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Cíntia dos Santos Costa

IMPACTO DE ACONSELHAMENTO DIETÉTICO REALIZADO NO PRIMEIRO ANO DE VIDA EM DESFECHOS DE SAÚDE ENTRE ESCOLARES E ADOLESCENTES

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RESUMO

Introdução: Evidências recentes sugerem que as práticas alimentares do primeiro ano de vida determimam hábitos alimentares da infância, e que estes podem se prolongar até a vida adulta, influenciando condições de saúde a curto e longo prazo. Intervenções precoces com foco na promoção do aleitamento materno e adequada alimentação complementar, dessa forma, apresentam potencial para influenciar condições de saúde durante toda a vida. **Objetivo:** Avaliar o impacto de visitas domiciliares para orientar mães sobre alimentação no primeiro ano de vida na resistência insulínica de crianças com 8 anos de idade e em parâmetros antropométricos, bioquímicos e dietéticos de adolescentes com 12 anos de idade. Como objetivo secundário, a associação independente entre ganho de peso desde o nascimento e resistência insulínica em escolares foi investigada. **Métodos:** Ensaio de campo randomizado realizado com mães de crianças que nasceram a termo e com peso ≥2500g entre outubro de 2001 e junho de 2002 em São Leopoldo, RS. Mães do grupo intervenção receberam aconselhamento sobre aleitamento materno e alimentação complementar baseado no Guia Alimentar "Dez passos para alimentação saudável para crianças menores de dois anos", do Ministério da Saúde. Alunos de graduação em nutrição realizaram o aconselhamento dietético em visitas domiciliares realizadas até o 10º dia de vida, mensais até o 6º mês e bimestrais até o 12º mês de vida das crianças. Entrevistadores coletaram dados sócio-demográficos, antropométricos e dietéticos no nascimento e nas idades de 1, 4, 8 e 12 anos de vida. Aos 8 e 12 anos, foi realizada coleta de sangue venoso para avaliação do perfil glicídico e resistência insulínica em escolares e avaliação de perfil lipídico, glicído e concentrações de PCR e cortisol em adolescentes. **Resultados:** Das 500 crianças (intervenção n=200; controle n=300) inicialmente recrutadas, avaliaram-se 397 no primeiro ano, 354 aos 4 anos, 315 aos 8 anos e 214 aos 12 anos. Não foi observado impacto da intervenção nas concentrações de glicose e insulina e nos valores do modelo de avaliação da homeostase (HOMA-IR) aos 8 anos de idade. Por outro lado, foi observada associação positiva entre valores de insulina e HOMA-IR aos 8 anos de idade e variação de IMC desde o nascimento. Em adolescentes, não foi observada diferença significativa entre os grupos intervenção e controle no estado nutricional, perfil lipídido e glicído, concentração sérica de PCR e cortisol e no consumo de produtos ultra-processados. **Conclusões**: Os resultados evidenciam que o ganho de peso acelerado desde o nascimento é preditor de alterações precoces na resistência insulínica em escolares, reforçando a importância de ações de monitoramento da velocidade de ganho de peso desde o nascimento para a prevenção de alterações metabólicas. Por outro lado, não foi observado efeito a longo prazo do aconselhamento dietético realizado durante o primeiro ano de vida das crianças nos níveis de glicose, insulina e HOMA-IR entre escolares e em parâmetos antropométricos, bioquímicos e dietéticos entre adolescentes. Programas de intervenção focados na promoção de hábitos alimentares saudáveis (e no monitoramento da velocidade de ganho de peso) devem iniciar precocemente e os achados deste estudo reforçam a importância da manutenção de ambientes saudáveis até a adolescência, para consolidação dos hábitos adquiridos, como uma das prioridades em programas de saúde pública.

Palavras-chave: ensaio clínico, aconselhamento, escolar, criança, resistência resistência à ação da insulina, índice de massa corporal.

ABSTRACT

Introduction: A growing body of evidence suggests that feeding practices during the first year of life provide the basis for food habits in childhood and tend to track later in life, influencing both short- and long-term health. Interventions focusing on complementary feeding practices may, thus, have the potential to influence health patterns throughout the life course. **Objective:** To assess the long-term effectiveness of dietary intervention accomplished during the first year of life on insulin resistance levels among school-age children and on anthropometric, metabolic and dietary variables among adolescents. A second objective was to investigate the relation between insulin resistance at schoolage and the weight changes over time. **Methods:** A randomized field trial was conducted with 500 mothers who gave birth to full-term infants babies with birth weight ≥2500g, between October 2001 and June 2002, in São Leopoldo, Brazil. Mothers in the intervention group received dietary counseling on breastfeeding and complementary feeding based on the "Ten Steps for healthy feeding from birth to two years of age" during the first year of life of their children. The dietary counseling was carried out by six couples of undergraduate students in Nutritional Sciences in home visits to the mothers within 10 days of the child's birth, monthly up to 6 months and with subsequent visits at 8, 10 and 12 months. Fieldworkers blinded to assignment assessed socio-demographic, dietary and anthropometric data during follow-up at ages 1, 4, 8 and 12 years. At 8 and 12 years old, fasting blood tests were performed to estimate serum glucose and insulin concentration and homeostasis model assessment index of insulin resistance (HOMA-IR) value among school-age children and complete lipid and glucose profile, and PCR and cortisol concentrations among adolescentes. **Results:** Of the 500 recruited children (intervention n=200, control n=300), 397 underwent the 1-year, 354 the 4-year, 315 the 8-year and 214 the 12-year assessment. There was no effect of the intervention on glucose and insulin concentrations or HOMA-IR values among children at the age of 8 years. A positive association was observed between BMI *z*-score variation from birth to 8 years and insulin and HOMA-IR values at 8 years old. Among adolescents, there were no significantly differences between intervention and control groups in regard to nutritional status, serum lipid and glucose profile, PCR and cortisol concentrations and ultra-processed products consumption. **Conclusions:** The results clearly show that accelerate weight gain from birth to 8 years old is a predictor of insulin resistance impairment, reinforcing the importance of monitoring the BMI trajectory in infancy and childhood at preventing early metabolic alterations. Nevertheless, a home-based maternal dietary counseling during the first year of life was not longeffective in improving glucose, insulin and HOMA-IR at 8 years old and on anthropometric, metabolic and dietary variables among adolescents. Intervention programs to promote healthy dietary pattern should initiate early in life and our findings suggest the importance of supporting a dietary safe environment (including weight gain velocity monitoring) throughout adolescence as a priority in public health strategies.

Keywords: controlled trial, counseling, childhood, adolescents, insulin resistance, body mass index.

LISTA DE ABREVIATURAS

BMI *Body Mass Index*

BMIz *BMI-for-age z-score*

HDL-c *high-density lipoprotein cholesterol*

HOMA-IR *Homeostasis model assessment index of insulin resistance*

LDL-c *low-density lipoprotein cholesterol*

RI *Resistência Insulínica*

TC *total cholesterol*

TG *triglycerides*

SUMÁRIO

1 APRESENTAÇÃO

Este trabalho consiste na Tese de doutorado “Impacto do aconselhamento dietético durante o primeiro ano de vida e desfechos em escolares e adolescentes”, a ser apresentada no Programa de Pós-graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre no ano de 2016. O trabalho foi elaborado a partir da análise de dados de projeto maior denominado "Impacto da implementação dos Dez passos da alimentação saudável para crianças: ensaio de campo randomizado", o qual foi iniciado no ano de 2001 na cidade de São Leopoldo, Rio Grande do Sul. O projeto foi desenvolvido pela equipe interdisciplinar e interinstitucional que compõem o Núcleo de Pesquisa em Nutrição (NUPEN), sob coordenação da Prof[a]. Dr[a]. Márcia Regina Vitolo. O NUPEN desenvolve estudos na área de nutrição infantil, especificamente com os temas obesidade e hábitos alimentares de crianças.

A atual situação epidemiológica das crianças brasileiras é caracterizada por prevalência de excesso de peso em torno de 30% e por diagnóstico frequente de alterações metabólicas, como resistência insulínica. Evidências de que hábitos alimentares inadequados são preditores da ocorrência de doenças crônicas são irrefutáveis. Intervenções para promoção da alimentação saudável ainda na infância, dessa forma, apresentam potencial para influenciar condições de saúde a curto e longo prazo. Nesse sentido, pretende-se neste estudo avaliar o impacto de visitas domiciliares para orientar mães sobre alimentação no primeiro ano de vida na resistência insulínica de crianças aos 8 anos de idade e em parâmetros antropométricos, metabólicos e dietéticos aos 12 anos de idade e, assim, estabelecer diretrizes que possam nortear políticas públicas voltadas para esta população.

O percurso desta tese começa com a apresentação, no primeiro capítulo, do referencial teórico. A seguir, no capítulo 2, são apresentados os objetivos do estudo. A concretização dos objetivos está apresentada em dois manuscritos apresentados nos capítulos 3 e 4. O capítulo 3 consiste no manuscrito “*Effect of maternal dietary counselling during the first year of life on glucose profile and insulin resistance at age eight: a randomized field trial*”, desenvolvido em parceria com o Professor Dr. Lambert Lumey, da Universidade de Columbia, EUA e aceito para publicação no periódico *British Journal of Nutrition*. O capítulo 4 consiste no manuscrito

"*Randomized controlled trial of dietary counseling intervention during the first year after birth: is it effective after twelve years?*", que será submetido para apreciação do corpo editorial da Revista *Preventive Medicine.* No capítulo 5, apresentamos a conclusão do trabalho e considerações finais acerca das potenciais implicações dos resultados encontrados para a pesquisa em nutrição e para o desenvolvimento de políticas públicas na promoção da saúde. Os documentos suplementares estão apresentados no último capítulo.

1. REVISÃO DE LITERATURA

1.1 Obesidade na infância e na adolescência.

A prevalência global de excesso de peso em crianças aumentou 47,1% no período entre 1980 e 2013 (DE ONIS *et al.*, 2010; NG *et al.*, 2014). Foi estimado que mais de 40 milhões de crianças apresentavam excesso de peso em todo o mundo no ano de 2013, sendo que 75% delas viviam nos países em desenvolvimento (WHO, 2015). Nestes países, a prevalência de excesso de peso variou de 8,1% em 1980, para 12,9% em 2013 nos meninos, e de 8,4% para 13,4% nas meninas, no mesmo período (NG *et al.*, 2014). Corroborando com esses dados, no Brasil, a prevalência de meninos de 5 a 9 anos de idade com excesso de peso mais que dobrou entre 1989 e 2009, passando de 15% para 34,8%, respectivamente; entre as meninas, a prevalência de excesso de peso aumentou de 11,9% para 32% no mesmo período e faixa etária, de acordo com dados do Instituto Brasileiro de Geografia e Estatística (IBGE, 2010).

