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PROGRAMA DE PÓS-GRADUAÇÃO EM PEDIATRIA: ATENÇÃO À SAUDE DA CRIANÇA E DO ADOLESCENTE

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Primary health care intervention reduces added sugar consumption during childhood: promising findings from a cluster randomized trial

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Dissertação submetida ao Programa de Pós-Graduação Pediatria: Atenção à Saúde da Criança e do Adolescente da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Mestre

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Aos meus pais, Simone e Marco, e meu irmão, Luigi, pelo apoio incondicional,

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RESUMO

Objetivos: Verificar o impacto da atualização dos profissionais de saúde sobre o consumo de açúcar adicionado e avaliar as principais fontes alimentares contribuintes para esse consumo entre crianças. **Metodologia:** Análise longitudinal aninhada a um ensaio de campo randomizado por conglomerados realizado com pares de mãe-criança residentes da cidade de Porto Alegre – RS. A randomização ocorreu entre as unidades de saúde participantes, alocadas em intervenção (n=11) e controle (n=9). A intervenção foi realizada com os profissionais de saúde e baseada nos "Dez Passos para Alimentação Saudável para Crianças Menores de Dois Anos". Gestantes no último trimestre de gestação foram entrevistadas quanto à situação socioeconômica, demográfica e de saúde. A ingestão dietética das crianças foi avaliada com auxílio do software Dietwin® aos 6 meses, 12 meses, 3 anos e 6 anos. O consumo de açúcar adicionado foi estimado por meio do recordatório alimentar de 24 horas, considerando a definição de açúcar adicionado da USDA. Os alimentos fonte de açúcar foram posteriormente classificados em (1) Bebidas açucaradas (2) Alimentos ultraprocessados (3) Preparações caseiras e (4) Açúcar de mesa. Todas as análises estatísticas foram realizadas pelo *Statistical Package for the Social Science*, versão 21.0. O impacto da intervenção na ingestão de açúcar adicionado foi verificado por meio da Equação de Estimativa Generalizada. Os dados de consumo em gramas foram apresentados como média, desvio padrão e porcentagem de contribuição para a energia total. A significância estatística foi estabelecida em $P < 0,05$. **Resultados:** Aos 12 meses, crianças do grupo intervenção apresentaram menor consumo de açúcar proveniente de preparações caseiras (diferença -1,43g/dia; IC95% -1,70 a -1,16; $p = 0,01$). O maior impacto foi observado aos 3 anos, com menor consumo de açúcar total e proveniente de alimentos ultraprocessados (diferença -6,36g / dia; IC95% -11,49 a -1,23 e diferença -4,38g / dia; IC95% -7,80 -0,96; $p = 0,012$ e $p = 0,015$, respectivamente). **Conclusão:** A intervenção realizada com os profissionais de saúde mostrou-se eficaz em reduzir o consumo de açúcar adicionado de preparações caseiras aos 12 meses e de alimentos ultraprocessados aos 3 anos. Ainda assim, o consumo de açúcar adicionado tem início precoce e está acima do recomendado, tendo como principais contribuintes os alimentos com alto grau de processamento e as bebidas açucaradas.

ABSTRACT

Objectives: Verify the impact of the health workers update on the added sugar consumption and evaluate the main food sources that contribute to this consumption among children. **Methods:** Longitudinal analysis nested to a cluster randomized trial conducted with pairs of mother-children in Porto Alegre - RS. Randomization occurred between participating health units, allocated to intervention (n = 11) and control (n = 9). The intervention was carried out with health workers and based on the "Ten Steps to Healthy Eating for Children Under Two Years". Women in the last trimester of pregnancy were interviewed regarding socioeconomic, demographic and health status. Children's dietary intake was assessed with the aid of the Dietwin® software at 6 months, 12 months, 3 years and 6 years. The consumption of added sugar was estimated using the 24-hour food record, considering the USDA definition. Sugar sources were further classified into (1) Sugar sweetened beverages (2) Ultra-processed foods (3) Homemade recipes and (4) Table sugar. All statistical analyzes were performed using the Statistical Package for the Social Science, version 21.0. The impact of the intervention was verified using the Generalized Estimation Equation. All consumption data were presented as mean, standard deviation and percentage of contribution to total daily energy. Statistical significance was established at $P < 0.05$. **Results:** At 12 months, children in the intervention group had lower consumption of sugar from homemade recipes (difference -1.43g/day; 95% CI -1.70 to -1.16; $p = 0.01$). The major impact was observed at 3 years of age, with lower consumption of total sugar and sugar from ultraprocessed foods (difference -6.36g/day; 95% CI -11.49 to -1.23 and difference -4.38g/day; CI95 % -7.80 -0.96; $p = 0.012$ and $p = 0.015$, respectively). **Conclusion:** The intervention performed with health workers proved to be effective in reducing the consumption of added sugar from to homemade recipes at 12 months and sugar from ultraprocessed foods at 3 years. Even so, the consumption of added sugar starts early and is above the recommended level, with the main contributors being foods with a high degree of processing and sweetened beverages.

LISTA DE ABREVIATURAS

AHA	American Heart Association
CI	Confidence Interval
COMA	Committee on Medical Aspects of Food Policy
DGCA	Dietary Guidelines Advisory Committee
EFSA	European Food Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition
GEE	Generalized Estimation Equation
NHANES	National Health and Nutrition Examination
NMES	Non-milk extrinsic sugars
SACN	Scientific Advisory Committee on Nutrition
SPSS	Statistical Package for the Social Science
US	United States
USDA	United States Department of Agriculture
%TE	Percentage of Contribution to Total Energy
WHO	World Health Organization

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1 INTRODUÇÃO

De acordo com o *United States Departament of Agriculture (USDA)*, açúcares de adição são aqueles adicionados aos alimentos como ingrediente pelo consumidor ou durante o processamento dos alimentos pelo fabricante (1). Os possíveis desfechos do consumo precoce de açúcar na infância têm sido associados à exposição precoce ao açúcar e posterior preferência pelo sabor doce (2,3), resultando na maior aceitação de alimentos açucarados (4) e consequentemente no desenvolvimento de sobrepeso (5), obesidade (6) e cárie dentária (7).

O consumo de açúcar de adição entre crianças difere entre países desenvolvidos e não desenvolvidos. Uma recente revisão sistemática envolvendo latino americanos revelou que no Brasil esse consumo pode chegar a 100g/dia ainda na adolescência (8). Entre os principais contribuintes para o aumento do consumo pode-se destacar os alimentos ultraprocessados, amplamente reconhecidos como fontes de sal, açúcar e gorduras (9) e as bebidas açucaradas, consideradas como a maior fonte de açúcar entre crianças nos Estados Unidos (10).

Na tentativa de estabelecer um limite para o consumo de açúcar na infância, a *World Health Organization (WHO)* recomenda que o consumo de açúcares adicionados não ultrapasse 10% da ingestão energética diária para crianças maiores de 24 meses (11). Antes dos 2 anos de idade, a *American Heart Association* sugere que o açúcar adicionado seja evitado (12). Para tanto, estudos de intervenção têm investigado formas de frear o consumo do nutriente por meio de taxação de bebidas açucaradas (13), subsídios para alimentos in natura (14) e mudanças na rotulagem de alimentos (15). Duas revisões sistemáticas que avaliaram os resultados de parte dessas intervenções concluíram que a qualidade e a eficácia das evidências ainda são moderadas e modestas, respectivamente (16), e que novas intervenções devem se concentrar na avaliação desses efeitos a longo prazo (17). Além disso, nenhum estudo incluído nas duas revisões avaliou os desfechos em crianças menores de 5 anos.

Considerando as evidencias acerca do prejuízo na saúde de crianças determinado pelo consumo precoce de açúcar adicionado e os parâmetros recomendados pelas organizações de saúde mundiais, o objetivo desta dissertação foi avaliar se um programa de atualização para profissionais de saúde obteve impacto no consumo de açúcar de adição por crianças atendidas em unidades de saúde no sul do Brasil.

2 REVISÃO DA LITERATURA

2.1 Açúcar: Definições, recomendações dietéticas e desfechos negativos na saúde

2.1.1. Definições

Embora o termo açúcar seja convencionalmente descrito como todo mono e dissacarídeo presente nos alimentos (18), órgãos responsáveis por emitir recomendações dietéticas tem utilizado diferentes nomenclaturas para descrever o açúcar adicionado aos alimentos (19), o que pode vir a confundir os consumidores e que comumente é objeto de discussão na literatura científica, assim como o limite de ingestão diária e os desfechos de saúde envolvidos em seu consumo.

Para o *Committee on Medical Aspects of Food Policy (COMA)*, o açúcar não naturalmente presente nos alimentos – exceto a lactose adicionada no leite e nos produtos lácteos – é chamado de *Non-milk extrinsic sugars (NMES)* (20). Para a *American Heart Association (AHA), European Food Safety Authority (EFSA), Dietary Guidelines Advisory Committee (DGCA)* e *United States Department of Agriculture (USDA)*, os açúcares e xaropes adicionados durante o processamento e preparação dos alimentos são atribuídos ao termo *Added sugar* (12,21,1). No ano de 2015, a *World Health Organization (WHO)* cunhou o termo *Free sugar* como mono e dissacarídeos adicionados a alimentos e bebidas pelo fabricante, cozinheiro ou consumidor, incluindo açúcares naturalmente presentes no mel, xarope, sucos de fruta e concentrado de suco de fruta (11). Posteriormente, as instituições *Scientific Advisory Committee on Nutrition (SACN)* e *European Society for Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition (ESPGHAN)* publicaram recomendações dietéticas utilizando o termo *Free sugar* (22,23).

2.1.2 Recomendações dietéticas

Os efeitos adversos do consumo de açúcar na infância têm sido uma questão de interesse público e científico. Na última década, diversas instituições se posicionaram sobre o limite de ingestão diária de açúcares entre crianças e adultos, em 2009, a *American Heart Association* propôs que o limite de consumo de açúcares adicionados não ultrapasse 36g para um homem adulto com ingestão energética diária de 2.200kcal (12). Um ano depois a *European Food Safety Authority* publicou que os dados disponíveis ainda não permitiam a definição de parâmetros de referência ou limite de ingestão diária para açúcares totais ou adicionados (21). Entre 2015 e 2017, *USDA, WHO e Dietary Guidelines Advisory Committee*

estabeleceram recomendações de que o consumo de açúcares adicionados e livres não deve ultrapassar 10% do consumo energético total entre crianças de 2 a 18 anos (1,11,24) e não mais que 5% desse consumo de acordo com *SACN e ESPGHAN* (22,23)

Em relação às recomendações dietéticas para crianças menores de dois anos, são poucas as evidências disponíveis na literatura. No Brasil, a primeira versão do Guia Alimentar para crianças menores de dois anos recomendava que o açúcar não fosse ofertado antes dos 12 meses de idade, salientando que a oferta de novos alimentos adicionados de açúcar diminui a aceitação de outras formas de preparação do alimento (25). A nova versão do material foi recentemente divulgada pelo Ministério da Saúde, estendendo a restrição do consumo de açúcar para até 2 anos de idade e salientando que não se deve (1) adoçar frutas e bebidas com nenhum tipo de açúcar: branco, mascavo, cristal, demerara, açúcar de coco e nem melado, mel ou rapadura ou (2) ofertar preparações que tenham açúcar como ingrediente, como bolos, doces, geleias e biscoitos doces e (3) ofertar alimentos ultraprocessados, visto que o açúcar está presente em grande parte destes alimentos; justificando que a recomendação contribui para a formação de hábitos alimentares mais saudáveis. Internacionalmente, a *American Heart Association* declarou que açúcares adicionados devem ser evitados antes dos dois anos de idade, alegando que não há espaço para calorias livres de nutrientes na dieta habitual de crianças pequenas (12).

