos: 1- el valor social del proyecto resulta del desarrollo de métodos de medición válidos y confiables para obtener información antropométrica, de actividad física, de hábitos de actividad, sociales, ambientales, y de entorno familiar en niños y adolescentes de seis ciudades de América del Sur. 2- El proyecto tiene validez científica, según se desprende del análisis del documento completo, incluyendo los antecedentes de los investigadores. 3- En el diseño no hay discriminación arbitraria de participantes; el estudio en Chile comprende pre escolares y escolares (3 grupos de 50 niños c/u) que asisten a escuelas municipales de Santiago. 4 - Se estimó que el consentimiento informado expresa la información necesaria en un lenguaje claro, incluyendo la protección de los derechos de las personas al establecer la posibilidad de no participar y de contactar a este comité en caso de algún reclamo sobre el trato recibido. 6- Los miembros del Comité no tienen ninguna relación con el patrocinante, ni con la investigadora que pudiera ser motivo de conflicto de interés.

En virtud de tales consideraciones el Comité otorga la autorización correspondiente para la realización del estudio dentro de las especificaciones señaladas en el Proyecto de Investigación y en el consentimiento informado.

Cualquiera modificación del protocolo debe ser autorizada por este Comité. Una vez finalizado el estudio, el comité deberá ser informado de los resultados de éste.

Atentamente,

Ana María Pino Z.
Presidenta