Excesso de peso na infância e adolescência é um importante fator de risco para o desenvolvimento da obesidade na idade adulta, ou seja, há um risco aumentado da criança obesa permanecer nessa condição quando adulto, se comparada aos indivíduos eutróficos (MUST *et al.*, 1992). Além disto, há evidências de que o tempo de exposição à obesidade está diretamente associado à morbimortalidade por doenças cardiovasculares (SRINIVASAN *et al.*, 1996). O aumento da prevalência da obesidade infantil é relevante sob o ponto de vista do indivíduo, dado o impacto negativo da doença nas condições de saúde e qualidade de vida ao longo da vida, mas também sob o ponto de vista coletivo, considerando a oneração econômica para a rede de saúde. Evidências recentes indicam que uma criança obesa aos 10 anos de idade irá gerar custo médio de vida adicional em torno US$ 12.660 em gastos com saúde, quando comparada a uma criança eutrófica da mesma idade (FINKELSTEIN *et al.*, 2014).

Em relação aos fatores de risco associados, nos últimos anos as variáveis mais estudadas no sentido de explicar o excesso de peso na infância são: baixo peso ao nascer seguido de ganho de peso acelerado (YANG e HUFFMAN, 2013), menor duração do aleitamento materno exclusivo (KRAMER e KAKUMA, 2012); maior escolaridade dos pais e maior nível socioeconômico da família (MENEZES *et* *al.*, 2011). Sabe-se também que fatores maternos ligados à gestação, especialmente sobrepeso pré-gestacional e ganho de peso excessivo na gravidez estão associados ao excesso de peso em crianças (DELLO *et al.*, 2013; ENSENAUER *et al.*, 2013; YANG e HUFFMAN, 2013) e adolescentes (SCHACK-NIELSEN *et al.*, 2010; LAITINEM *et al.*, 2012). Considerando levantamentos recentes, indicando que mais de dois terços das mulheres em idade reprodutiva apresentam excesso de peso (OGDEN *et al.*, 2006; ARTAL *et al.*, 2010), faz-se necessário o monitoramento do estado nutricional pré e durante a gestação como componente essencial dos cuidados em atenção primária para prevenção da obesidade infantil (MACAULAY *et al.*, 2014; KAPADIA *et al.*, 2015).

Há relativo consenso sobre as causas do excesso de peso infantil, admitindo-se que sua ocorrência pode ser determinada por um ou pela soma de fatores genéticos, ambientais e comportamentais. Neste sentido, as consequências da exposição a ambiente obesogênico é bastante explorada na literatura (LOBSTEIN *et al.*, 2015). Consumo de alimentos e bebidas hipercalóricos e com baixa densidade de nutrientes (SCHARF e DEBOER, 2016), aumento do tempo de tela e sedentarismo (MARSH *et al.*, 2013) são hábitos que contribuem para o acúmulo de peso excessivo em crianças. A manutenção do padrão alimentar (MADRUGA *et al.*, 2012) e comportamento sedentário (BIDDLE *et al.,* 2010) da infância para adolescência já foi evidenciada em revisões sistemáticas. Achados recentes sugerem que a exposição da criança a ambiente obesogênico inicia-se precocemente, já nos primeiros dois anos de vida (YANG e HUFFMAN, 2013). Esta situação é preocupante, dado que a formação da preferência alimentar é estabelecida nesta faixa etária (BIRCH e DOUB, 2014). Resultados de pesquisas de intervenção indicam que tentativas de modificar hábitos alimentares na infância são mais eficazes se focadas em crianças pequenas (antes dos dois primeiros anos de vida) (SILVA-SANIGORSKI *et al.*, 2010; REIFSNIDER *et al.*, 2013; BARBER *et al.*, 2015). Se o padrão de vida obesogênico é estabelecido nos primeiros anos de vida, e se tais hábitos não forem modificados, a tendência é que sejam mantidos com consequente impacto negativo para as condições de saúde a curto e longo prazos.

Assim, parece razoável postular que a primeira infância é uma oportunidade única para o estabelecimento de comportamento e estilo de vida que promovam a saúde e minimizem o risco para o desenvolvimento de obesidade e doenças crônicas associadas (LANIGAN *et al.*, 2009). Atualmente, a literatura científica tem explorado os efeitos dos primeiros mil dias de vida, período entre a concepção e os dois anos de idade, para o crescimento e o desenvolvimento saudáveis da criança (WHO, 2013). Esse período é considerado uma *janela de oportunidade* que irá influenciar a formação dos hábitos alimentares e, por um processo de programação – como alterações no ambiente intrauterino e pós-natal – refletir no estado nutricional e saúde adulta (PLAGEMANN *et al.*, 2005; HORTA *et al.*, 2007; LANIGAN *et al.*, 2009).

Concomitante ao aumento da prevalência do sobrepeso na infância, o diagnóstico de alterações no perfil lipídico (DING et al, 2016; DATHAN-STUMPF et al., 2016), resistência insulínica (VAN DER *et al.*, 2015), diabetes tipo II (FARSANI *et al.*, 2013; DABELEA *et al.*, 2014), hipertensão (XI *et al.*, 2015; YANG *et al.*, 2015) e outras complicações metabólicas são cada mais incidentes em crianças. Dados do *The Bogalusa Heart Study* mostraram que a distribuição da gordura abdominal em crianças e adolescentes com idade entre 5 e 17 anos foi associada com concentrações aumentadas de TG, LDL-C e insulina, em uma análise transversal (FREEDMAN *et al.*. 2007). Corroborando com estes dados, revisão sistemática recente concluiu que o acúmulo excessivo de gordura abdominal em crianças e adolescentes aumenta o risco de desenvolvimento de doenças crônicas nestas faixas etárias (KELISHADI *et al.*, 2015). Essa situação é preocupante, dado as evidências consolidadas de que a ocorrência precoce de fatores de risco na infância é preditora do desenvolvimento de doenças crônicas na vida adulta (JUONALA, *et al.*, 2013; SUOMELA *et al.*, 2016). Estimativas científicas indicam que 60% dos sujeitos com diagnóstico de obesidade na primeira década de vida (concomitante ou não ao diagnóstico de disfunção metabólica) irão apresentar pelo menos uma alteração metabólica na vida adulta (SRINIVASAN *et al.*, 2002).

Considerando o exposto acima, fica evidente a necessidade de implementação e avaliação de ações que previnam o desenvolvimento de obesidade, particularmente no início da vida e, consequentemente, retardem ou evitem a ocorrência de morbidades relacionadas à obesidade e doenças crônicas relacionadas. É necessário desenvolver e testar abordagens de prevenção precoces da obesidade infantil que sejam eficazes, mas também práticas, viáveis e sustentáveis por longo prazo (LEUNISSEN *et al.,* 2009; TAVERAS *et al.*, 2009; BROTMAN *et al.*, 2012; HEARST *et al.*, 2013). A promoção de hábitos alimentares saudáveis é elemento essencial dessas intervenções (KAVANAGH *et al.*, 2008;

HONG, 2010). Uma vez que os pais desempenham papel importante na formação de padrões alimentares em crianças pequenas (BIRCH e DOUB, 2014), intervenções para esta faixa etária devem sempre envolvê-los. Somente desta forma é possível alcançar a meta do *US Task Force on Childhood Obesity* (2010) de reduzir a prevalência da obesidade infantil para 5% até 2030.

1.2 Resistência à ação da insulina na infância.

O conceito da RI (resistência à ação da insulina), inicialmente proposto em 1936 (HIMSWORTH, 1936), refere-se à resposta subnormal da insulina em promover a adequada captação periférica da glicose (TEN e MACLAREN, 2010). A causa mais comum da RI é a obesidade, embora a ocorrência de RI em sujeitos magros também seja possível. O acúmulo excessivo de tecido adiposo, especialmente de tecido adiposo visceral, promove liberação aumentada de ácidos graxos livres, o que afeta a sinalização da insulina a nível celular, prejudica a translocação da proteína transportadora de glucose-4 (GLUT-4) para membrana plasmática das células, diminuindo a captação de glicose nos tecidos (MLINAR e MARC, 2011).

A etiologia da RI inclui principalmente fatores ambientais como padrão alimentar inadequado e sedentarismo (TEN e MACLAREN, 2010). Por outro lado, o aumento fisiológico da RI pode ser observado em determinadas fases do desenvolvimento humano: na adolescência (devido ao efeito de esteroides sexuais e do hormônio do crescimento), na gestação (por efeito do lactogênio placentário) e no envelhecimento (em função da sarcopenia e redistribuição do tecido adiposo) (ZEITLER *et al.*, 2014).

Evidências recentes indicam que o ganho de peso acelerado na infância está relacionado com a ocorrência de RI em adultos, em países desenvolvidos (SLINING *et al.*, 2011; FABRICIUS-BARRIER *et al.*, 2011) e em desenvolvimento (NORRIS *et al.*, 2012; ADAIR *et al.*, 2013). Além disto, evidências indicam que RI na infância é preditora de RI em adultos. Estudo conduzido por Sinaiko e colaboradores mostrou que RI aos 13 anos de idade prediz RI aos 19 anos, independente do IMC atual (SINAIKO et al., 2006). A hiperinsulinemia, decorrente da RI, está associada ao desenvolvimento de diversas alterações metabólicas: hipertensão, dislipidemia, esteatose hepática não alcoólica, aterosclerose e síndrome metabólica (ECKEL *et* *al.*, 2005; TEN *et al.*, 2010). Crianças que apresentam RI tem um risco aumentado de desenvolverem diabetes melitos tipo II e doenças cardiovasculares quando adultas (KUMANYIKA *et al.*, 2008). Estudo de coorte recente mostra que valores aumentados de insulina sérica em crianças de 3 a 6 anos foi associado ao diagnóstico de diabetes tipo II em adultos, indepente do IMC atual e da história familiar da doença (SABIN *et al.*, 2015). Ainda neste sentido, RI em crianças de 6 a 9 anos de idade foi preditora de aumento nos níveis de pressão arterial em adolescentes (TAITTONEN *et al.*, 1996).