2.1.3 Desfechos negativos na saúde

Essas recomendações baseiam-se na estreita relação entre exposição precoce ao açúcar e formação dos hábitos alimentares nos primeiros anos de vida (2,3,26), aumentando a palatabilidade e por consequência a aceitação de alimentos ultraprocessados e bebidas açucaradas ao longo dos anos. Sabe-se que crianças nascem com preferência inata pelos sabores doce, salgado e umami e rejeição dos sabores azedo e amargo, além de serem geneticamente predispostas a preferir alimentos densos em energia (27). A experiência com esses sabores começa no útero, por meio do líquido amniótico e, mais tarde, na amamentação, em que os sabores da dieta da mãe são experimentados (28). A soma dos fatores genéticos à introdução precoce de açúcar adicionado pode promover o desenvolvimento de paladar adocicado acentuado, e refletir em outros fatores alimentares.

Ainda que consolidada a relação entre exposição precoce e preferência ao sabor doce, os resultados publicados são, na sua maioria, restritos aos desfechos de cárie bucal (7). Entretanto, existem evidências suficientes para acreditar na associação entre o consumo

excessivo de açúcar e o aumento de ingestão energética diária (29), maior adiposidade corporal (30), peso corporal (5) e obesidade (6) além de forte evidência entre o consumo de açúcar adicionado e o aumento do risco de doenças cardiovasculares (12).

2.2 Alimentos ultraprocessados

2.2.1 Classificação NOVA

Com o aumento global no consumo de açúcares adicionados, a identificação dos principais alimentos fonte desse consumo tem sido objetivo de estudos de base populacional. Resultados têm evidenciado a preferência por produtos ultraprocessados em detrimento aos alimentos in natura, minimante processados e preparações realizadas em casa (31,32), devido à alta conveniência e hiper palatabilidade desses produtos. Essa preferência caracteriza mudança nos padrões alimentares da população, e torna necessária uma forma de avaliação dietética em que o nível de processamento dos produtos não seja negligenciado.

Nesse contexto, uma nova classificação de alimentos foi proposta por um grupo de pesquisadores brasileiros, atribuindo os gêneros alimentícios a três grupos de acordo com a extensão e finalidade do processamento utilizado em sua produção (33). Essa proposta foi atualizada e reconhecida como ferramenta válida para pesquisa (9). A nova versão sugere que a classificação dos produtos alimentícios se dê em quatro grupos:

Grupo 1 - Alimentos não processados ou minimamente processados: Partes comestíveis de plantas (sementes, frutos, folhas, caules, raízes) ou de animais (músculo, miudezas, ovos, leite) e também fungos, algas e água, após a separação da natureza.

Grupo 2 - Ingredientes culinários processados: Substâncias obtidas diretamente dos alimentos do grupo 1 ou da natureza por processos como prensagem, refinação, moagem e secagem por atomização.

Grupo 3 - Alimentos processados: Produtos relativamente simples feitos pela adição de açúcar, óleo, sal ou outras substâncias do *Grupo 2*. A maioria dos alimentos processados tem dois ou três ingredientes. Os processos incluem vários métodos de conservação ou cozimento e, no caso de pães e queijos, fermentação não alcoólica.

Grupo 4 - Alimentos ultraprocessados e produtos para beber: Formulações industriais tipicamente com cinco ou mais ingredientes. Tais ingredientes frequentemente incluem

aqueles também usados em alimentos processados, como açúcar, óleos, gorduras e sal, além de antioxidantes, estabilizadores e conservantes.

De acordo com os autores, a formulação e os ingredientes dos produtos contemplados no *Grupo 4* tornam esses alimentos altamente convenientes (prontos para o consumo), atrativos (hiper palatáveis), rentáveis (ingredientes de baixo custo) e, portanto, altamente competitivos em relação à alimentos que estão naturalmente prontos para consumir e refeições preparadas na hora. Como resultado de sua formulação, os produtos pertencentes a esse grupo de alimentos são intrinsecamente desequilibrados em nutrientes e tendem a ser consumidos em grandes quantidades (9).

2.2.2 Consumo na infância

Estudos de análise populacional têm se beneficiado da classificação *NOVA* para avaliar a disponibilidade e o consumo de alimentos de acordo com os diferentes níveis de processamento. Recentemente, o mesmo grupo de pesquisadores mostrou que a disponibilidade destes produtos no domicílio varia em média de 10,2%, 13,4%, 46,2% a 50,4% em Portugal, Itália, Alemanha e Reino Unido, respectivamente. O estudo conclui que entre os dezenove países avaliados, alimentos ultraprocessados representam 26,4% do total dos alimentos adquiridos (34). Outros estudos corroboram que essa aquisição independe da condição socioeconômica da família (35), pois tem sido alta em todos os grupos socioeconômicos (36).

Embora o percentual de alimentos ultraprocessados na dieta infantil também atinja altos parâmetros, são poucos os estudos que avaliam o consumo desses produtos ainda na infância. No Brasil, o período crítico para introdução de alimentos ultraprocessados está entre o terceiro e o sexto mês (37), com prevalência de consumo de 43% já no final do primeiro ano de vida (38). Uma análise transversal realizada com 1185 crianças participantes de um estudo de corte de nascimento da cidade de São Luís, Maranhão, mostrou que o consumo de ultraprocessados já representa em média 24,5% da ingestão energética diária em crianças de apenas 13 a 35 meses (39).

A partir dos dois anos, a escolha dos alimentos que serão consumidos passa a envolver a autonomia da criança e os hábitos alimentares já adquiridos nos primeiros anos de vida, além da influência do que é ofertado pelos pais e cuidadores. Por esse motivo, os resultados encontrados nessa faixa etária espelham a preferência alimentar adquirida, com maior chance

de ser reportada ao longo da vida. Estudos de análise transversal e de coorte realizados no México, Chile e Brasil mostraram que o consumo de ultraprocessados colabora com 30%, 38,6% e 49,2% da energia total consumida em um dia (40,41,42), com consumo acentuado especialmente entre pré-escolares (41).

O consumo de ultraprocessados também tem sido avaliado sob a ótica de um alimento fonte de açúcar de adição. Nessa perspectiva, 38,4% de açúcares adicionados consumidos por crianças entre o primeiro e o quarto ano de vida são provenientes de alimentos com alto grau de processamento (43). Entre crianças australianas com idade entre 2 e 3 anos, o grupo de alimentos que contemplava bolos, biscoitos, doces e produtos à base de massa também obteve a maior parcela de contribuição no consumo de açúcar adicionado (15,8% e 20,3%) em dois estudos semelhantes (44,45). Latasa e colaboradores realizaram uma análise detalhada ao longo das duas últimas décadas e verificaram que alimentos ultraprocessados são fonte de 80,4% dos açúcares adicionados consumidos pelas famílias (46). Para Steele e colaboradores esse percentual chega a 89,7%, sugerindo que a diminuição do consumo de alimentos ultraprocessados pode ser uma maneira eficaz de reduzir a ingestão excessiva de açúcares adicionados nos Estados Unidos (47).

2.2.3 Desfechos negativos na saúde

Fatores dietéticos

Com o aumento do consumo de alimentos ultraprocessados reconhecidamente adicionados de açúcar, óleos, gorduras e sal (9), o teor de carboidratos, açúcares livres, gorduras totais, gorduras saturadas e sódio na dieta de crianças e adolescentes aumenta significativamente (48,36) enquanto o conteúdo de proteína, fibra e potássio diminui (49,50), resultando em um perfil nutricional não saudável (51). A participação dos alimentos ultraprocessados também determina a qualidade nutricional geral e impacta de forma negativa nas dietas infantis (49,48).

Em busca de uma nova perspectiva, uma análise transversal aninhada a ensaio de campo randomizado por conglomerados conduzida por Sangalli e colaboradores avaliou a ingestão dietética de crianças de 2 a 3 anos de idade residentes da região sul do Brasil, e revelou baixa prevalência de crianças com consumo insuficiente de micronutrientes entre aquelas que possuíam elevado consumo de alimentos ultraprocessados, revelando até mesmo risco de consumo excessivo de micronutrientes fornecidos por esses alimentos (52).

Fatores clínicos

A composição nutricional desequilibrada somada ao consumo excessivo dos alimentos ultraprocessados tem sido descrita como possível mecanismo gerador de desfechos de saúde desfavoráveis. Uma revisão sistemática encontrou associações positivas entre o consumo desses alimentos e aumento da gordura corporal durante a infância e a adolescência (53). Além disso, há evidências suficientes para acreditar que grupos com maior consumo de alimentos ultraprocessados apresentam maior chance de apresentar excesso de peso e obesidade, quando comparados a grupos que possuem menor consumo (54,55).

Um estudo realizado por Rauber e colaboradores mostrou que o consumo de ultraprocessados na idade pré-escolar foi fator preditor para o aumento do colesterol total e LDL na idade escolar, salientando o risco de consumo excessivo de energia, aumento de peso, e por consequência desenvolvimento de doenças crônicas não transmissíveis relacionadas à dieta (42). Outra análise envolvendo a mesma amostra de crianças evidenciou que o consumo de alimentos ultraprocessados também esteve associado ao aumento na circunferência da cintura, sugerindo que o consumo precoce de alimentos ultraprocessados desempenha um papel no aumento da obesidade abdominal em crianças (53).

2.3 Bebidas açucaradas

2.3.1 Definição

Outro importante contribuinte na ingestão de açúcares de adição em crianças e adultos são as bebidas açucaradas (10). Por definição, são consideradas bebidas açucaradas aquelas adicionadas de adoçantes calóricos (sacarose, xarope de milho rico em frutose e concentrados de suco de frutas), incluindo refrigerantes, bebidas à base de frutas, bebidas esportivas, bebidas energéticas e com vitaminas, chá gelado adoçado, polpa e xarope de fruta, com exceção de suco natural de fruta (56). Apesar de composição nutricional semelhante, bebidas naturalmente adoçadas devem receber análise diferenciada devido ao teor de potássio, cálcio, vitaminas A, C e fortificação de vitamina D, mesmo que se assemelhem na quantidade de açúcares e de valor energético (57).