O padrão ouro para o diagnóstico da RI é o *clamp euglicêmico-hiperinsulinêmico* (LEVY-MARSHAL *et al.*, 2010). No entanto, este é um procedimento de alto custo e de difícil manejo na pesquisa epidemiológica (DEFRONZO *et al.*, 1979; MATSUDA *et al.*, 1999; OTTEN *et al.*, 2014). Como alternativa, muitos autores utilizam modelos matemáticos que predizem a sensibilidade à insulina pela medida da glicemia e insulina de jejum. O método HOMA-IR (do inglês, *homeostatic model assessment*; em português, modelo de avaliação da homeostase) foi inicialmente descrito em 1985 e é calculado pela fórmula: HOMA-IR = glicemia de jejum (mmol/l) x insulina basal (uU/ml)/22,5 (MATHEWS, 1985). O HOMA-IR foi comparado com o *clamp euglicêmico-hiperinsulinêmico* e os resultados evidenciam alto grau de correlação em crianças (SCHWARTZ *et al.*, 2008). O HOMA-IR é, portanto, uma alternativa simples e economicamente viável cujas evidências suportam a utilização como ferramenta em estudos epidemiológicos.

Recentemente, VAN DER e colaboradores (2015) publicaram artigo de revisão cujo objetivo foi investigar a prevalência global de RI em crianças e adolescentes. A revisão incluiu 18 estudos originais realizados em 13 países. As prevalências de RI encontradas variaram de 3,1% a 44%, como pode ser observado na Figura 1. Os autores concluíram que diferenças nas características das populações estudadas (idade, peso, etnia, estágio puberal, etc.) podem parcialmente explicar a grande variação nas diferenças nas prevalências econtradas. No entanto, a utilização de diferentes métodos e os diferentes valores de corte para determinar RI podem também justificar a amplitude das prevalências de RI. Os autores sugerem a realização de estudos longitudinais para a definição de ponto de corte único para o diagnóstico de RI em crianças.

Estudos avaliando a distribuição de valores de glicose, insulina e HOMA-IR em crianças pré-puberes já foram publicados, sendo que alguns autores sugeriram pontos de corte para esta faixa etária (GRANT *et al.*, 1967; WENNLOF *et al.*, 2005; BARJA *et al.,* 2011; MELLERIO *et al.*, 2012; ARADILLAS-GARCIA *et al.*, 2012). Todavia, todos estes estudos são limitados quanto à amostra estudada, uma vez que utilizam dados nacionais e tamanhos de amostra pequenos para modelagem estatística de valores de referência. Nenhum dos estudos incluiu mais de 1000 pré-puberes, com exceção de um corte transversal mexicano com cerca de 2500 crianças de idade entre 6-10 anos (ARADILLAS-GARCIA *et al.*, 2012). Recentemente, pesquisadores do IDEFICS (*Identification and prevention of dietary and lifestyle induces health effects in children and infants*) sugeriram curvas com valores de referência padronizados para a população infantil, considerando amostra de mais de sete mil crianças europeias com idade entre 3 e 10 anos de idade (PEPLIES *et al.*, 2014).

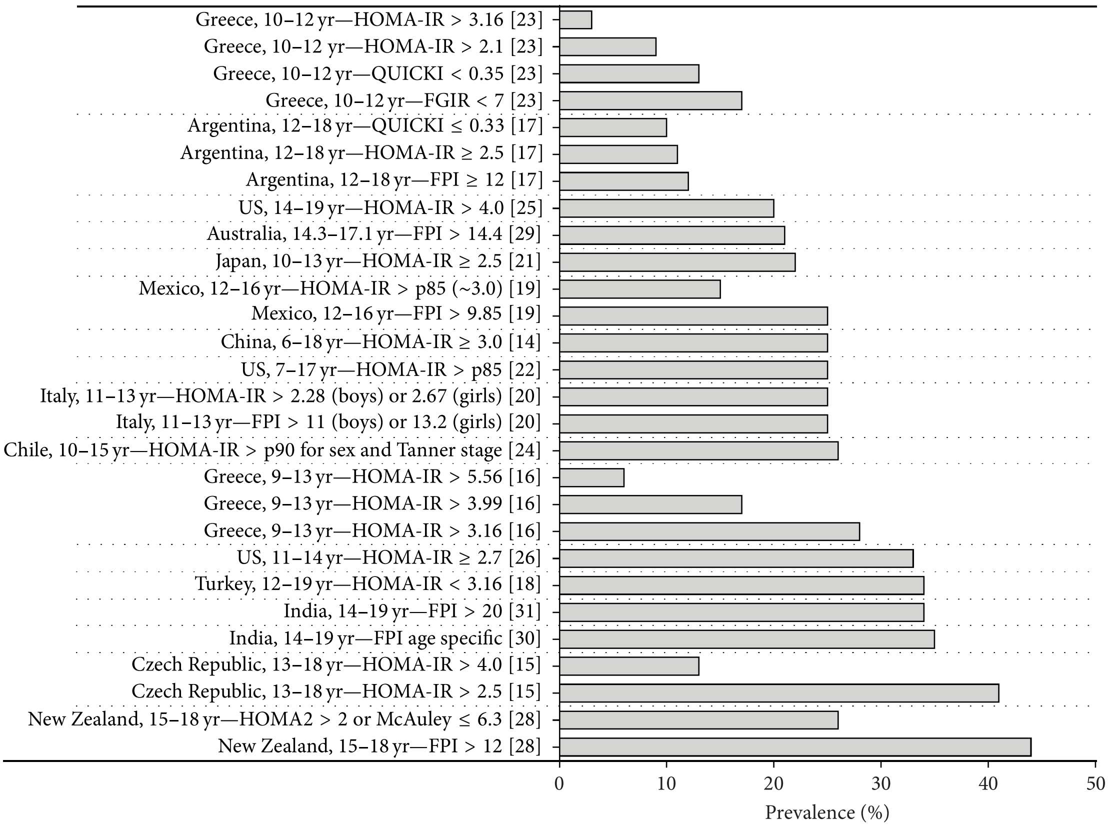

FIGURE 2: The overall prevalence rates (%) of IR in the included studies.

Figura 1: Prevalência global de RI em crianças e adolescentes. Adaptado de VAN DER *et al., 2015.*

Embora os sintomas da RI sejam mais facilmente manifestados em adultos, a prevenção da RI deve iniciar na infância, quando mudanças no estilo de vida podem reduzir a incidência e severidade de doenças crônicas relacionadas a esta alteração metabólica ao longo da vida. Considerando que a prevalência de RI tem aumentado significativamente na população infantil (CHIARELLI *et al.*, 2008; VAN DER *et al.*, 2015), destaca-se a necessidade de estudos que avaliem estratégias de intervenções precoces e viáveis para a prevenção desta complicação metabólica nesta população.

Evidências atuais mostram a importância do padrão alimentar adequado para prevenção da RI, em crianças e adolescentes. Estudo recente realizado com criancas mexicanas mostrou que dieta caracterizada por consumo exagerado de bebidas adocicadas e produtos industrializados e baixo consumo de vegetais e de frutas frescas está positivamente associado com RI, mesmo após ajuste para IMC (ROMERO-POLVO et al., 2012). Resultado semelhante foi encontrado em outro estudo realizado com crianças e adolescentes gregos, o qual evidenciou que padrão alimentar caracterizado por consumo exagerado de margarina, doces e produtos processados foi associado com risco 2,51 (95% IC 1,30-4,90) vezes maior de desenvolvimento de RI, em análise multivariada (KARATZI et al, 2014).

1.3 Alimentos ultra-processados: classificação, consumo e consequências.

A avaliação do impacto do consumo alimentar na saúde depende de sistemas de classificação utilizados para caracterizar os alimentos. Classificações convencionais agrupam os alimentos de acordo com seu perfil de nutrientes. Entretanto, é de senso comum que os indivíduos não ingerem nutrientes isolados, mas refeições compostas por diferentes alimentos que fornecem diversos nutrientes e compostos químicos que interagem entre si. Desde 1998, a Organização Mundial da Saúde sugere que o consumo alimentar seja avaliado com base em alimentos em detrimento de nutrientes (WHO, 1998).

No Brasil, foi recentemente proposto um novo sistema de classificação de alimentos baseado na extensão e no propósito do processamento empregado nos alimentos. O processamento de alimentos, tal como entendido por esta classificação, denominada NOVA, envolve processos físicos, biológicos e químicos que ocorrem após a colheita do alimento. O sistema NOVA considera todos os métodos e técnicas utilizados pela indústria para a transformação de alimentos in natura em produtos alimentícios. A fundamentação teórica e a caracterização dos grupos de alimentos definidos nessa classificação foram descritas pela primeira vez em 2010 (MONTEIRO *et al.,* 2010). Desde então, vem sendo detalhada e aprimorada (MONTEIRO *et al.,* 2012; MONTEIRO *et al.,* 2014). A classificação divide os alimentantos em quatro grupos: 1) alimentos *in natura* ou minimamente processados; 2) ingredientes culinários processados; 3) alimentos processados e 4) alimentos e bebidas ultraprocessados.

Grupo 1: Alimentos in natura e minimamente processados.

Os alimentos in natura são aqueles de origem vegetal (folhas, caules, raízes, tubérculos, frutas, nozes, sementes) ou de origem animal (carnes, ovos, leite) consumidos logo após a colheita ou o abate. Alimentos minimamente processados são os alimentos in natura que foram submetidos a um processamento industrial mínimo antes de serem adquiridos e consumidos. Esse processamento não adiciona outros produtos ou substâncias. Envolve limpeza, remoção de partes não comestíveis, fracionamento, pasteurização, redução de conteúdo de gordura, refrigeração, congelamento, desidratação ou procedimentos similares, conservando grande parte das propriedades nutricionais do alimento original e aumentando sua disponibilidade e segurança. Em combinações apropriadas, todos os alimentos desse grupo são a base para uma alimentação saudável.

Grupo 2: ingredientes culinários processados.

São as substâncias extraídas dos alimentos in natura ou da natureza, tais como óleos vegetais, sal e açúcar. O processamento inclui prensagem, moagem, pulverização, refinação, hidrogenação e outros métodos. Os ingredientes culinários não são consumidos isoladamente e são utilizados na preparação de outros alimentos, com refeições feitas à base de alimentos in natura ou minimamente processados.