2.3.2 Consumo na infância

Bebidas açucaradas são atualmente a maior fonte de açúcar de adição e a principal fonte de energia diária entre crianças nos Estados Unidos (10). Um estudo que avaliou

tendências de consumo entre os anos de 1990 e 2016 encontrou aumento significativo na disponibilidade global dessas bebidas, além de se tornarem acessíveis com maior rapidez entre países de baixa e média renda (58). Dentre os possíveis determinantes desse consumo, a ingestão de lanches do tipo *fast food* apresenta-se como importante preditor do consumo de bebidas adoçadas, enquanto o hábito de tomar café da manhã é considerado fator protetor (59). Em relação à influência familiar, pais e cuidadores consumidores de bebidas açucaradas exercem influência positiva no consumo dessas bebidas por parte dos filhos, enquanto pais com maior nível de escolaridade têm menor chance de ofertar esses produtos aos seus filhos. O consumo também varia de acordo com a idade da criança, acentuado entre a faixa etária pré-escolar (59,60)

Em relação ao consumo de bebidas açucaradas como fonte de açúcar adicionado, um estudo de análise transversal avaliou a ingestão dietética de crianças de 2 a 8 anos e categorizou o consumo de açúcar adicionado em tercis. No tercil mais baixo, os três maiores contribuintes foram cereais prontos para consumo (14,8%), produtos doces de padaria (14,7%) e bebidas açucaradas (12,4%). Nos tercis intermediário e alto, as bebidas açucaradas passaram a ocupar o primeiro lugar (22,8% e 36,8%) (61). Refrigerantes e bebidas de frutas também foram as principais fontes de consumo de açúcares adicionados entre as 9092 crianças avaliadas por Afeiche e colaboradores. No estudo, bebidas açucaradas contribuíram com 43%, 28% e 7% da ingestão total de açúcar no México, Estados Unidos e China, respectivamente (62). Entre crianças não amamentadas do primeiro ao quarto ano de vida, mais da metade do consumo de açúcares adicionados foi proveniente do grupo de bebidas açucaradas (*regular soda, fruit, flavored, sports, and energy drinks, sweetened coffee and tea, aguas frescas and homemade SSBs e sweetened milk and milk beverages*) (43).

As tendências na ingestão e nos alimentos fonte de açúcares adicionados entre crianças e adolescentes norte-americanos de 2 a 18 anos foram avaliadas em um importante estudo de Slining e Popkin, que utilizou cinco pesquisas representativas dos Estados Unidos (*CSFII 96*, n=8259; *NHANES 04*, n=3435; *NHANES 06*, n=3652; *NHANES 08*, n=2594 e *NHANES 10*, n=2763). Nos resultados apresentados, bebidas açucaradas foram os maiores contribuintes para o consumo de açúcar de adição em todas as etapas avaliadas (7,5%, 8,1%, 6,4%, 5,7% e 4,9%). Embora o consumo de açúcares adicionados tenha diminuído entre 1994-1998 e 2009-2010 ($p<0,05$), os autores concluem que a ingestão média diária continua a exceder os limites recomendados em 18 a 28% do consumo total de energia (63). Malik e colaboradores também encontraram diminuição moderada do consumo de bebidas

açucaradas entre os americanos, no entanto, salientam que entre nos países em desenvolvimento o consumo tem aumentado rapidamente (64).

No Brasil, o consumo de bebidas adoçadas inicia majoritariamente antes do primeiro ano de vida e em menor parte antes dos seis meses de idade (37). Em termos de acessibilidade, Popkin e Howkes revelaram que o país está enfrentando crescimento nas vendas de bebidas açucaradas, estando entre os dez países que mais compram bebidas dessa categoria em uma comparação a nível mundial; com destaque para o consumo de refrigerantes (65).

2.3.3 Desfechos negativos na saúde

As evidências entre consumo de bebidas açucaradas e obesidade têm se acumulado desde a última década: para cada porção adicional dessas bebidas, tanto o índice de massa corporal quanto a frequência de obesidade aumentam, mesmo após ajuste para variáveis antropométricas, demográficas, dietéticas e de estilo de vida. (66). O consumo de bebidas açucaradas superior a 120ml ao dia também esteve associado ao maior risco de obesidade e hipertrigliceridemia entre crianças de 7 a 18 anos (67).

Acredita-se que o principal promotor do ganho de peso gerado pelo consumo de bebidas açucaradas seja o excesso de calorias. No entanto, o risco do desenvolvimento de doenças cardiovasculares e diabetes mellitus tipo 2 pode aumentar de forma independente, por meio de resposta glicêmica adversa e efeitos metabólicos exclusivos, como aumento da lipogênese hepática, acúmulo de adiposidade visceral e ectópica e produção de ácido úrico (64). Nesse contexto, além de apresentar maior consumo de açúcar, crianças que consomem bebidas adoçadas também apresentaram maiores níveis de glicemia e triglicerídeos, quando comparadas as que não consomem, mesmo após ajuste para o índice de massa corporal (68). Duas revisões sistemáticas com meta análise evidenciaram que o consumo habitual de bebidas açucaradas está associado a alta incidência de diabetes tipo 2 (69), independentemente da adiposidade corporal (70).

2.4 Estratégias de intervenção para diminuir consumo de açúcar

Diferentes ações para reduzir o consumo de açúcar foram propostas nos últimos anos, incluindo taxação de bebidas açucaradas (71), modificações na rotulagem nutricional (72) e a proposta de bebidas alternativas às opções açucaradas, que podem incluir água, suco natural

de frutas, café, chá, e bebidas adoçadas artificialmente (73). Entre os resultados observados, a substituição de uma porção de bebida açucarada por uma porção equivalente de água foi significativamente associada a menor ganho de peso (74) e menor risco de ter diabetes (75).

Dessa forma, considerando a importância dos primeiros anos de vida na formação dos hábitos alimentares, intervenções para promover práticas alimentares saudáveis devem ser encorajadas ainda na infância (76). Estudos de intervenção tem utilizado diferentes estratégias para diminuir o consumo de açúcar em crianças, que podem incluir reuniões presenciais, websites informativos e mensagens de texto (77) o que se mostrou eficiente na redução do consumo de bebidas açucaradas nos filhos e perda de peso corporal nas mães (78). Famílias que aceitaram participar de estudo randomizado e controlado receberam visitas de profissional formado em nutrição e dietética para auxiliar na otimização de dieta e hábitos de atividade física em cerca de 10 intervenções (79), resultando no menor consumo de carboidratos e açúcares de adição quando comparados ao grupo controle (80).

Com dados da mesma população deste estudo, Vitolo e colaboradores concluíram que a intervenção foi efetiva em aumentar a prevalência de aleitamento materno exclusivo e reduzir alimentos de alta densidade energética (balas, refrigerantes, salgadinhos e chocolates) no grupo intervenção (81). Apesar dos resultados promissores, uma intervenção com maior enfoque no consumo de alimentos fonte de açúcar poderia vir a trazer benefícios ainda maiores. Ainda, os aspectos culturais, comportamentais, escolaridade, nível socioeconômico e acompanhamento (82) deverão ser considerados no desenvolvimento de novas estratégias para que maiores resultados possam ser alcançados.

3 OBJETIVOS

3.1 Objetivo Principal

Avaliar o impacto de um programa de atualização em alimentação infantil para profissionais de saúde no consumo de açúcar adicionado em crianças aos 6 meses, 12 meses, 3 anos e 6 anos de idade.

3.2 Objetivos Secundários

- Verificar a participação das bebidas açucaradas, alimentos ultraprocessados, preparações caseiras e açúcar de mesa no padrão alimentar das crianças entre a faixa etária avaliada.
- Estimar a contribuição do açúcar adicionado proveniente desses alimentos para a ingestão energética diária das crianças avaliadas.

4 HIPÓTESES

Hipótese nula: Não houve impacto da intervenção realizada com os profissionais de saúde no consumo de açúcar adicionado pelas crianças avaliadas.

Hipótese alternativa: Houve impacto da intervenção realizada com os profissionais de saúde no consumo de açúcar adicionado pelas crianças avaliadas.

5 CONCLUSÕES

Os resultados desta dissertação possibilitam concluir que a intervenção realizada com os profissionais de saúde foi efetiva em reduzir o consumo de açúcar adicionado pelas crianças pertencentes ao grupo intervenção aos 12 meses e aos 3 anos de idade. Os alimentos que forneceram o consumo de açúcar nessa população, especialmente ultraprocessados e bebidas açucaradas, devem fazer parte do enfoque de futuras intervenções de caráter populacional que busquem melhorar o padrão de consumo alimentar infantil detectado por este estudo. Nesse contexto, o padrão alimentar dos primeiros dois anos de vida, determinante na formação das preferências alimentares ao longo da vida, deve ser tema de atualização para os profissionais de saúde atuantes na atenção básica, visando o aprimoramento dos conhecimentos acerca da alimentação infantil. O aleitamento materno exclusivo até os seis meses de idade e o início adequado da introdução alimentar após esse período podem ser aliados para resultados promissores. No entanto, para que os parâmetros recomendados pela Organização Mundial da Saúde e pela Academia Americana de Pediatria sejam alcançados, a restrição ao consumo de açúcar adicionado por crianças menores de dois anos deverá ser claramente priorizada.

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ANEXOS

ANEXO A – ARTIGO ORIGINAL

Será submetido ao *The Journal of Nutrition*

Primary health care intervention reduces added sugar consumption during childhood: promising findings from a cluster randomized trial

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Short Title: Primary care intervention reduces sugar in children

List of Abbreviations: US – United States; USDA – United States Department of Agriculture; AHA – American Heart Association; EPIC – European Prospective Investigation into Cancer and Nutrition; SPSS – Statistical Package for the Social Science; GEE – Generalized Estimation Equation; %TE – Percentage of Contribution to Total Energy; NHANES – National Health and Nutrition Examination; CI – Confidence Interval.

Abstract

Background: The early introduction to added sugar reinforces the preference for sweet taste and increases sugar consumption in childhood, widely associated with overweight, obesity and dental caries. Educational strategies that aim to avoid added sugar early in life could positively impact eating habits and provide a healthy start to food choices.

Objective: We aimed to evaluate the impact of a primary care intervention on added sugar intake and assess the major food sources contributing to this consumption.

Methods: A cluster field trial (ClinicalTrials.gov NCT00635453) was conducted at health centers in Porto Alegre, Brazil, randomly assigned to intervention (n=9) or control (n=11) group. At the intervention sites, health workers joined a training session on the Ten Steps for Healthy Feeding for Children from Birth to Two Years of age. Eligible pregnant women from these centers were enrolled for the follow-up of their children. Dietary data was collected using 24-h recalls among children between 6 months and 6 years of age. Added sugar sources were further classified based on NOVA classification. The impact of the intervention on added sugar intake was verified using the Generalized Estimation Equation. The consumption data in grams were presented as mean, standard deviation and percentage of contribution to total daily energy. Statistical significance was established at $P<0.05$.

Results: At 12 months of age, children in the intervention group had lower consumption of sugar from homemade recipes (difference -1.43g/day; 95% CI -1.70 to -1.16; p = 0.01). The major impact was observed at 3 years of age, with a decrease in total sugar and sugar from ultraprocessed foods (difference -6.36g/day; 95% CI -11.49 to -1.23 and difference -4.38g/day; CI95 % -7.80 -0.96; p =0.012 and p = 0.015, respectively).

Conclusions: These findings suggest that health workers training in a primary care setting is a promising and feasible intervention strategy to reduce added sugar consumption in childhood.

Introduction

Dietary sugars are important contributors to the overall intake and may promote a positive energy balance (1), representing up to 30% of daily energy intake in children at 1 to 8 years of age in Europe (2), 27% at 2 to 5 years of age in the United States (3) and being a large source of calories among Asian children at 6 and 12 months of age (4). Recent data have also evaluated added sugar consumption by Brazilian children (5,6) which mostly begins before 6 months of age (7) and is consumed by 95% of them in the first year of life (8).

According to current recommendations, children over two years of age should not consume more than 10% (1) or 5% (9) daily energy from free sugars. The same upper limit was established for added sugars (10), which should be avoided in the first two years of life (11). Those recommendations are based on the know association between early sugar exposure and later preference for sweet tastes (12,13,14), contributing to higher acceptance of ultra-processed foods and sugar-sweetened beverages. In addition, several conditions such as increased energy intake (15), higher body fat index (16), higher body weight (17), obesity (18) and caries development (19) has been associated with excessive sugar consumption in childhood, being able to remaining throughout adult life.