Grupo 3: alimentos processados.

Os alimentos processados são fabricados a partir da adição de substâncias como o óleo, açúcar ou o sal nos alimentos in natura ou minimamente processados, bem como por processos que os tornam mais duráveis e palatáveis. Mesmo que algumas características do alimento original sejam mantidas nos alimentos processados, as técnicas utilizadas alteram substancialmente o perfil nutricional destes alimentos. Para o processamento são utilizadas técnicas como a salga, salmoura, defumação, cura, acondicionamento dos alimentos em vidros ou latas.

Grupo 4: alimentos e bebidas ultraprocessados
Os alimentos e bebidas ultraprocessados são produzidos de modo predominante ou unicamente a partir de ingredientes industriais, com pouco ou nenhum alimento integral em sua composição, com a proposta de serem produtos duráveis, acessíveis e convenientes, palatáveis, atraentes e rentáveis. São alimentos prontos ou quase prontos para serem consumidos por si só ou em combinação com outro produto ultraprocessado (por exemplo, biscoito recheado e refrigerante). Os componentes dos produtos ultraprocessados são, em grande maioria, aditivos como conservantes, estabilizantes, emulsionantes, solventes, agentes de ligação, adoçantes, além de realçadores sensoriais, de sabores e cores. O processamento inclui a hidrólise, a hidrogenação, a extrusão, a moldagem, a fritura e o cozimento prévios, além da adição de micronutrientes para fortificação do alimento.

Neste contexto, o Ministério da Saúde publicou em 2014 a nova edição do “Guia alimentar para a população brasileira” (MINISTÉRIO DA SAÚDE, 2014), que se constitui em uma das estratégias para implementação da diretriz de promoção da alimentação adequada e saudável. A nova publicação tem como princípios básicos a orientação para redução do consumo de alimentos ultraprocessados, baseados de acordo com a classificação proposta, ressaltando a importância do processamento industrial dos alimentos e seu impacto na saúde. O novo guia brasileiro é um marco internacional, pois adota a classificação dos alimentos de acordo com o nível de processamento como base para as diretrizes de saúde pública e nutrição para a população do país (MONTEIRO *et al.*, 2015; MONTEIRO *et al.*, 2016).

Considerando-se o padrão alimentar da população, de forma geral, observa-se progressiva substituição dos alimentos *in natura* ou minimamente processados e de ingredientes culinários por alimentos ultraprocessados (MOUBARAC *et al.*, 2013; MARTINS *et al.,*2013). Recente relatório da Organização Pan Americana da Saúde (OPAS, 2015) revelou o aumento de 48% nas vendas de produtos ultraprocessados no período de 2000 a 2013 em países latino-americanos. No Brazil, pesquisas nacionais de aquisição de gêneros alimentícios para consumo domiciliar mostram que a participação de alimentos ultraprocessados no total de calorias adquiridas aumentou de 20,0%, em 2002-2003, para 25,4%, em 2008-2009 (MARTINS *et al.*, 2013).

Fatores associados a este aumento podem estar relacionados às características destes produtos (MONTEIRO, 2010). Alimentos ultraprocessados são convenientes, práticos e de fácil transporte. Na maioria das vezes, são desenvolvidos para que possam ser consumidos em qualquer local, como no ambiente de trabalho ou meios de transporte, diante da televisão, dispensando o uso de pratos e talheres. Em geral, são vendidos como produtos prontos ou semiprontos para consumo e podem facilmente substituir refeições baseadas em alimentos *in natura* ou minimamente processados. Além disso, são produtos hiperpalatáveis, sendo que as técnicas de processamento utilizadas podem danificar os processos endógenos que sinalizam a saciedade e controlam o apetite provocando o consumo excessivo e “desapercebido” de calorias (LUDWIG, 2011; OGDEN *et al.,* 2013). O conjunto das características desfavoráveis dos alimentos ultraprocessados é amplificado por um *marketing* agressivo e sofisticado, modificando padroes de consumo, especialmente entre os consumidores vulneráveis, como as crianças e adolescentes (MALLARINO et al., 2013).

Os alimentos ultraprocessados apresentam perfil nutricional desfavorável e impactam negativamente na qualidade nutricional da alimentação. Produtos ultra-processados apresentam maior densidade energética, concentrações excessivas de açúcar livre, sal e gorduras saturadas e trans, e contém pouca ou nenhuma fibra alimentar em sua composição (LOUZADA *et al.*, 2015; STEELE *et al.*, 2016). Isso foi documentado em estudos de diferentes países utilizando dados de pesquisas de compras de alimentos (CROVETTO *et al.,* 2014; MOUBARAC *et al.,* 2013), inquéritos de consumo alimentar individual (BARCELOS *et al.,* 2014; BIELEMANN *et al.,* 2015) e análises de produtos disponíveis em supermercados (LUITEN *et al.,* 2015).

A classificação dos alimentos ultraprocessados tem sido utilizada para avaliar os possíveis impactos do consumo destes produtos à saúde humana. No entanto, estudos epidemiológicos com objetivo de avaliar o efeito do consumo de alimentos ultra processados na saúde de populações ainda são raros, dado sua recente definição. Estudo realizado pela OPAS evidenciou associação entre o volume de vendas de alimentos ultraprocessados por habitante e a prevalência de obesidade entre os adultos. Este levantamento indica que países como Bolívia e Peru, onde as vendas de alimentos ultraprocessados são pequenas e a alimentação tradicional ainda é predominante, apresentam as menores médias de IMC. Por outro lado, México e Chile, onde as vendas de alimentos ultraprocessados são elevadas, apresentam os maiores valores de IMC (OPAS, 2015).

A classificação dos alimentos ultraprocessados tem sido utilizada para avaliar os possíveis impactos do consumo destes produtos à saúde humana. CANELLA *et al.* (2014), em estudo realizado com amostra representativa da população brasileira, demonstraram associação positiva e independente entre a disponibilidade domiciliar de produtos ultraprocessados e a obesidade. Um estudo transversal realizado com adolescentes em um município do Rio de Janeiro mostrou que o consumo de produtos processados e ultraprocessados teve associação com a síndrome metabólica (TAVARES *et al.*, 2012). Recentemente RAUBER *et al.* (2015), em estudo longitudinal com crianças de baixa condição socioeconômica no sul do país revelaram que o consumo de produtos ultraprocessados na idade pré-escolar é um preditor do aumento das concentrações de colesterol total e LDL-colesterol na idade escolar.

1.4 Intervenções dietéticas no primeiro ano de vida.

Os primeiros anos de desenvolvimento da criança estão diretamente relacionados com a saúde futura (WHO, 2014; BIRCH & DOUB, 2014; LANGLEY-EVANS, 2015). Hábitos alimentares não-saudáveis nos primeiros anos de vida constituem-se em relevante problema de saúde pública, estando associadas a desfechos negativos de saúde não apenas na infância, mas também na idade adulta (HANSON *et al.*, 2012; BRANDS *et al.*, 2014). Falta de conhecimento e informação dos pais ou cuidadores e restrições impostas por tradições, crenças e mitos são fatores associados a realização de práticas alimentares inadequadas nos primeiros anos de vida (GULDAN *et al*, 2000; MELLO *et al*, 2016). Dessa forma, intervenções focadas na promoção do conhecimento adequado sobre alimentação infantil para mães, pais e cuidadores tornam-se fundamentais para mudança de comportamento e melhora do padrão alimentar de crianças pequenas (SHI e ZANG, 2010).

Há inúmeros fatores limitantes que interferem no sucesso das ações precoces de educação nutricional, como a idade da criança no início da intervenção, a complexidade dos comportamentos a serem modificados e o contexto social e econômico da comunidade na qual a intervenção será realizada (SANTOS *et al*, 2001; SHI e ZANG, 2010). Além destes, há fatores relacionados à metodologia, frequência e duração da intervenção. O delineamento ideal para cada ação educacional depende da cultura, dos recursos, da infraestrutura e dos canais de comunicação existentes no local, bem como das possibilidades para modificá-los. Intervenções educacionais efetivas ocorrem em tempo suficiente para promoção de mudanças nas práticas alimentares e usam número limitado de mensagens direcionadas para ações que sejam viáveis de serem adotadas pela população-alvo (DEWEY e ADU-AFARWUAH, 2008). Em geral, as mensagens utilizadas em programas que focam na alimentação complementar abordam: duração do aleitamento materno exclusivo e aleitamento materno, uso de alimentos ou preparações ricas em nutrientes ou alimentos específicos, consistência da alimentação, alimentação na presença de doenças e higiene na preparação e armazenamento dos alimentos (DEWEY e ADU-AFARWUAH, 2008).

Nos últimos cinco anos, estudos de intervenção focados no aconselhamento dietético para mães de crianças pequenas foram publicados e adicionaram evidências sobre seus efeitos na promoção do aleitamento materno (EKSTROM *et al.*, 2012; HAROON *et al.*, 2013; FU *et al.*, 2014; SIKANDER *et al.*, 2015), melhoras na prática da alimentação complementar (SHI *et al.*, 2010; OLAYA *et al.*, 2013; DANIELS *et al.*, 2014) e melhora do estado nutricional das crianças (IMDAD et al., 2011; LASSI et al, 2013). Dois estudos recentes de meta-análise avaliaram o efeito de ensaios de campo randomizados realizados em países em desenvolvimento. As evidências dão suporte à hipótese de que intervenções de aconselhamento dietético com componentes educacionais relacionadas à alimentação complementar podem ser efetivas na promoção do aleitamento materno e adequada alimentação complementar, além de acelerar a velocidade de ganho de peso e estatura das crianças nos primeiros dois anos de vida (IMDAD *et al.*, 2011; LASSI *et al.*, 2013).

A situação epidemiológica atual, contudo, acentua a necessidade de estratégias de ação focadas em problemas relacionados ao ganho de peso excessivo e doenças crônicas relacionadas. A maioria dos programas de prevenção de obesidade na infância são direcionadas para crianças em idade escolar (BIRCH e

VENTURA, 2009; CORVALAN *et al.*, 2009). CIAMPA et al (2010), em revisão sistemática recente, encontraram apenas doze artigos cujos objetivos foram avaliar o efeito de intervenções precoces para prevenção da obesidade em crianças menores de 2 anos, sendo que nenhuma intervenção causou impacto significativo no peso dos sujeitos estudados. Recentemente, estudo de revisão concluiu que faltam estudos randomizados controlados que evidenciem os efeitos a longo prazo do aleitamento materno e da adequada introdução dos alimentos na prevalência de obesidade, especialmente em países em desenvolvimento (YANG e HUFFMAN, 2013).

Estudos que avaliem o efeito de intervenções precoces em desfechos metabólicos a logo prazo são ainda mais raros na literatura científica. O *Special Turku Coronary Risk Factor Intervention Project (Strip Study)* é um ensaio clínico randomizado iniciado no ano de 1990, em que famílias finlandesas com crianças de cinco meses da idade foram recrutadas e randomizadas para intervenção de aconselhamento dietético em domicílio (SIMELL *et al.*, 2009). O grupo intervenção recebeu aconselhamento dietético duas vezes por ano, enquanto o grupo controle recebeu aconselhamento dietético uma vez por ano, ambos até a adolescência. Os resultados evidenciam melhora no padrão alimentar nos primeiros dois anos de vida das crianças do grupo intervenção (LAGSTROM *et al.*, 1997). Em relação aos desfechos metabólicos, os autores encontraram melhora na sensibilidade à insulina no grupo intervenção em relação ao grupo controle nas idades de 6 anos (KAITOSAARI *et al.*, 2006) e dos 15 aos 20 anos de idade (ORANTA *et al.*, 2013) e melhora no perfil lipídico durante todos os anos de seguimento (NIINIKOSKI *et al.*, 2007). Por outro lado, o *Promotion of Breastfeeding Intervention Trial* (PROBIT) é um ensaio randomizado de *cluster*, cuja intervenção para promoção de adequadas práticas alimentares aconteceu nos 12 primeiros meses de vida. Os resultados mostram que não houve diferença entre os grupos intervenção e controle para os desfechos glicose e insulina sérica aos 11,5 anos de idade (MARTIN *et al.*, 2014).

Em 2002, o Ministério da Saúde brasileiro e a Organização Pan-americana da Saúde publicaram a primeira edição do “Guia alimentar para crianças menores de dois anos” (MINISTÉRIO DA SAÚDE, 2002). Ele foi baseado nas diretrizes alimentares propostas pela Organização Mundial da Saúde para este ciclo da vida e elaborado de acordo com o contexto socioeconômico e cultural e os problemas de saúde locais relacionados à alimentação da época de seu planejamento, após amplo levantamento de dados existentes no país. A partir desse estudo, foram elaboradas recomendações publicadas no manual técnico “Dez passos para alimentação saudável para crianças menores de dois anos” com o objetivo de nortear os conteúdos das mensagens a serem repassadas para a população alvo (MINISTÉRIO DA SAÚDE, 2002). Seus principais objetivos são: o fomento do aleitamento materno exclusivo até o sexto mês, complementar até o segundo ano de vida e a adequada introdução da alimentação complementar (MINISTÉRIO DA SAÚDE, 2002).

Os "dez passos" consistem em: (1) dar somente leite materno até aos seis meses, sem oferecer água, chás ou qualquer outro alimento; (2) a partir dos seis meses, oferecer, de forma lenta e gradual, outros alimentos, mantendo o leite materno até os dois anos de idade ou mais; (3) a partir dos seis meses, dar alimentos complementares (cereais, tubérculos, carnes, leguminosas, frutas e legumes) três vezes ao dia, se a criança receber leite materno, e cinco vezes ao dia, se estiver desmamada; (4) a alimentação complementar deve ser oferecida sem rigidez de horários, respeitando-se sempre a vontade da criança; (5) a alimentação complementar deve ser espessa desde o início e oferecida de colher, começar com consistência pastosa (papas/purês) e, gradativamente, aumentar a consistência até chegar à alimentação da família; (6) oferecer à criança diferentes alimentos ao dia (uma alimentação variada é uma alimentação colorida); (7) estimular o consumo diário de frutas, verduras e legumes nas refeições; (8) evitar açúcar, café, enlatados, frituras, refrigerantes, balas, salgadinhos e outras guloseimas nos primeiros anos de vida e usar sal com moderação; (9) cuidar da higiene no preparo e manuseio dos alimentos e garantir o armazenamento e conservação adequados; (10) estimular a criança doente e convalescente a se alimentar, oferecendo seus alimentos preferidos, respeitando a sua aceitação.

No Brasil, Vitolo e colaboradores (2005) realizaram estudo randomizado para avaliar o efeito de orientações alimentares baseadas nas diretrizes dos “Dez passos para alimentação saudável para crianças menores de dois anos” em domicílio com mães durante o primeiro ano de vida das crianças. Os resultados mostraram impacto positivo da intervenção na proporção de aleitamento materno exclusivo aos 4 meses, aleitamento materno aos 12 meses e na redução do consumo de guloseimas no grupo intervenção aos 12 meses. No mesmo grupo de crianças, estudos demonstraram que, em idade pré-escolar, a prevalência de crianças com dieta de baixa qualidade, avaliada pelo *Heathy Eating Índex (*KENNEDY *et al., 1995)*, foi menor no grupo intervenção em relação ao grupo controle (VITOLO *et al.*, 2010). A eficácia a longo prazo do aconselhamento dietético no primeiro ano de vida foi também observada no perfil sérico de crianças em idade escolar: meninas do grupo intervenção apresentaram maiores valores de HDL-colesterol e menores concentrações de triglicerídeos, comparadas ao grupo controle (LOUZADA *et al.*, 2012).

Os estudos disponíveis que avaliam o efeito de intervenções dietéticas precoces são, portanto, consistentes com a hipótese de que intervenções de aconselhamento dietético com componentes educacionais relacionadas à alimentação complementar poderiam ser efetivos na melhora do padrão alimentar na infância e na pré-escola. Nota-se, entretanto, lacuna na literatura cientifica quanto aos possíveis efeitos destas intervenções em idades avançadas. Além disto, também são raros estudos que avaliem os efeitos de intervenção de cunho educacional em desfechos metabólicos. Após intervenção realizada por nosso grupo de pesquisa no primeiro ano de vida de crianças do sul do país, continuamos coletando dados socio-demográficos, antropométricos, bioquímicos e dietéticos até que os sujeitos de pesquisa completassem 12 anos. Desta forma, é possível avaliar o efeito a longo-prazo de intervenções focadas na promoção do conhecimento adequado sobre alimentação infantil, sendo este o propósito central desta tese. Além disso, a disponibilidade de dados não apenas antropométricos e dietetéticos, mas também laboratoriais oportuniza análise inédita quanto aos efeitos a longo prazo de intervenção focada na promoção do conhecimento adequado sobre alimentação infantil em desfechos metabólicos.

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2.OBJETIVOS

2.1 Objetivo geral

Avaliar o impacto de aconselhamento dietético realizado durante o primeiro ano de vida em desfechos metabólicos em escolares e em desfechos antropométricos, metabólicos e dietéticos em adolescentes.

2.2 Objetivos específicos

- Comparar as concentrações séricas de glicemia e insulina e os valores de HOMA-IR entre os grupos controle e intervenção aos 8 anos de idade;
- Investigar a associação entre o ganho de peso desde o nascimento e alterações nas concentrações de glicose e insulina e nos valores de HOMA-IR aos 8 anos de idade;
- Comparar as medidas de altura z-escore para idade, IMC z-escore para idade, soma das pregas cutâneas triciptal e subscapular, percentual de gordura, massa de gordura e massa livre de gordura entre os grupos intervenção e controle aos 12 anos de idadade;
- Comparar os valores séricos de colesterol total, LDL, HDL, n-HDL, triglicerídeos, razão HDL/TG, glicose, insulina, HOMA-IR, cortisol e Proteína C-reativa entre os grupos intervenção e controle aos 12 anos de idade;
- Comparar o consumo de produtos ultraprocessados entre os grupos intervenção e controle aos 12 anos de idade;
- Verificar se a efetividade da intervenção é diferente entre os sexos masculino e feminino, em adolescentes.

3. ARTIGO ORIGINAL 1

Effect of maternal dietary counselling during the first year of life on glucose profile and insulin resistance at age eight: a randomized field trial.

Dietary counselling and insulin resistance

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KEYWORDS: intervention studies, randomized controlled trial, dietary counselling, childhood, insulin resistance, HOMA-IR, BMI gain.

Clinical Trial Registry: the study has been registered at www.clinicaltrials.gov (identifier NCT00629629).

ABSTRACT

Education interventions that stimulate complementary feeding practices can improve the nutritional status of children and may protect against future chronic diseases. We assessed the long-term effectiveness of dietary intervention during the first year of life on insulin resistance levels, and investigated the relation between insulin resistance and the weight changes over time. A randomized field trial was conducted among 500 mothers who gave birth to full-term infants between October 2001 and June 2002 in a low-income area in São Leopoldo, Brazil. Mother-child pairs were randomly assigned to intervention (n=200) and control groups (n=300) and the mothers in the intervention group received dietary counselling on breastfeeding and complementary feeding of their children during the first year of life. Fieldworkers blinded to assignment assessed socio-demographic, dietary and anthropometric data during follow-up at ages 1, 4 and 8 years. Blood tests were performed in 305 children at age 8 to measure fasting serum glucose and insulin concentrations and the Homeostasis Model Assessment index of Insulin Resistance (HOMA-IR). At age 8 years, the intervention group showed no changes in glucose and insulin concentration, or HOMA-IR values (change 0.07; 95%CI: -0.06 to 0.21 for girls; and change -0.07; 95%CI: -0.19 to 0.04 for boys) compared to study controls. Insulin resistance was highly correlated however with increases in BMI between birth and age 8. Although this dietary intervention had no impact on glucose profile at age 8, our findings suggest that BMI changes in early childhood can serve as an effective marker of insulin resistance.

KEYWORDS: intervention studies, dietary counselling, childhood, insulin resistance, BMI gain.

INTRODUCTION

The number of overweight children under the age of five in 2013 was estimated to be over 42 million. 75% of these are living in developing countries[1,2]. The relation between body weight and insulin resistance in children is therefore an important research topic[3-5]. A growing body of evidence suggests that excessive weight gain in infancy and in childhood is likely to be associated to insulin resistance in adults[6-8]. The impact of early weight gain on insulin resistance in children has not been clearly identified however. Insulin resistance in childhood is of great clinical importance as it may lead to diabetes type 2, hypertension, hepatic steatosis, endothelial dysfunction[9-11], cardiovascular diseases and to metabolic syndrome[12] later in life. The prevalence of insulin resistance in childhood has shown a steadily increase in recent years[13,14] and this points to the need for appropriate and early preventive intervention strategies.