To avoid excessive consumption of added sugar, effective interventions should start early in life. Two recent systematic reviews have shown some strategies to reduce sugar consumption by parental involvement component (20) and home based interventions (21). However, the quality and effectiveness of evidence was moderate and modest, respectively, and the authors suggest that further interventions should focus on assessing long term effects. In addition, no trials included in the systematic reviews evaluated children under 5 years of age.

Our team has conducted a cluster randomized trial in a primary care clinic setting, updating health workers on the national dietary guidelines (22). The outcomes of the study were verified in children from 6 months to 6 years of age, with successful results on improving breastfeeding (23), complementary feeding practices (24) and reducing functional constipation (25) at 6 years of life. Thus, considering the high frequency of sugar consumption early in life worldwide and the new recommendations restricting this practice, the objective of the current analysis was to verify the impact of the clinic interventions on added sugar intake and assess the major food sources contributing to this consumption.

Methods

Trial design and participants

We conducted a longitudinal analysis based on a cluster-randomized field trial targeting selected primary health care centers in Porto Alegre, South of Brazil. The trial included health centers that provide primary care services predominantly to low-income families. Of the 52 municipal health centers, 21 were excluded for met at least one exclusion criteria (≤ 100 infant patient visits in 2006, staff sharing between clinics, or participation in other contemporaneous, community-based dietary programs).

The randomization process took place in the university research office. Of the 31 eligible health centers, 16 were initially selected via a witnessed draw, by the principal investigator, of labeled markers from an opaque container, such that two health centers would be included from each of the city's eight geo-administrative districts. Following a stratified randomization scheme, the health centers were block-randomized by district, with one health center per district allocated to the intervention group and another to the control group. To increase statistical power, four additional health centers from the original 31 were randomly drawn. To maintain a balanced number of births by intervention group, the additional four health centers were block-randomized at a 1:3 ratio. This yielded 9 intervention and 11 control group health centers.

The sample size calculations were based on the calculation for the primary outcome of the original trial, exclusive breastfeeding rate. Six hundred mother-child pairs were needed to detect a statistical difference between a 40% prevalence of exclusive breastfeeding up to four months of age in the intervention group (22) and a 25% prevalence in the control group, with 90% power, α of 0.5, and a design effect of 1.5. We therefore recruited 715 pregnant women to obtain the required study power, taking into account an anticipated loss to follow-

up of 20%. Thus, the sample size of this analysis varied according to the availability of data from the initial trial.

Interviewers visited the intervention and control health centers from April to December 2008 to identify and enroll women who were in the last trimester of pregnancy and reporting a negative HIV test. Of 736 pregnant women who were eligible, 715 (97.1%) agreed to participate and answered a questionnaire about their socioeconomic status and expected due date. Addresses and telephone numbers were obtained in order to schedule subsequent home visits. Births from May 2008 to February 2009 were included for study.

Intervention

Physicians, nurses and administrative staff of all intervention health centers participated in a training session in January 2008 based on the Ten Steps for Healthy Feeding for Brazilian Children from Birth to Two Years of Age guideline (26). The main researcher conducted a training session for the health care team to outline the Ten Steps recommendations and strategies and to provide suggestions how best to incorporate these into the consultations. Printed materials were provided to the Health Care Centers for use by these professionals and for access to the Brazilian Ministry of Healthy Nutrition Department´s website. Health staff members received a pocket guide for use during the appointments.

The Ten Steps recommend: (1) providing exclusive breastfeeding until 6 months of age; (2) continued breastfeeding until two years of age with the gradual introduction of complementary foods; (3) starting complementary feeding (grains, meat, fruits) at 6 months of age 3 times daily while continuing breastfeeding; (4) providing meals at regular intervals, adjusted to the child's internal hunger cues; (5) making new foods gradually thicker until the child is able to eat a normal family meal, but foods should never be liquefied; (6) providing a variety of healthy foods every day; (7) providing a daily intake of different fruits and

vegetables; (8) avoiding sugar, sweets, soft drinks, salty snacks and processed/fried foods; (9) using good hygiene practices for food preparation and handling; and (10) providing appropriate, responsive feeding when the child is ill.

The study provided flyers to health workers hand to mothers during the clinic appointments, encouraging them to ask more information from the health worker if needed. In addition, two colorful posters were provided by us for display in the waiting rooms sessions of health care centers with (1) information about complementary meals and (2) ultra-processed foods commonly consumed by children (e.g. cookies, coffee, jelly, salty foods, candy, soft drinks and snacks). The posters remained in the clinics throughout the intervention period and reinforcing the training sessions carried out with health professionals.

Control group

In the healthcare centers randomized to the non-intervention group, the guideline update program was not carried out and the staff continued their routine assistance, providing the standard care without any interference of the research team. None of the materials developed to the intervention group were provided to these clinics. All mothers were encouraged to maintain normal pediatric visits for their children during the study period.

Data collection

When pregnant women in the last trimester of pregnancy attended consultations at health centers, the research team invited them to participate and obtained home address, telephone number and expected due date, as reported above. When children achieve 6 months of age, the team made phone calls to schedule the first visit and obtained the baseline data. Up to 3 years of age, data collection continued at home. At six years old, when data collection included laboratory and dental exams, the team carried out the home visit and invited mothers

to go to the research center of the study to obtain the data of the final stage. During the six-year follow-up all participants were advised to seek the health care centers if any questions or doubts concerning an infant's nutrition, diet or any health problems were raised during the interviews. Data collection by the interviewers was verified through telephone call backs to a 5% random sample of interviewed mothers. At all waves of the study, tracing and re-enrollment was attempted of children who were lost to follow-up at previous examinations.

Dietary assessment

At six months of age, the 24-hour recall was collected during the single home visit. At 12 months and 3 years of age, the first 24-hour recall was sustained and a second recall was added, requiring a second home visit with an interval of 2 weeks to 1 month from the first interview. At six years of age, the first recall was carried out at home and the second at the research center, during complementary exams.

Details about food types, amounts, recipes and brands were collected in all waves. Common household measures (e.g. teaspoons, tablespoons, cups and serving sizes) were used to help mothers report the amounts of food given to their children and to standardize portion sizes, and then converted to grams using a table for assessment of food consumption in home measures (27).

For children at 6 and 12 months of age, breast milk intake was estimated using methods developed by Dewey (28), based on the total number of breastfeeding frequency. For child who received breast milk 6 times a day or more, the estimated volume was 130 ml/feed; 3 to 5 feeds a day, 89 ml/feed; and less than 3 feeds a day, the volume assigned was 53 ml/feed. Breast milk energy density was taken to be 67kcal/100ml.

Dietary energy intake was estimated using the Dietwin® software program (version 2008 professional; Dietwin®), based on US Department of Agriculture food composition

tables (USDA, Agricultural Research Service, 1998). Manufacturers' information on industrial products and national food composition tables was included to the program when available.

Assessment of added sugars

From a biochemical point of view, sugars correspond to a dietary monosaccharides (glucose, fructose, and galactose) and disaccharides (sucrose and lactose) which may be intrinsically or extrinsically present on food composition. However, several authors has made distinctions between non-milk extrinsic sugars (i.e. not contained within the cellular structure of a food except lactose in milk and milk products) (29), added sugars (i.e. added to foods by the manufacturer, cook or consumer) (10) and free sugars (i.e. added sugars plus sugars naturally present in fruit juices) (1). As added sugar is the most comprehensive term, and the main difference between added and free sugars consist on sugars naturally present in fruit juices, we decided to use added sugars. Examples of this term may include white and brown sugar, cane sugar, dextrose, molasses, honey and all types of syrups, according to USDA definition (10).

Information on added sugar intakes were obtained from food reported on 24-hour recalls. Added sugars values for these food items were determined using the USDA definition (10). In most cases, sugars contents on this food items were estimated using the food nutrition label providing by the manufacturers. For added sugars not found in the nutrition label, the Nutrition Composition Tables of Foods Consumed in Brazil (30) and USDA Food Composition Table (31) were used. When data on sugar content were not available, we estimate by comparison with similar products.

Additionally, the mean daily consumption of added sugar was used to verify percentage of energy from added sugars according to USDA recommendation, from the

Dietary guidelines for Americans (10). For inappropriate consumption, we consider AHA guidelines, in which children should not consume added sugars before age two (11).

Food sources of added sugar

To investigate main food sources of added sugars on this population, we classified all sugar sources into four groups (1) Sugar-sweetened-beverages (2) Ultraprocessed food (3) Homemade recipes and (4) Table sugar. *Sugar sweetened beverages* included sodas, processed and concentrated juices, juice boxes, yogurts and dairy drinks. *Ultraprocessed foods* included ready-to-eat foods, noodles, powder soups, mayonnaises, processed meat, breads, chips, crackers, biscuits, stuffed biscuits, cookies, chocolate powder, candies, fruit jellies, breakfast cereals, enriched flours, *petit suisses* and gelatins. Some examples of *Homemade recipes* are lasagna pasta and another types of pasta, homemade breads, cookies, puddings and jellies. *Table sugar* considers white sugar, brown sugar and honey. These four groups were based on NOVA, a classification that considers the extent and purpose of industrial food processing (32). Artificially sweetened beverages were not included on this analysis.

Statistical methods

Usual dietary intake of sugar was estimated using the Multiple Source Method, a web-based tool developed by researchers at the European Prospective Investigation into Cancer and Nutrition (EPIC) for estimating usual dietary intakes of nutrients and foods consumed by populations and individuals, available at https://msm.dife.de/.

All statistical analyzes were performed using Statistical Package for the Social Science, version 21.0 (SPSS Inc.). All data were double entered independently by different staff members for subsequent validation in EPI-INFO, version 6·4 (CDC) and later resolution

240 of any discrepancies. The presence of outliers was investigated using box-plot graphs and 25
241 cases were excluded based on added sugar intake: 8 at age 6 months (4 intervention vs. 4
242 control), 9 at 12 months (6 intervention vs. 3 control), 8 at 3 years (3 intervention vs. 5
243 control) and at 6 years (1 intervention vs. 2 control). The Kolmogorov–Smirnov test was used
244 to assess distributions of normality where applicable.

245 Maternal and family characteristics who were lost to follow-up and those who
246 remained in the study were compared using the Student's t test. To verify the impact on added
247 sugar intake at intervention and control groups, we performed a Generalized Estimation
248 Equation (GEE). The role consumption data were presented as means, standard deviation and
249 percentage of contribution to total energy (%TE). Statistical significance was set at $P<0.05$.

Results

From 715 pregnant women enrolled at baseline, 633 infants presented nutritional assessments at ages 6 months, 545 at 12 months, 476 at 3 years and 387 at 6 years. For the purpose of this study, at age 6 months, children exclusive breastfeeding were not considered into analysis (n=20), as well as those with genetic and congenital diseases (n=14) **(Figure 1)**. During the intervention, no adverse events were reported. Dietary data were available for 591 children (306 intervention *vs.*285 control) at ages 6 months, 511 (272 intervention *vs.* 240 control) at 12 months, 438 (230 intervention *vs.* 208 control) at 3 years and 347 (185 intervention *vs.* 162 control) at 6 years. Losses to follow up were mainly due to withdrawal from the study or inability to locate. Pattern of those losses was previously checked and defined as similar into both groups of randomization **(data not shown)**. There was no difference between randomization groups for selected baseline characteristics. Overall, family monthly income was low for most families (approximately US$600/month) and school age was less than eight years in 30% of mothers (**Table 1**).

At the first wave of randomization, children from intervention and control groups presented a similar consumption of added sugar, reaching 17.58 and 20.48 grams per day, respectively. At 12 months of age, the intervention significantly decreased the intake of added sugar from homemade recipes (difference -1.43g/day; 95% CI -1.70 to -1.16; p=0.01). The major impact of this study was observed on children at 3 years of age, when intervention group consumed less added sugar from ultraprocessed foods (difference -4.38g/day; 95% CI -7.80 to -0.96; p=0.015) and total added sugar (difference -6.36g/day; 95% CI -11.49 to -1.23; p=0.012), compared to the control group. At the last wave, children of 6 years old consumed more than 100 grams of added sugar on the day the 24-hour record was collected. All means of added sugar consumption by age and randomization group are available on **Table 2**.

Regarding the contribution of added sugar consumption to daily energy intake, we observed an upward trend from 8% of total daily energy at 6 months to 23% at 6 years of age, representing a threefold increase over the period (**Table 2**). Besides that, nearly one third of children at 6 months of age already exceeded the USDA recommended limit of 10% daily energy from added sugars. This proportion raise up to 66.7% of children at 12 months, 90.4% and 94.5% at 3 and 6 years of age, respectively.

Added sugars from ultraprocessed food – such as baby cereals and biscuits and *petit suisses* – account for 56.31% of total added sugar in control group and 52.44% in intervention group (p=0.09) at 6 months, and remains as the principal contributor for added sugar intake at 12 months (53.44% intervention and 51.26% control; p=0.77) and 3 years of age (48.17% intervention vs. 50.53% control; p=0.90). When children achieve 6 years of age, sugar sweetened beverages becomes the major contributor to increase added sugar consumption in this population (46.23% intervention vs. 41.16% control; p=0.68).

Discussion

The impact of dietary counseling on health care workers on infant feeding in the first year of life was effective in reducing added sugar intake, with statistical differences at 12 months and 3 years of age.

The lower added sugar consumption from homemade recipes at 12 months of age suggests that mothers and caregivers were sensitized by health workers, adding less sugar to the meals prepared for family and consequently for children. We believe that the widespread use of baby cereals, which was consumed by almost 37% of this sample, may have negatively influenced the impact of the intervention on other variables at this age. Nowadays, we know that these products are the major source of added sugar in children's consumption (33), but they were not included in the list of foods not recommended at the time. Future interventions should, therefore, emphases the high energy density and sugar content present in these products (34).

The major impact of the intervention was observed at 3 years of age, with a 9% reduction in total added sugar intake. This finding can be explained, in part, by the lower consumption ultra-processed foods among children in the intervention group already recognized in previous analyzes (data not published). The Step 8, that was reinforced among the health care workers during the training, suggested avoiding the consumption of energy dense foods – such as sweets, soft drinks, salty snacks and processed/fried foods – in the first 2 years of life (26).

In addition to significant results, we found a lower consumption of added sugar in another 13 of 20 variables analyzed, although did not reach statistical significance. In this regard, we would like to emphasize that added sugar was not a primary objective throughout the intervention carried out. Moreover, we have to deal with the widespread use of baby bottles and baby cereals (36). After the first two years of life, however, when dairy products

313 are no longer the main contributors to total daily energy and the pattern of food preferences
314 is already established (37), the impact of the intervention could be observed.

315 Despite promising results, it is important to emphasize that the added sugar intake
316 remained above the recommendations in both randomization groups. In general, sugar
317 consumption by children at age 6 months has already reached 20g/day, representing more
318 than 5% of total daily energy. At age 12 months, children already exceeded the
319 recommendations of American Heart Association of no more than 25g per day (11) and the
320 World Health Organization of 10 % of total daily energy (1).

321 Comparing these results to studies with similar methods and the same definitions of
322 added sugar we found that sugar consumption was higher than estimated in developed
323 countries. Data from the National Health and Nutrition Examination (NHANES) 2011-2012
324 showed that the intake of added sugar starts with 3.5g/day in the first year of life, reaching
325 100g/day only in early adulthood (38). According to recent publication from NHANES, in
326 2014, the percentage of energy supplied by added sugars among children aged 2 to 19 years
327 remained below 15% (39). In development countries, sugar consumption intensifies and
328 reaches around 110g/day even during adolescence (40). A possible explanation for similar
329 results observed in American countries is the social determinants involved the consumption
330 of added sugar, which involves socioeconomic disadvantage, accessibility to the place of
331 food purchase, the family's eating habits and peers influence (38).

332 The low cost, long shelf life and hyper-palatability of products with a high degree of
333 processing (41) make sugary drinks and ultra-processed foods the main contributors to added
334 sugar from birth to six years of age. These results are in
335 accordance with a recent study that evaluated children, adolescents and adults, where sugary
336 beverages and foods were the major contributors to sugar consumption at all ages evaluated
337 (42).

The same characteristics that raise ultra-processed foods consumption also represent some of the major challenges to avoid these products by children. To avoid excessive consumption, several countries are taxing food and beverages (43), subsidizing fresh foods (44) and changing food labeling (45). In this scenario, the Brazilian Ministry of Health reviewed and published the new "Food Guide for Brazilian Children up to Two Years" (46). The new guide contains "12 Steps to Healthy Eating", with focus on not offering sugar or preparations and products containing added sugar to children up to two years of age. This document supports the performance of health workers and can provide an important tool to change the infant feeding scenario presented in this study.

Our study has some limitations. First, it was not possible to discriminate added sugar in sucrose, fructose, glucose or high fructose corn syrup. So far, in Brazil, this information is not available in national food composition tables or in nutritional information provided by manufacturers. Thus, our intention was to verify the impact on added sugar in general terms, as well as the consumption of added sugar and the foods that contributed to this consumption. Second, this study was carried out in a group of low-income children, which may limit the applicability of the study findings for more privileged populations. In the other hand, the less privileged socioeconomic groups in Brazil are larger and more socially vulnerable. However, the study has also two important strengths: first, the longitudinal design that allows us to verify the long-term impact of intervention strategies and, second, the use of the NOVA system, internationally recognized as a tool with the potential to assist in the development of future public policies.

Conclusion

The intervention was carried out with health workers proved to be effective in reducing the consumption of added sugar from homemade preparations at 12 months and

ultra-processed foods at 3 years of age. Even so, the consumption of added sugar starts early and is above recommended, having as main contributors foods with a high degree of processing and sugary drinks.

Statement of authors' contributions to manuscript

M.R.V designed and conducted research; P.S.B and C.N.S. analyzed data; and P.S.B., C.N.S. and J.L.V. wrote the paper. P.S.B. had primary responsibility for final content. All authors read and approved the final manuscript.

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Figure 1. Flow diagram of the study.

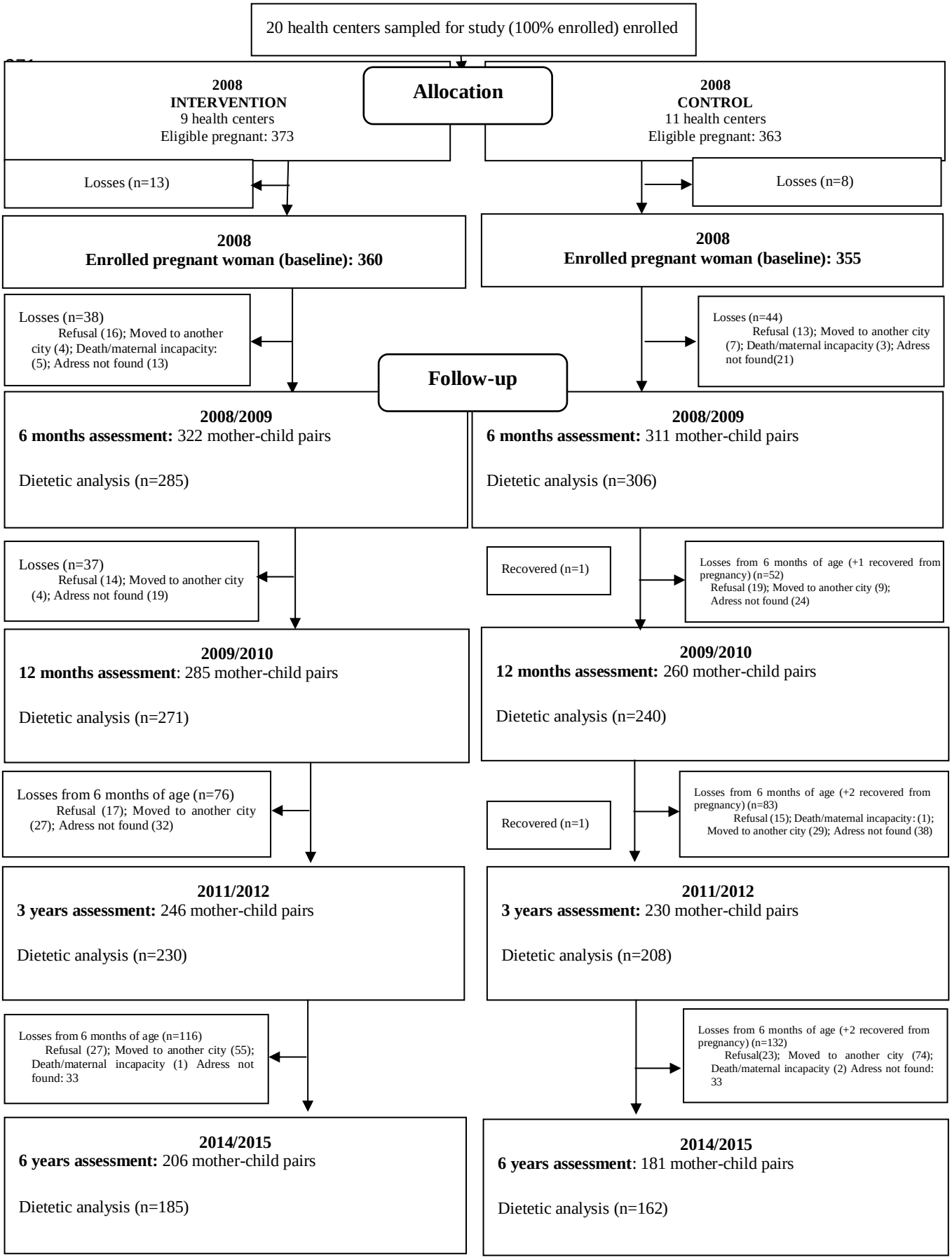

Table 1 – Baseline characteristics of children and family in the intervention and control groups.

Characteristics	**Intervention** n=306	**Control** n=285
Age, (months), mean (SD)	5.9(0.6)	5.9 (0.6)
Boys, n (%)	52.8 (168)	52.6 (152)
Mother		
Age<20 years, n (%)	72 (20.0)	76 (21.4)
Education < 8 years, n (%)	27.8 (100)	35.3 (102)
BMI >25, n (%)	114 (36.2)	115 (37.2)
Unemployment, n (%)	65.0 (234)	70.6 (204)
Father		
Education <8 years, n (%)	27.0 (92)	31.2 (86)
Unemployment, n (%)	9.9 (34)	12.5 (35)
Economic status		
Monthly family income (US$), mean (SD)	613.22±434.22	567.01±366.10

*7mean (SD): mean (standard deviation); BMI: body mass index; * significant difference P<0.05*

Table 2. Added sugar consumption in children at 6 months, 12 months, 3 years and 6 years of age in intervention and control groups.