Systematic reviews suggest that educational interventions including breastfeeding and complementary feeding practices may be effective in improving the nutritional status of infants and young children[15,16] and thereby also protect against future chronic diseases[17]. We have shown in a field trial that maternal counselling for infant feeding in the home can stimulate infant breastfeeding at 4, 6, and 12 months of age[18] which could provide some long-term protection against the development of insulin resistance[19,20]. In the same field trial, maternal counselling during the first year of life was effective in reducing children's energy-dense food consumption at 12 months[18,21], and in improving diet quality at 4 years[22] and lipid profile in daughters at 8 years[23]. We are not aware however of any studies of the potential benefits of maternal counselling on infant feeding in reducing insulin resistance in childhood, particularly in developing countries. We expect that efforts to prevent insulin resistance, especially if started early enough, could delay the progress of metabolic complications and optimize healthier outcomes[24]. We therefore investigated if the effects of our home-based infant nutrition interventions were still seen after the study population had reached the age of 8 years. For this study, our focus is on selected metabolic parameters related to insulin resistance, including serum glucose, insulin and the HOMA-IR measure. To further clarify the relation between body size and insulin resistance in children, we also examined the relation between the metabolic parameters and weight changes over time in children of mothers who did and did not receive dietary counselling.

SUBJECTS AND METHODS

The randomized field trial was conducted from the maternity wards of a hospital in a low-income population setting in the city of São Leopoldo, Brazil. Mothers of healthy, singleton, full-term (> 37 weeks) and normal birth weight (≥ 2500 g) babies were invited to participate. We excluded women with HIV-positive mothers, infants with congenital malformations or admitted to neonatal intensive care unit, and individuals with breast-feeding impediments. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the Universidade Federal de Ciências da Saúde de Porto Alegre. All parents provided written informed consent. We followed the Consolidated Standards for Reporting Trials (CONSORT) guidelines to report on this randomized field study[25]. The study has been registered at www.clinicaltrials.gov (Identifier NCT00629629).

A total of five hundred mother-child pairs were recruited by fieldworkers at the maternity, representing 89.5% of all invited mothers. To guarantee blinding of the intervention assignment, an investigator not involved in the recruitment conducted the randomization procedure during the trial. Block randomization was used to avoid imbalances during the randomization process. Mothers who agreed to participate were sequentially listed based on their time of delivery, grouped in blocks of 5, and their names separated in opaque, sealed envelopes. Two mothers from each block were randomly assigned to the intervention group and the remaining three mothers were allocated to the control group. At the end of the randomization, 200 children were allocated to the intervention group, and 300 to the control group. We included more mother-child pairs in the control group as we expected greater losses to follow-up in controls because of a lower frequency of follow-up home visits.

For the original trial, we calculated that a sample of 363 infants would be required to detect a 65% increase in the frequency of exclusive breastfeeding up to 4 months of age in the intervention group (with 80% power and alpha=5%), assuming a 21.5% frequency of exclusive breastfeeding in the control group. For the current follow-up at age 8 years, 128 children would be required to detect a HOMA-IR change of 0.5 SD units, with 80% power and alpha= 5%.

Intervention

The intervention consisted of dietary advice about breastfeeding and complementary feeding based on the "Ten steps for the healthy feeding for Brazilian children from birth to two years of age"[26]. It was carried out between October 2001 and June 2002 by home visits within 10 days of the child's birth, on a monthly basis up to 6 months old and at 8, 10, and 12 months old. The main purpose of the program was to promote exclusive breastfeeding for 6 months followed by healthy complementary foods. During each home visit mothers received dietary advice in accordance with the baby's age. Mothers were advised against the addition of sugars (cane sugar, honey) to fruit, porridge, juices, milk, or other liquids. They were encouraged to avoid fried food, soft drinks, sweets, and salty snacks and to use salt in moderation. Advice on hygiene practices in food preparation and handling was provided. A simple coloured leaflet with food pictures composing a healthy meal was used to guide the dietary advice and was given to the mother as a reminder. The writing material was simplified to take into consideration the mothers' level of education. During each visit of about 40 to 60 minutes, the fieldworkers clarified and reinforced recommendations while respecting the mother's level of cognition as well as cultural and economic background. The dietary intervention summary and the main counselling strategies applied during each home visit have been described elsewhere in detail[(23)]. The counselling was carried out by paired undergraduate students in nutrition science. The fieldworkers who carried out the dietary advice received 8 hours of theoretical training. During the intervention program, quality control was ensured by weekly scheduled meetings with all fieldworkers and the coordinator of the program to discuss all dietary advice provided to mothers. Mothers were encouraged to report any adverse events that occurred with children during the intervention.

Control Group

Mothers in the control group received the recommended standard care. They were interviewed twice during the first year after childbirth (at 6 and 12 months of age), for data collection only. All mothers were encouraged to maintain normal paediatric visits for their babies during the study period. Nutritional diagnoses were provided to mothers and they were advised to talk to the health professionals about the nutritional diagnosis that we provided to them. After the first year, children in intervention and control groups were followed up during childhood and new data were collected at 4 and 8 years old.

Data Collection

Identification and data required for locating the families in the community were collected at the time of recruitment. Trained fieldworkers, not involved in the intervention and unaware of group allocation, conducted face-to-face structured interviews during home visits with the mothers at 1 year, 4 years and 8 years following the infants' birth. Every month, 10% of the questionnaires were selected randomly and followed up by telephone calls to the mothers to verify the authenticity of the collected data. Children's gender, skin colour, birth weight and mode of delivery were obtained from hospital records. Pre-pregnancy weight and maternal weight at the end of pregnancy were self-reported and mothers' height was measured during home visits by fieldworkers when the children were six months old. Pre-pregnancy BMI was calculated as pre-pregnancy weight divided by the square of height (kg/m^2). Gestational weight gain was calculated by subtracting pre-pregnancy weight from weight at the end of pregnancy. Household income and duration of exclusive breastfeeding data were collected during home visits. When the children were 1 year old, the mothers were asked if their infants had received regular health care services in the first year after birth. At 8 years old, for diet pattern analyses, two multiple-pass 24-h dietary recalls were collected for each child on two randomly selected and non-consecutive days upon home visits to the families. The mean nutritional composition of the two 24-h dietary recalls for each child was classified according to the Healthy Eating Index (HEI)[27], which is an instrument that attributes scores to the diet quality of individuals. Details have been described elsewhere[28, 29]. In order to assess a sedentary lifestyle marker among 8-year-old children, mothers were asked to report the total (hours and minutes) night-time sleep duration and the total (hours and minutes) screen time duration (including television, computer and video-game) on the preceding day of the interview.

Anthropometric Measurements

At 1 year of age, all children were weighed without clothing on a portable digital scale (Techline, São Paulo, Brazil), and their length was measured by using an infant stadiometer (Serwital Inc, Porto Alegre, Brazil). At ages 4 and 8 years old, children were weighed to the nearest 0.1 kg in light clothing without shoes on a digital scale (Techline), and standing height was measured to the nearest 0.1 cm using a stadiometer (SECA, Hamburg, Germany). All measures were converted into z-scores of BMI-for-age based on World Health Organization Growth Standards[30,31]. Changes in growth measurements from birth to 8 years old were analysed as BMI-for-age *z*-score variation, considering three periods: from birth to 1 year, from 1 year to 4 years and from 4 to 8 years old.

Glucose Profile

At 8 years of age, venous blood samples were obtained from the right arm after an overnight fast to measure serum glucose and insulin concentrations and calculate the HOMA-IR index. Analyses were performed at the laboratory of Cardiology Institute of Rio Grande do Sul by technicians who were unaware of study assignments. Glucose and insulin were estimated using an automatic analyser (Cobas Integra®, Roche, São Paulo, Brazil). HOMA-IR was calculated as (insulin μU/ml x glucose mmol/l) / 22.5[32].

Statistical Analyses

Analyses were performed by intention to treat and by gender. Non-normally distributed variables were log-transformed for all statistical procedures. Untransformed values are presented in all tables for ease of clinical interpretation. Student's *t* test was used to evaluate the effect of the intervention on independent continuous variables. Univariate and multivariate linear regressions were performed to examine the relation of intervention and selected anthropometric covariates of special interest on glucose and insulin concentration and HOMA-IR values at age 8 years, including pre-pregnancy BMI, gestational weight gain, child's birth weight, and BMI *z*-score from birth to 1, 4, and 8 years old. In univariate models, the reported beta-coefficients represent separate models for each listed variable; and in multivariate models, beta-coefficients are adjusted for all variables in the model. We also examined the effect of adjustment for baseline social, demographic and breastfeeding variables: child's gender (male/female) and skin colour (white/not-white), maternal schooling, total household income, mode of delivery (normal/caesarean), exclusive breastfeeding (≥ 4 months) and breastfeeding, on our estimates of treatment effects. Finally, we further analysed the effect of adjustment for total HEI score, total sleep and screen time duration at 8 years old, on our estimates of treatment effects. Collinearity was checked in all models. All statistical analysis were performed using SPSS 16.0 (SPSS IBMInc., Chicago, IL USA) and statistical significance was set at $p<0.05$, two-sided.

RESULTS

Among the 500 children initially recruited, 396 underwent anthropometric assessments at age 1, 345 at age 4, and 309 at age 8 (Fig 1). A total of 305 children underwent glucose assessment and 303 children underwent insulin and HOMA-IR assessment at 8 years old. No adverse events were reported during the intervention. The proportion of overweight children (BMI > 1 SD) was 36.1% (n=143) at age 1, 20.6% (n=71) at age 4, and 27.5% (n=85) at age 8 years. There were no differences in overweight prevalence proportions between intervention and control groups for the three periods (1, 4 and 8 years) and this result persisted after analyses by gender. The median duration of exclusive breastfeeding was 3.5 months (95%CI: 0.5 to 6.5) in the intervention group and 1.5 months (95%CI: 0.5 to 6.5) in the control group; the median duration of breastfeeding was 12.5 months (95%CI: 0.5 to 12.5) in the intervention group and 10.5 months (95%CI: 0.5 to 12.5) in the control group.