	6 months			12 months			3 years			6 years		
	Control n=(285)	Intervention n=(306)		Control n=(240)	Intervention n=(271)		Control n=(208)	Intervention n=(230)		Control n=(162)	Intervention n=(185)	
Source of added sugar	**Mean±SD**	**Mean±SD**	**Difference (95% CI)**	**Mean±SD**	**Mean±SD**	**Difference (95% CI)**	**Mean±SD**	**Mean±SD**	**Difference (95% CI)**	**Mean±SD**	**Mean±SD**	**Difference (95% CI)**
Sugar sweetened beverages (g)	1.58±3.82	1.26±5.31	-0.31 (-1.22;0.60)	6.16±5.92	6.16±6.00	0.00 (-1.38;1.39)	26.56±15.71	24.18±14.92	-2.38 (-5.05;0.29)	43.79±24.83	46.31±30.50	2.52 (-3.85;8.89)
Ultraprocessed food (g)	10.74±12.18	9.90±13.50	-0.84 (-3.91;2.22)	19.03±12.05	17.54±10.87	-1.28 (-4.02;1.45)	36.71±18.06	32.32±15.47	-4.38† (-7.80;-0.96)	44.66±18.58	41.14±17.61	-3.52 (-7.47;0.42)
Homemade recipes (g)	0.26±2.22	0.79±9.55	0.52 (-0.47;1.53)	2.22±1.07	0.79±1.65	-1.43† (-1.70;-1.16)	2.57±2.74	2.93±2.77	0.36 (-0.02;0.74)	9.40±7.05	9.01±7.21	-0.39 (-2.23;1.43)
Table sugar (g)	7.88±15.37	5.61±12.35	-2.27 (-5.08;0.54)	10.70±17.78	7.76±13.57	-2.94 (-6.35;0.46)	7.52±12.20	7.64±12.73	0.11 (-2.61;2.85)	6.28±11.38	5.58±12.87	-0.69 (-4.14;2.75)
Total added sugar (g)	20.48±22.96	17.58±25.80	-2.89 (-8.57;2.78)	37.42±24.89	33.21±20.72	-4.21 (-9.91;1.48)	73.34±25.26	66.98±22.51	-6.36† (-11.49;-1.23)	104.44±32.77	102.50±35.70	-1.93 (-11.02;7.14)
Energy intake (kcal)	957.70±384.13	900.26±333.56	-57.43 (-115.06;0.19)	1034.66±488.12	1004.47±438.35	-30.19 (-110.01;49.62)	1564.46±491.57	1444.91±377.95	-119.54 (-200.76;-38.33)	1880.47±532.58	1831.22±547.95	-49.25 (-163.18;64.68)
Total added sugar as percentage of energy intake (%)	7.81±7.14	6.85±7.35	-0.95 (-2.71;0.79)	14.91±7.21	13.64±6.47	-1.27 (-2.90;0.35)	19.53±5.77	19.02±5.58	-0.50 (-1.74;0.72)	22.86±5.94	23.08±6.53	0.22 (-1.00;1.53)

† P<0.05; SD, Standard Deviation; CI, Confidence Interval; Total sugar as percentage of energy intake: energy intake/total sugar x 100%; Complete dietary data were available for 591 children at age 6 months, 511 at 12 months, 438 at 3years and 347 at 6 years.

ANEXO B – APROVAÇÃO NO COMITÊ DE ÉTICA EM PESQUISA DA UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO FACULDADE FEDERAL DE CIÊNCIAS MÉDICAS DE PORTO ALEGRE
COMITÊ DE ÉTICA EM PESQUISA
APROVADO PELA CARTA Nº 880/2004-CONEP/CNS/MS
RUA SARMENTO LEITE, 245 – FONE: (51) 3224.8822
CEP 90050-170 – PORTO ALEGRE – RS - cep@fffcmpa.edu.br

Of. 532/07-CEP

Porto Alegre, 06 de novembro de 2007.

Ilma. Sra.
Profa. Márcia Regina Vitolo
Nesta Faculdade

Senhora Professora

Informamos que seu projeto intitulado "Implementação dos Dez Passos da Alimentação Saudável para Crianças Menores de Dois Anos nas Unidades Básicas de Saúde.", Processo nº 226/07, foi aprovado por este Comitê, na reunião de 14 de junho 2007, conforme parecer consubstanciado nº 471-07.

Atenciosamente,

ANEXO C – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

O presente estudo **IMPLEMENTAÇÃO DOS DEZ PASSOS DA ALIMENTAÇÃO SAUDÁVEL PARA CRIANÇAS MENORES DE DOIS ANOS NAS UNIDADES BÁSICAS DE SAÚDE** visa comparar as condições de crescimento e desenvolvimento de crianças cujas mães foram submetidas a orientações de práticas alimentares adequadas por profissionais de saúde especificamente treinados e capacitados com crianças atendidas de acordo com a rotina da rede básica de saúde.

Utilizaremos questionário para fazer-lhe perguntas sobre sua família, o qual conterá: condições de vida (sociais e econômicas), moradia, práticas alimentares de seu filho, atividades diárias e presença de doenças. Será realizada avaliação aos 6 e 12 meses de idade por meio das medidas de peso, altura, quantidade de gordura corporal as quais não conferem riscos nem dor para seu filho. Em data marcada com o pesquisador, ao final dos doze meses de acompanhamento, será realizada coleta de sangue por profissional treinado com agulhas descartáveis, sem risco de contaminação, para realização de hemograma, análise dos níveis de ferritina e proteína C reativa. A criança sentirá um pequeno desconforto no momento da picada, porém não haverá riscos a sua saúde. Entretanto, não há outra forma de verificação que possa fornecer resultados mais precisos. Essas informações serão transformadas em números e a identidade da sua família não será divulgada em nenhum momento.

Este estudo é importante para prevenção de deficiências nutricionais no primeiro ano de vida, diminuição da freqüência de morbidades, aumento do tempo de aleitamento materno exclusivo e redução da prevalência de crianças com deficiência de ferro. Além disso, propõe a implementação de uma política pública que possa ser reproduzida em toda a rede básica de saúde, dentro da prática de Atenção Primária em Saúde. A senhora receberá todos os resultados das avaliações e orientações ou encaminhamentos se necessário para o melhor bem estar seu e de seu filho. A senhora também terá toda a liberdade de interromper a entrevista em qualquer momento ou de pedir maiores esclarecimentos caso tenha alguma dúvida. Assinará duas cópias desse consentimento, ficando uma em seu poder e a outra com a responsável do programa.

Eu, ...(responsável pela criança) fui informado dos objetivos da pesquisa acima de maneira clara e detalhada. Recebi informação a respeito do tratamento recebido e esclareci minhas dúvidas. Sei que em qualquer momento poderei solicitar novas informações e modificar minha decisão se assim eu o desejar. Fui certificado (a) de que todos os dados desta pesquisa referentes ao meu filho serão confidenciais e terei liberdade de retirar meu consentimento de participação na pesquisa, face a estas informações. Caso tiver novas perguntas sobre este estudo, posso chamar a pesquisadora responsável no telefone 81629929 ou 33038798. Para qualquer pergunta sobre os meus direitos como participante deste estudo ou se penso que fui prejudicado pela minha participação, posso chamar o Comitê de Ética em Pesquisa da FFFCMPA, localizado na Rua Sarmento Leite, 245 .Telefone: (51) 3303-8798 .

Declaro que recebi cópia do presente Termo de Consentimento.

Nome da criança	Assinatura do responsável	Data
Nome do pesquisador	Assinatura do pesquisador	Data

Termo de Consent... Versão aprovada CEP/SMS

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ANEXO D – NORMAS PARA PUBLICAÇÃO

Manuscript Preparation

JN is limited in the number of pages that can be published each year, and article length is a consideration in the editorial process. Manuscripts up to 5000 words maximum from Introduction through Discussion, will be considered. Maximum word count does not include the Title Page, Abstract, Acknowledgments, Author Contributions, References, Figure Legends, or Tables. Authors are encouraged to be clear and concise. Papers must be completely double spaced. Number the lines continuously (not per page) beginning with the abstract and ending before the references, tables, and figures. Number pages consecutively in the upper right-hand corner of each page, beginning with the title page. Manuscript submissions that are not formatted correctly may be returned to authors. For a succinct list of formatting requirements, please see the Quick List Formatting on page 14. Foreign authors are advised to have their manuscripts reviewed by a colleague who is fluent in English.

JN encourages authors to provide the names, fields of interest, addresses, telephone and fax numbers, and e-mail addresses of 4–6 unbiased and qualified potential expert reviewers who do not have a conflict of interest.

Include in your research manuscript:

Title Page

Abstract page

Introduction

Methods

Results

Discussion

Acknowledgments and statement of authors' contributions to manuscript

References

1. Title page

The title page must include:

A title that is composed as a single declarative statement and focused on the results presented in the manuscript. The title should include the animals, participants, or cells studied. Please do not use a colon or semicolon in the title. Keep the title as generally applicable as possible. It usually is not necessary to include the exact study location or a specific study name in the title, because this information can be included in the abstract.

The names of all authors (first name, middle initial, last name)

Author Affiliations (departmental and institutional) at the time the research was done.

Sources of support
The word count for the entire manuscript (introduction through discussion).

Conflict of Interest and Funding Disclosure - List any existing financial arrangements between an author and a company whose product figures prominently in the submitted manuscript or between the author and any company or organization sponsoring the research reported in the submitted manuscript. If an author has no conflicts of interest, list the author's name, followed by "no conflicts of interest." For detailed guidelines on possible conflicts of interest, see the ASN Journals Conflict of Interest Guidelines (5).

Corresponding Author name, mailing address, telephone number, and e-mail address

The word count for the entire manuscript (introduction through discussion).

The number of figures (to print, not Supplementary data).

The number of tables (to print, not Supplementary data).

Supplementary data submitted.

A running title of 50 or fewer characters and spaces.

a list of abbreviations and their definitions for all abbreviations used in the text if there are 3 or more

2. Abstract page

A properly constructed and informative abstract is helpful for the initial editorial review of the submitted manuscript. Research articles must include a structured abstract that contains no more than 300 words, is written in complete sentences, includes information pertinent to any clinical trial registry in which a trial is registered, and uses the following headings:

Background. Provide 1 or 2 sentences that explain the context of the study.
Objective. State the precise objective, the specific hypothesis to be tested, or both.
Methods. Describe the study design, including the use of cells, animal models, or human subjects. Identify specific methods and procedures.
Results. Report the most important findings, including key data and results of statistical analyses.
Conclusions. Summarize in 1 or 2 sentences the primary outcomes of the study, including their potential importance (avoid generalizations). Include the participants, animals, or cells studied.

Review articles, special articles, and reports should include an unstructured abstract (no more than 300 words) that states the purpose of the article and emphasizes the major concepts and conclusions. Any abbreviations used in the abstract should be defined in the abstract at first mention.

Below the abstract, provide and identify 5–10 keywords or short phrases, including the subject group, that will help to increase the discoverability of your manuscript; do not use

adjectives. Terms that are fundamental to your manuscript but are not included in your manuscript title or abstract are especially important to include to increase discoverability by indexing services such as PubMed.

Please note that during manuscript submission, you will be asked to supply keywords to assist the editors in locating suitable reviewers for your manuscript. Keywords for reviewer searches should include the terms most fundamental to your manuscript, and may differ from your list of keywords for publication.

Authors submitting original research, RANS, or critical reviews also are required to submit a lay summary as part of the article, in addition to the main text abstract. The lay summary should clearly summarize the focus and findings of the article for non-expert readers, and will be published as part of the article online and in PDF. The lay summary should be submitted for peer review as part of the main manuscript file, under the heading "Lay summary," before the article's main text. The lay summary should be no longer than 200 words. As with a main abstract, avoid citations and define any abbreviations.

3. Introduction

Describe clearly the background to the research conducted and the specific objectives. This should not be a comprehensive review of the literature, however. State the specific objective or hypothesis of the study.

4. Methods

Documentation of methods and materials used should be sufficient to permit replication of the research. Describe clearly the experimental design including the control and experimental groups. State the source of specialized materials, diets, chemicals, and instruments and other equipment, with model or catalog numbers, where appropriate. Specify kits, analyzers, and commercial laboratories used. Cite references for methods whenever possible and briefly explain any modifications made.

Human and animal research. Reports of human studies must include a statement that the protocol was approved by the appropriate institutional committee or that it complied with the Helsinki Declaration as revised in 1983. Registration is required for all clinical trials that began after July 1, 2010. When preparing reports of randomized, clinical trials, refer to the checklist published in the CONSORT Statement (6). Include a CONSORT flow diagram as a manuscript figure summarizing participant flow with the sizes (n) of initial (recruited, enrolled) and final groups. Indicate in both the abstract and the manuscript text whether the outcomes reported are primary or secondary outcomes of the study. For systematic reviews and meta-analyses, refer to the PRISMA checklist and include a PRISMA flow diagram as a figure in the manuscript (7). CONSORT and PRISMA checklists can be uploaded as supplemental material for the benefit of reviewers and editors.

Research on animals should include a statement that the protocol was approved by the appropriate committee or complied with the Guide for the Care and Use of Laboratory

Animals (8). Compliance with the ARRIVE guidelines is encouraged and the checklist can be uploaded as supplemental material (9). Describe how animals were euthanized. Describe control and experimental animals or participants, giving age, weight, sex, race, and for animals, breed or strain. Include the supplier of experimental animals.

Diets. Composition of control and experimental diets must be presented. When a diet composition is published for the first time in *JN*, provide complete information on all components in a table. If previously described in *JN* or *AJCN*, a reference may be used. State specifically any modifications made to the published diet compositions. The proximate composition of closed formula diets should be given as amounts of protein, energy, fat, and fiber. Express components as g/kg diet. Vitamin and mineral mixture compositions should be included using *JN* units and nomenclature. For a discussion of the formulation of purified animal diets, refer to Baker (10) and to a series of ASN publications (11–14). The experimental diets should differ from the control diets only in the nutrient(s) being investigated. Nonpurified diets generally should not be used as control diets; animals fed these diets should be included for reference only and their data should not be included in the statistical analysis.

Statistical methods. Describe all statistical tests utilized and indicate the probability level (P) at which differences were considered significant. If data are presented in the text, state what they represent (e.g., mean ± SEM). Indicate whether data were transformed before analysis. Specify any statistical computer programs used.

Present the results of the statistical analysis of data in the body of each table and on figures per se. Use letters or symbols to indicate significant differences; define these in a table footnote or the figure legend. Provide the appropriate statistics of variability with an estimate of the error variance (SD or SEM) of group means. Standard ANOVA methodology assumes a homogeneous variance. If error variance is tested and found to be heterogeneous, transform data before ANOVA, or use nonparametric tests. For a discussion of variability calculations and curve-fitting procedures, see Baker (10).

If non-significant P values are reported, use only 2 digits past the decimal (e.g., P=0.15). Present significant P values to a maximum of 4 decimal places (e.g., P<0.0001); using fewer is acceptable. Present coefficients to a maximum of 2 decimal places (e.g., r=0.87, R^2=0.16, etc.).

5. Results

Report the results of the study without repeating the methodology, Introduction, or content in the Discussion section. Do not duplicate data from tables or figures in the text.

6. Discussion

In the Discussion, explain the importance of the findings, putting them into the context of the existing literature. Clearly state the overall conclusions.

7. Acknowledgments

Technical assistance and advice may be acknowledged in a section at the end of the text. Only named individuals should be included in this section. Authors are responsible for obtaining written permission from everyone providing a personal communication or acknowledged by name in the manuscript and for providing to the Editor a copy of the permission, if requested.

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Authors must indicate their contribution(s) to the manuscript in the Acknowledgments section. Use the relevant descriptors listed below unless the author performed a function that clearly is not covered by one of these. All manuscripts, including reviews, must indicate who is responsible for design, writing, and final content and must include a statement affirming that all authors have read and approved the manuscript. The initials of all authors must be included.

designed research (project conception, development of overall research plan, and study oversight).

conducted research (hands-on conduct of the experiments and data collection).

provided essential reagents, or provided essential materials (applies to authors who contributed by providing animals, constructs, databases, etc., necessary for the research).

analyzed data or performed statistical analysis.

wrote paper (only authors who made a major contribution).

had primary responsibility for final content.

other (use only if categories above are not applicable; describe briefly).

All authors have read and approved the final manuscript. For single-authored research papers and reviews, please state: The sole author had responsibility for all parts of the manuscript.

Please do not include "obtained funding." The initials of authors who received grants may be included in the footnote on the title page regarding Support.

An example is:
A. X., R. F. G., and P. G. Y. designed research; R. F. G. and Q. C. conducted research; P. T. analyzed data; and A. X., P. G. Y. and Q. C. wrote the paper. P. G. Y. had primary responsibility for final content. All authors read and approved the final manuscript.

8. References

Consecutively number references, including web citations, in the order in which they are first mentioned in the text. Number references cited for the first time in tables or figure

legends in order, based on the first citation of the table or figure in the text. Identify references in the text, tables, and legends for figures by Arabic numbers in parentheses.

Only published papers and accepted papers that are "in press" may be included in the References section. "In press" papers must be submitted as supplemental files in PDF format at the time of manuscript submission. Personal communications from others and unpublished data of the authors, including submitted manuscripts, should appear parenthetically in the text. Include the full name and affiliation of the person providing a personal communication.

JN reference format is consistent with the International Committee of Medical Journal Editors (ICMJE) recommended format for bibliographic citations (15) with the following exception: list the names of all authors, unless there are more than ten, in which case list the first ten plus "et al." The ICMJE states, "as an option, if a journal carries continuous pagination throughout a volume (as many medical journals do) the month and issue number may be omitted." *JN* follows this optional style. If you are using software such as EndNote or Reference Manager that inserts this additional material, it will be automatically deleted during production of accepted manuscripts. Abbreviate journal names according to the National Library of Medicine (NLM) journal abbreviations list (16).

Authors may add to a reference, the DOI ("digital object identifier" number unique to the publication) for articles in pressIt should be included immediately after the citation in the References. An example is:

Kimokoti RW, Judd SE, Shikany JM, Newby PK. Metabolically healthy obesity is not associated with food intake in White or Black Men. J Nutr 2015 Sep 30 (Epub ahead of print; DOI: *doi:10.3945/jn.115.221283*).

Examples of citations to sources on the internet and to books can be found in the References in JN Instructions for Authors (page 13). Monographs can be cited in the following format:

Gibson RS, Ferguson EL. An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries. HarvestPlus Technical Monograph 8: Washington, DC and Cali, Colombia: International Food Policy Research Institute and International Center for Tropical Agriculture. 2008.

There is no limit on the number of citations allowed; cite recent literature comprehensively. Begin the list of references on a new page. Note that there should be no line numbers on the row with the “References” heading or throughout the References section.

Units of Measure

Metric units are required (e.g., m, kg, and L for height, weight, and volume, respectively), as is the Celsius scale (°C). For reporting data, use of SI units (le Systeme Internationale d’Unites) (17) is preferred (e.g., mmol/L,g/L) but not mandatory. Conventional units such as mg/dL and mg/mL are acceptable, using L, not l, for liter. Use units for the same

analyte/compound consistently throughout the manuscript. Placing an alternate unit parenthetically in the text or giving conversion factors in table footnotes or figure legends is acceptable. Units should not be pluralized (e.g., wk, not wks) or followed by a period.

Useful websites are:
SI conversions: Online Conversion
SI conversions: Wikipedia - Conversion of Units
Clinical SI conversions: JCS Clinical Laboratory Units Conversion

Significant Digits

The number of significant figures presented for a variable should be correct and consistent. Use no more than 3 significant figures (fewer, if appropriate) or justify the greater precision.

Base the number on the precision of the analytical method and round accordingly. In some cases, change the submultiple; e.g. change 1038 mg/d to 1.04 g/d.

Five rules govern significant figures (18):

Non-zero digits are always significant; 1.121 has four significant digits.

Any zeros between two significant digits are significant; 1.08701 has six significant digits.

Zeros before the digits are placeholders and not significant; in the number 0.00254 , only the 2, 5, and 4 are significant, meaning the number has 3 significant digits.

Zeros after the decimal point and after figures are significant; in the number 0.2540, the 2, 4, 5, and last 0 are significant digits5. Exponential digits in scientific notation are not significant; $1.12x10^6$ has three significant digits, 1, 1, and 2.

A tutorial on the use of significant digits is available (19).

Abbreviations

Use standard abbreviations in JN papers without definition in the text. Standard abbreviations, however, should be defined at first mention in the abstract. An abridged list is in Table 1 (20). Other common standard abbreviations are listed in Scientific Style and Format (4).

Each nonstandard (author-defined) abbreviation should be defined in the abstract and text at first mention. If three or more nonstandard abbreviations are used in the text, prepare an abbreviation footnote. The footnote should be associated with the first abbreviated term in the text and should be an alphabetized listing of all author-defined abbreviations and their definitions. Group designations should be defined parenthetically at first mention [for example, “control (CON) and high-fat (HF) groups”] and included in the abbreviation footnote. Abbreviations (other than units such as min, h, m, kg) should be pluralized where appropriate (e.g., The n-3 PUFAs are…) but should not be followed by

a period. Use the standard abbreviations for SI prefixes found in Young (19) and in Table 2 and those for units of measure in Table 3 (21).

All nonstandard abbreviations, including group or treatment designations, used in a table or table title must be defined alphabetically in a footnote to the table title. If the footnote to the table title contains multiple items, the definitions of the abbreviations should be the last item. If a table contains only one abbreviated term in the body of the table, then a separate footnote placed after that abbreviation should be used to define that term. Similarly, all nonstandard abbreviations, including group or treatment designations, used in a figure or figure legend must be defined alphabetically at the end of the figure legend.

Genes and Proteins

Full gene names are not required for tables and figures in which a database identifier number is given. A full citation to the database used should be in the References and the sequential reference number to the citation provided in the text, figure legend, or table footnote. If the genes are listed in online Supplementary data (e.g., supplemental tables and figures), the citation can be given as a table footnote or in the figure legend [e.g., National Center for Biotechnology Information (NCBI) Entrez Gene (22) or Unigene (23)].

All gene symbols should be italicized throughout the text, tables, and figures. The use of prefixes to designate species is not allowed. For rodent genes, the first letter should be uppercase with the rest in lowercase letters (e.g., for PPARγ, Pparg). For human genes, all letters should be uppercase (e.g., PPARG). Messenger RNA (mRNA) and complementary DNA (cDNA) use the same gene symbol and formatting conventions. Protein designations are the same as the gene symbols, are in all uppercase letters (even rodents), and are not italicized (e.g., PPARG). For the genes of other species, follow the convention for abbreviating human gene and protein names. Further information on gene and protein nomenclature rules can be found as indicated in the list below.