No differences were found between children who were lost to follow-up and those who remained on study at 8 years of age in terms of pre-pregnancy BMI (p=0.48), gestational weight gain (p=0.89), mode of delivery (p=0.88), weight at birth (p=0.55), maternal age at child's birth (p=0.22), and maternal level of education (p=0.66). There were no differences between intervention and control groups on selected baseline characteristics (Table 1).

There were no differences between the intervention and control group comparing glucose and insulin concentrations and HOMA-IR indices at 8 years old, for both genders (Table 2). In addition, there were no significant differences between intervention and control groups comparing BMI *z*-score changes from birth to 1 year, from age 1 to 4, and from age 4 to 8 years, in girls and boys.

The linear regression analyses evaluating the associations between anthropometric covariates and glucose, insulin and HOMA-IR values at 8 years old are shown in Table 3. In multivariate analysis, birth weight and the increase in BMI *z*-scores between 1-4 years and between 4-8 years all contributed significantly to glucose and insulin concentrations at age 8 and to rises in HOMA-IR. The increase in BMI during the first year of life was positively associated with rises in insulin and HOMA-IR. These findings persisted after adjustment for child's gender and skin colour, total family income, maternal schooling, mode of delivery, and exclusive or partial breastfeeding. Additional analyses were performed after further adjustment for total sleep and screen time duration and total HEI score at 8 years old and all results persisted.

DISCUSSION

To the best of our knowledge, this is the first randomized trial to examine the potential effects on children of maternal dietary counselling during the first year of life on insulin resistance at age 8. Contrary to our expectation, we did not see any changes in insulin resistance at this age.

We considered two possibilities that may explain the lack of effectiveness of the trial to affect metabolic outcomes in childhood. First, the change in dietary practices observed in this population[(18,21,22)] might not be large enough to have any long-term impact on glucose metabolism at age 8 years. This supports the need to continue the dietary counselling even after the infancy period, to achieve adequate public policies. Second, any early changes in dietary practices may have been overridden by dietary and weight gain changes between the ages 1-8 years, after the study intervention had ceased at age 1. The focus of the "Ten steps for the healthy feeding for Brazilian children from birth to two years of age" intervention plan is on dietary counselling, and monitoring excessive weight gain is not emphasized by the plan. As insulin resistance is closely related to body weight[(3,4,33)], the lack of effectiveness of the trial to affect metabolic outcomes in older children could be related to weight gains between the ages 1-8 years of all study participants, irrespective of dietary counselling in the first year of life. Although the effectiveness of breastfeeding and complementary feeding interventions to improve child nutrition, growth, and development is well documented in many settings[(34-37)] less attention has been paid in clinical trials to the potential impact of weight gains in infancy and childhood as a contributor to the development of adverse metabolic outcomes, including insulin resistance in children.

In the absence of an intervention effect, we therefore also analysed the impact of selected anthropometric variables on the glucose profile and insulin resistance at ages 1, 4, and 8 years. Our findings show that increased birth weight and higher weight gain velocities from birth to 8 years old all had a negative impact on the glucose profile and insulin resistance among school-aged children, irrespective of the dietary counselling in the first year of life. These findings support other observations that higher weight gain velocities at either 3 to 5 years old[(38)] or 4 to 9 years old[(39)] can lead to relative larger increases in fat mass and insulin resistance, either through overproduction of non-esterified fat or through increased synthesis and release of pro-inflammatory cytokines[40]. Recently, a positive relation was seen between BMI *z*-score increases and adverse insulin and HOMA-IR outcomes in Brazilian children followed from 4 to 10 years, although no impact was found on glucose concentrations[41]. Weight gains from birth to 2 years old were already related to insulin resistance among young adults in several cohort studies conducted in developing countries[42]. There is also evidence that increases in fasting insulin between the ages 3-6 years can be associated with a greater risk of type 2 diabetes, irrespective of adult BMI and parental history[43]. Although major metabolic diseases related to early insulin resistance may not become symptomatic until adulthood, our findings suggest that the detection and prevention of risk factors for insulin resistance should begin in childhood, when changes in lifestyle, including those related to weight gain can still reduce the risk and severity of metabolic disease later in life.

This study has some limitations. While we experienced losses to follow-up, we found no differences between the baseline characteristics of the children who remained in the study and those who were lost. Secondly, the mothers may have been aware of the intervention group to which they were assigned. This may have affected responses to study due to social desirability bias because it is not possible to blind patients in studies that evaluate dietary advices. To minimize such bias, fieldworkers not involved in the intervention carried out the assessments. A limitation with respect to generalizability of the study findings is the choice of the study population, including only children between birth and 8 years of age from a low-income population. In response, we note that this target population is of specific interest for the introduction and evaluation of large-scale dietary interventions to improve population health in regional or national health programs. There could be self-report bias of pre-pregnancy weight; however, studies conducted in high[44] and low-income countries[45], including a study in Brazil[46], have demonstrated that the BMI obtained using self-reported pre-pregnancy weight strongly correlated with those obtained using anthropometrics measured. Considering the outcome of this study (insulin resistance), another limitation is the fact that gestational diabetes data were not obtained and children born from those mothers could present metabolic alterations. However, this trial was conducted with healthy newborn babies and the analyses were adjusted for pre-pregnancy weight, gestational weight gain and birth weight, variables highly related to gestational diabetes melittus. As a further limitation, we were not able to measure insulin resistance prior to age 8 years and other studies will be needed to fill this gap. This study clearly shows however that understanding the metabolic impact of dietary interventions and growth trends in infancy and childhood is extremely important to formulate public health strategies aimed at preventing chronic diseases and that the long-term follow-up even of study populations recruited at birth can be extremely important.

In summary, our study shows no impact of dietary counselling in the first year of life on metabolic profiles and insulin resistance at the age of 8. Our results do point however to the crucial relation between infant and childhood weight gain and insulin resistance at age 8 in school children from a low-income community in Brazil. We found that weight gains between birth and age 1, age 1-4 years, and age 4-8 years all contributed significantly to adverse changes in insulin resistance at age 8. Our findings suggest that preventing excessive gain weight since early life is a relevant key to prevent insulin resistance in childhood.

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Conflict of Interest: none.

Authorship: CSC: formulating the research question, analysed and interpreted the data, performed the statistical analysis and wrote the manuscript; PDBC: conducted the research and critically reviewed the manuscript; LHL: interpreted the data and statistical analysis and critically reviewed the manuscript; MRV: designed and conducted the research, formulating the research question, interpreted the data and the statistical analysis and critically reviewed the article. All authors: read and approved the final manuscript.

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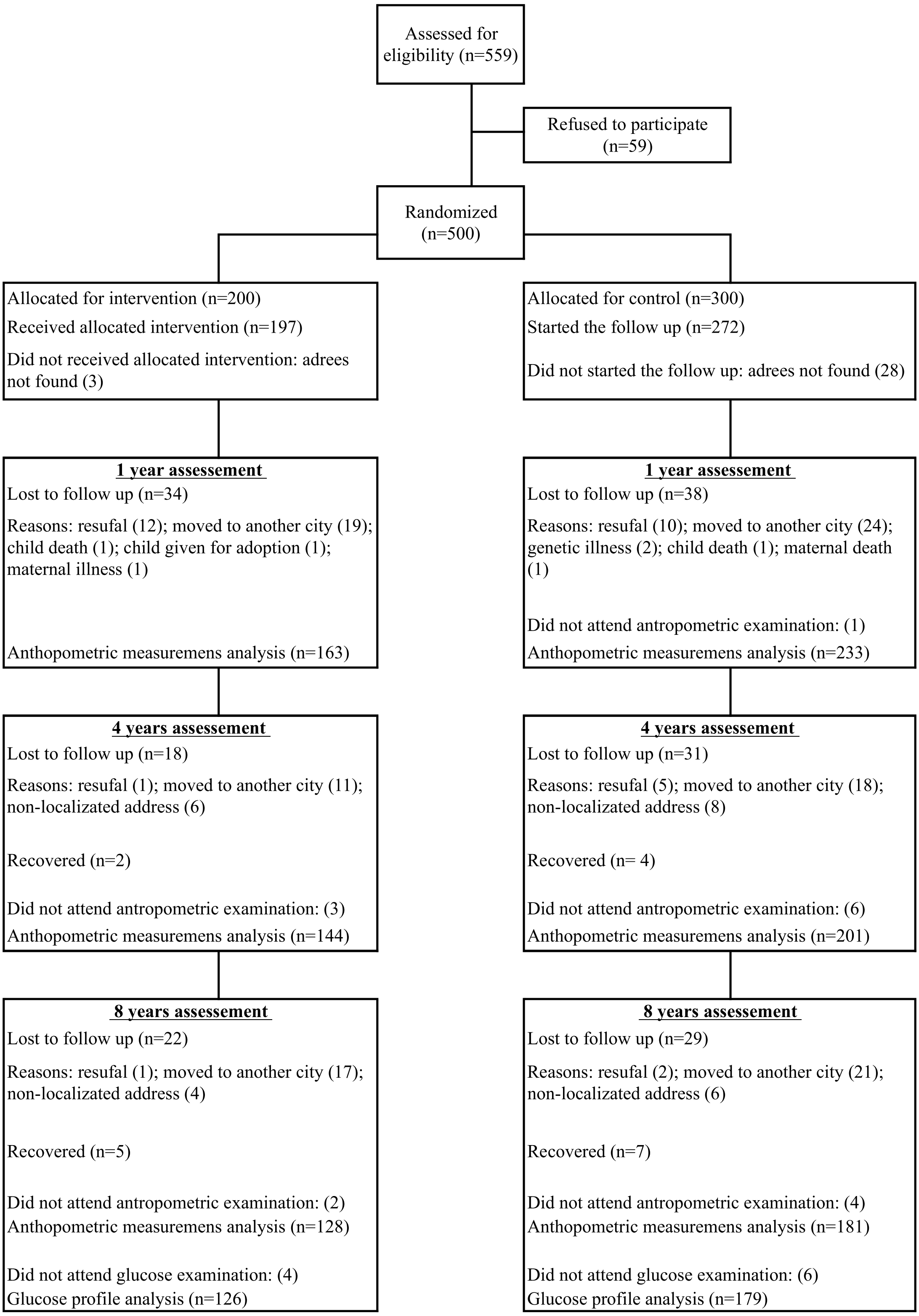

Figure 1: Trial profile of participation in the randomized controlled trial from recruitment of mother-child pairs through the assessment at 8 years of ag

Table 1: Characteristics of children and their households at baseline according to the group, at 1-year old[a].