Human: HUGO Gene Nomenclature Committee (HGNC) (24)

Mouse: Mouse Genome Nomenclature Committee (MGNC) (25)

Rat: Rat Genome and Nomenclature Committee (RGNC) (26)

Bovine: Bovine Genome Database (BGD) (27) Chicken and other avians: Chicken Gene

Nomenclature Committee (CGNC) (28)

Xenopus and other amphibian (29)

Zebrafish and other piscine: Zebrafish Model Organism Database (ZFIN) (30)

Drosophila: (31)

Porcine: No official genome nomenclature committee statements or annotation resources are available online to date. The International Society for Animal Genetics (ISAG) publishing guidelines defer to the HUGO Name (24) when applicable. Use NIH Gene (22) or HUGO (24) to confirm names. Where one-to-one human orthology cannot be

established, an unofficial gene symbol can be used if it is supported by a previous literature assignment. Clade- specific or species-specific genes will be designated with an official gene symbol upon completion of the genome.

Equine, ovine, canine: No official genome nomenclature committee statements or annotation resources are available online to date; see porcine guidelines.

With respect to defining gene and protein symbols, please follow the instructions in the list below.

Text: Define all gene and protein symbols (abbreviations) at first use in the abstract and text.

Tables and figures: Define protein symbols or abbreviations. Full gene names are not required for tables and figures in which a database identifier number is given. Therefore, either use NM_ or other database identification numbers or define gene symbols.

Abbreviation footnote: Define protein symbols (abbreviations). Do not define genes for which a database identifier number is given in the text, tables, or figures. Define gene names for which a database identifier number is not given.

Nomenclature

Chemical and biochemical terms and abbreviations and identification of enzymes generally should conform to the recommended usage of the International Union of Biochemistry and Molecular Biology (32). Names for vitamins, related compounds, and abbreviations for amino acids should follow the ASN nomenclature policy (33, 34).

For fatty acids, use the ω or n system consistently with a colon to separate the chain length and number of bonds (e.g.,18:2n–6, 20:5n–3, 18:2ω-6, 20:5ω-3). In the text, refer to n–3 fatty acids, ω3 PUFA, the ratio of n–3/n–6 fatty acids, etc. Regardless of which system is used in the text, include the alternative form in parentheses in the abstract. Use common names and systematic names together at first mention, and then use the common name throughout. In general, there is no need to use the abbreviations “c” and “t” to denote cis and trans after first usage [e.g., cis-9, trans-11 CLA (18:2c9,t11)]. If, however, the article includes many references to systematic names including cis and trans designators, it may be more economical to use the “c” and “t” designators.

Ethical Considerations

Individuals who are asked to review a manuscript should decline the solicitation if they have a conflict of interest. Detailed guidelines on conflicts of interest for reviewers can be found at ASN Journals Conflict of Interest Guidelines (5).

JN strongly encourages registration in an appropriate public trials registry of all clinical trials and observational studies. Beginning in 2015, this will be required.

Before acceptance, all papers will be screened for similarity to previously published papers using iThenticate. Selected papers will be screened at earlier stages of the review

process. Those with disproportionate similarity to published papers will, at the Editor's discretion, be rejected outright or returned to authors for rewriting followed by re-review before a final decision is made.

The following are considered inappropriate re-use of material (plagiarism):

Copying the published words of other authors or modifying only slightly, with or without citation of the original work.

Reusing the author's own previously published words, with or without citation (self-plagiarism).

Failure to quote and/or acknowledge by citation substantially similar ideas, content, tables, or illustrations that have been published or copyrighted by others.

Most published work is copyrighted. Thus, all text in the submitted paper must be original, including the Methods section. Frequently, the previous publication can be cited and thereby the length of the Methods section can be reduced. Review articles also must be original; they cannot repeat verbatim or include only minimally changed words from previous reviews or original research papers by the author or others. The NIH's Office of Scientific Integrity's piece, "Avoiding Plagiarism, Self-Plagiarism, and Other Questionable Writing Practices: A Guide to Ethical Writing" (35) is highly recommended.

As recommended in the Committee on Publication Ethics Code of Conduct for Journal Publishers (36), and supported by the International Committee of Medical Journal Editors (37), when ASN is made aware of cases of suspected research and publication misconduct, ASN holds the right to publish an Expression of Concern during an investigation, and, depending on the outcome of the investigation, to retract articles.

Tables and Figures

See current print or electronic papers in *JN* for examples of table and figure styles. Cite tables and figures sequentially in the text with the first citation of each table and figure in bold font. Tables or figures adapted or reproduced from another source must acknowledge that source in a table footnote or the figure legend and be accompanied by written proof that the copyright bearer has granted permission to reproduce or adapt the table or figure. To obtain permission, authors may need to reference the information found at the page Permission to Re-Publish Copyrighted Content in *JN* (38).

Authors of supplement and symposium manuscripts

Include 1 of 3 statements in all figure and table captions:

Reproduced with permission from (reference X),

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Original to this manuscript.

Tables

Tables must be included in the text file, and each table should begin on a new page. Please do not upload tables as separate files. Each table should have a title that clearly but concisely describes the treatments and experimental animals or participants. Information concerning methods or explanatory material can be included in footnotes to the table, but repetition of methodology should be minimized. Clearly indicate units of measure after the variable in rows, above the first value in each column, or centered over all columns to which the unit applies. Show statistics of variability (e.g., SD, pooled SEM) and the significance of differences among the data. Omit internal horizontal and vertical rules before submitting your tables.

For an illustrated table quality checklist, visit the Table Checklist (38).

Figures

Submit each figure in a separate file. Image files (TIF, EPS) and Microsoft PowerPoint (PPT,) are acceptable figure files. Figures prepared as Word, PNG, or JPEG will not be accepted.

Compile figure titles and legends on one or more pages in the manuscript's .doc file rather than on the figure itself, but include figure keys on figures, within the bounds of the graphs or on X-axes, not in legends. Figure titles should concisely describe the species or participants and treatments but are not required to call out panels. Each legend should contain enough detail, including an explanation of the results of statistical tests shown to ensure that the figure is interpretable without reference to the text. For figures with 2 or more panels, describe each panel in the legend, beginning with the panel letter. Minimize repetition of methodology, but specific assay conditions can be given.

Submit all panels of a multipanel figure on a single page, aligning the panels horizontally and/or vertically with one another. Minimize white space within and between panels. Label each panel, A, B, C, D, etc., without the word, “figure,” or the figure number, in the upper-left corner of the panel.

Label axes clearly with variables and where appropriate, units of measure. Show significant differences using symbols or letters. Remove outer boxes from figures and figure panels.

Size all text on figures proportionately and large enough to be legible after reduction to 1 column width of <8.5 cm or, in rare cases, 2 column widths. Preferred text size is 7 points.

1 column: 18p0 / 3 inches / 7.6 cm

1.5 column: 27p0 / 4.5 inches / 11.5 cm

Maximum width (to span 2 columns): 34p0 / 5.7 inches / 14.4 cm

Maximum height: 53p0 / 8.8 inches / 22.4 cm

Because of the expense of color printing, which is in part subsidized by *JN*, please submit no more than two color figures per manuscript. Only those figures that are essential to display in color will be printed in color. Avoid unnecessary color for histograms, line drawings, etc. When color is deemed unnecessary, the editors may ask authors to resupply figures in black and white or in gray tone. When possible, prepare multipanel figures that

group all color images into a single figure. Color reproduction costs will be charged to the author. Color may be used in Supplementary data.

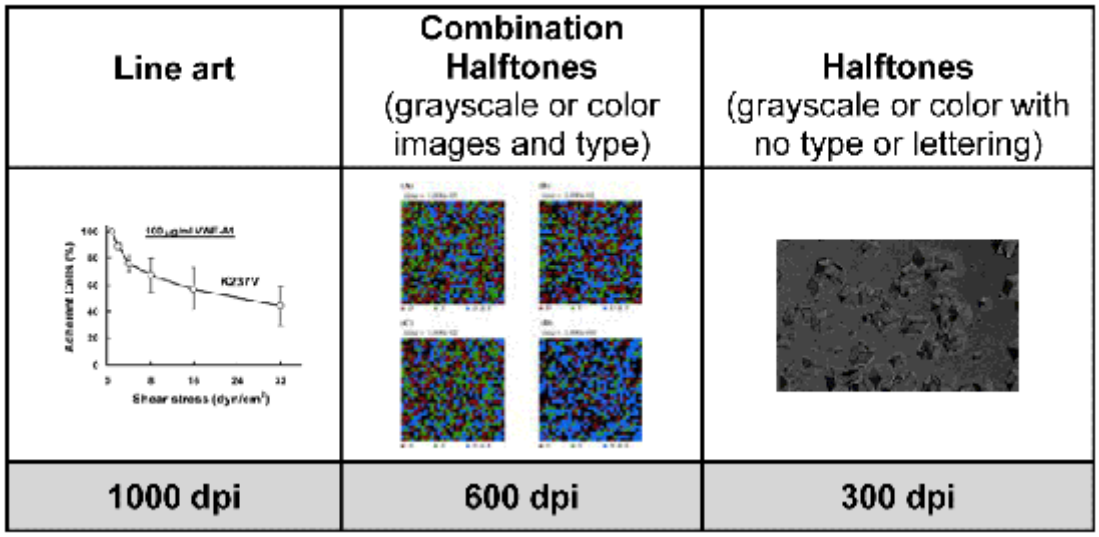
Image resolution

If a figure is very small in the manuscript submission system–generated PDF file, the resolution of the figure file was likely not high enough. Upload a higher resolution figure before approving the PDF. Files must conform to the following minimum resolution specifications:

Line art. 1000 dpi

Combination halftones. 600 dpi (grayscale or color images and type)

Halftones. 300 dpi (grayscale or color with no type or lettering)



For an illustrated figure quality checklist, visit the Figure Quality Checklist (40). For video on preparing digital images for publication, visit the Preparing Digital Images for Publications series (41).

Supplementary Data

Unusually lengthy descriptions of experimental procedures, extensive data, extra figures, etc. may be published as online-only attachments to published articles. For example, if several primers were used, they should be listed in a supplemental table. Supplementary data should be integral to the manuscript but impractical to include in the printed article. References to the availability of supplemental data on authors' websites will not be included in published articles.

Please note the following:

For revised submissions, do not use yellow highlighting to show changes made to your Supplementary data. Remember that Supplementary data are not copyedited before publication and will be published exactly as you upload the supplemental file(s).

Supplemental text, tables, and figures can be single spaced and should not include line numbers, but should otherwise be in JN format.

Label the top of all Supplementary data pages with the header, "Supplementary data."

Name and label each unit of supplemental material as appropriate (e.g., Supplemental Table 1, Supplemental Table 2, Supplemental Figure 1, Supplemental References, etc.)

Call out all Supplementary data parenthetically in the manuscript text; e.g.: (Supplemental Fig. 1).

Place supplemental figure legends immediately under the supplemental figure.

If the citations in the Supplementary data do not appear elsewhere in the printed paper, they should NOT be added to the paper's References section. Instead, prepare a Supplemental References section [beginning with (1), (2), etc.] and include it at the end of supplemental materials.

Format the footnote to the manuscript title with specific information about the Supplementary data available. For example: Supplemental Table 1 and supplemental methods are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents available on the Journal homepage.

Submit Supplementary data video files in the native format. To avoid excessive delays in downloading the files, limit videos to no more than 5 MB in size and 60 s in length. Authors are encouraged to use QuickTime's "compress" option when preparing files to help control file size.