Characteristics	*n* [a]	Intervention	Control	P[b]
Child				
Boys, n (%)	397	93 (57.1)	131 (56.0)	0.83
Skin colour (white), n (%)	331	58 (41.1)	85 (44.7)	0.51
Birth weight (g), mean (SD)	390	3375.34 (464.51)	3354.30 (466.70)	0.66
Birth length (cm), mean (SD)	390	48.74 (1.92)	48.84 (2.12)	0.62
Households at baseline				
Maternal pre-gestational BMI, mean (SD)	367	24.72 (5.32)	23.90 (4.15)	0.10
Gestational age, mean (SD)	375	39.34 (1.31)	39.38 (1.19)	0.76
Delivery by caesarean section, n (%)	356	51 (36.7)	94 (43.3)	0.21
Maternal age at child's birth <20 years, n (%)	397	29 (17.8)	46 (19.7)	0.64
Mother's education <8 years, n (%)	396	99 (61.1)	131 (56.0)	0.31
Father's education <8 years, n (%)	369	93 (61.6)	120 (55.0)	0.21
Mother's employment, n (%)	391	58 (35.8)	76 (33.2)	0.59
Father's employment, n (%)	361	130 (88.4)	195 (91.1)	0.40
Annual household income ≤ U$300, n (%)	395	122 (74.8)	165 (71.1)	0.18

BMI: body mass index; g: grams; cm: centimetres. [a] n indicates the number of responses recorded for each characteristic. [b] Student's t test or x were used (depending on categorical or continuous variable).

Table 2: Intervention effect on glucose, insulin and HOMA-IR at 8 years old and on BMI *z*-score variation from birth to 8 years old, according to sex.

	Intervention		Control		Difference (95% CI)	p
	n	Mean(SD)	n	Mean(SD)		
Girls						
Glucose mmol/l[a]	52	4.22 (0.42)	80	4.24 (0.38)	0.02 (-0.12 to 0.16)	0.80
Insulin uU/ml[a,b]	52	5.57 (3.12)	79	7.32 (7.02)	0.07 (-0.06 to 0.21)	0.28
HOMA-IR[a,b]	52	1.06 (0.62)	79	1.41 (1.40)	0.07 (-0.06 to 0.21)	0.29
Δ BMI *z*-score birth to 1y[a]	70	0.17 (1.59)	99	0.28 (1.18)	0.11 (-0.31 to 0.53)	0.61
Δ BMI *z*-score from 1y to 4y[a]	60	-0.44 (0.82)	86	-0.45 (1.00)	-0.01 (-0.32 to 0.30)	0.94
Δ BMI *z*-score 4y to 8y[a]	49	0.04 (0.87)	79	0.19 (0.82)	0.15 (-0.14 to 0.45)	0.32
Boys						
Glucose mmol/l[a]	74	4.46 (0.42)	99	4.46 (0.42)	-0.01 (-0.13 to 0.12)	0.97
Insulin uU/ml[a,b]	73	5.74 (4.32)	99	4.53 (3.14)	-0.07 (-0.19 to 0.03)	0.17
HOMA-IR[a,b]	73	1.15 (0.87)	99	0.92 (0.62)	-0.07 (-0.19 to 0.04)	0.17
Δ BMI *z*-score birth to 1y[a]	93	0.10 (1.22)	127	0.03 (1.25)	-0.07 (-0.40 to 0.27)	0.69
Δ BMI *z*-score from 1y to 4y[a]	82	-0.21 (1.32)	110	-0.28 (1.05)	-0.07 (-0.41 to 0.27)	0.67
Δ BMI *z*-score 4y to 8y[a]	72	0.24 (1.11)	93	-0.06 (1.18)	-0.29 (-0.65 to 0.06)	0.10

[a]Student't test was used; [b] Nonnormally distributed variables were log-transformed; HOMA-IR: homeostatic model assessment of insulin resistance (insulin μU/ml x glucose mmol/l) / 22.5); y: year(s).

Table 3. Linear regressions analysis of glucose, insulin and HOMA-IR at 8 years old and independent variables (n = 305).

	Univariate model*		Multivariate model**	
	B (95% IC)	p	B (95% IC)	p
Glucose (mmol/l)				
Intervention	0.03 (-1.72 to 1.78)	0.98	-0.31 (-2.18 to 1.48)	0.71
Pre-pregnancy BMI, kg/m^2	0.17 (-0.03 to 0.36)	0.09	-0.03 (-0.24 to 0.18)	0.77
Gestational weight gain, kg	0.03 (-0.11 to 0.18)	0.63	-0.02 (-0.18 to 0.13)	0.77
Birth weight, kg	1.11 (-0.78 to 3.00)	0.25	2.79 (0.44 to 5.14)	0.02
Δ BMI *z*-score birth to 1y	-0.25 (-0.94 to 0.43)	0.46	0.64 (-0.24 to 1.52)	0.15
Δ BMI *z*-score 1y to 4y	1.17 (0.36 to 1.99)	0.01	1.46 (0.51 to 2.41)	0.01
Δ BMI *z*-score 4y to 8y	1.61 (0.78 to 2.43)	<0.001	1.79 (0.89 to 2.70)	<0.001
Insulin (μU/ml) (log)				
Intervention	0.01 (-0.08 to 0.10)	0.81	-0.03 (-0.11 to 0.07)	0.56
Pre-pregnancy BMI, kg/m^2	0.01 (0.00 to 0.02)	0.04	0.00 (-0.01 to 0.01)	0.98
Gestational weight gain, kg	-0.00 (-0.01 to 0.01)	0.72	-0.00 (-0.01 to 0.00)	0.47
Birth weight, kg	0.00 (-0.09 to 0.10)	0.98	0.12 (0.01 to 0.24)	0.03
Δ BMI *z*-score birth to 1y	0.01 (-0.02 to 0.04)	0.48	0.08 (0.04 to 0.12)	<0.001
Δ BMI *z*-score 1y to 4y	0.08 (0.04 to 0.12)	<0.001	0.12 (0.08 to 0.17)	<0.001
Δ BMI *z*-score 4y to 8y	0.09 (0.05 to 0.13)	<0.001	0.11 (0.07 to 0.15)	<0.001
HOMA-IR (log)				
Intervention	0.01 (-0.08 to 0.10)	0.82	-0.03 (-0.12 to 0.06)	0.54
Pre-pregnancy BMI, kg/m^2	0.01 (0.00 to 0.02)	0.03	0.00 (-0.01 to 0.01)	0.99
Gestational weight gain, kg	-0.00 (-0.01 to 0.01)	0.77	0.00 (-0.01 to 0.00)	0.45
Birth weight, kg	0.01 (-0.09 to 0.11)	0.88	0.14 (0.02 to 0.30)	0.02
Δ BMI *z*-score birth to 1y	0.01 (-0.03 to 0.04)	0.55	0.08 (0.04 to 0.18)	<0.001
Δ BMI *z*-score 1y to 4y	0.09 (0.05 to 0.13)	<0.001	0.13 (0.04 to 0.14)	<0.001
Δ BMI *z*-score 4y to 8y	0.10 (0.06 to 0.14)	<0.001	0.12 (0.05 to 0.14)	<0.001

IC: confidence interval; mo: months; BMI: body mass index; HOMA-IR: homeostatic model assessment of insulin resistance (insulin μU/ml x glucose mmol/l) / 22.5); y: year(s). *univariable model: B coefficients for a separate model for each listed variable; **multivariate model adjusted for all variables together.

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4 ARTIGO ORIGINAL 2

Randomized controlled trial of dietary counseling intervention during the first year after birth: is it effective after twelve years?

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ABSTRACT

Objective: To assess the long-term impact of dietary intervention accomplished during the first year of life on anthropometric, metabolic and dietary variables among adolescents.

Method: A randomized field trial was conducted among 500 mothers who gave birth to full-term infants between October 2001 and June 2002 in a low-income area in São Leopoldo, Brazil. Mother-child pairs were randomly assigned to intervention (n=200) and control groups (n=300) and the mothers in the intervention group received dietary counseling on breastfeeding and complementary feeding of their children during the first year of life. Fieldworkers blinded to assignment assessed socio-demographic, anthropometric and dietary data in 214 adolescents (age 12 years old). Blood tests were performed in 214 adolescents to measure complete lipid and glucose profile, and PCR and cortisol concentrations.

Results: At 12 years old, there were no significantly differences between intervention and control groups on nutritional status, serum lipid and glucose profile, and PCR and cortisol concentrations, for both genders. Similarly, intervention and control groups showed no changes comparing ultra-processed products consumption (percentage of total energy intake; boys: change 0.82%; 95% CI: -2.97 to 4.62; girls: change 0.56%; 95% CI: -3.31 to 4.43).

Conclusions: The favorable effect of this dietary counseling intervention observed among infants and children, in a low-income community in Brazil, may not be maintained until adolescence. Intervention programs to promote healthy eating should initiate early in life and our findings suggest the importance of supporting a dietary safe environment throughout adolescence as a priority in public health strategies.

Clinical Trial Registry: the study has been registered at www.clinicaltrials.gov (identifier NCT00629629).

INTRODUCTION

A growing body of evidence suggests that feeding practices during infancy tend to track later in life (Beauchamp et al, 2009; Birch e Doub, 2014). Therefore, the establishment of early healthy food habits is of great public health concern. Moreover, recent systematic reviews suggest that educational interventions including breastfeeding and complementary feeding practices may be effective in improving the nutritional status (Imdad et al, 2011; Lassi et al, 2013) and the dietary quality (Shi and Zhang, 2011) among infants and children, and thereby also protect against future chronic diseases (Laningan e Singhal, 2009). In addition, recently, the role of epigenetic changes caused by diet in early life have been related to individual differences in the risk of obesity and related metabolic outcomes throughout one's lifetime (Lillycrop & Burdge, 2011).

We have previously shown in a field trial that maternal counseling for infant feeding can stimulate breastfeeding at 4, 6, and 12 months of age (Vitolo et al, 2005), decrease children's energy-dense food consumption at 12 months (Vitolo et al, 2005; Vitolo et al, 2012) and improve diet quality at 4 years (Vitolo et al, 2010). In the same field trial, dietary counseling during the first year of life had a long-term impact in improving lipid profile in daughters at 8 years (Louzada et al, 2012). We are not aware however of any studies that had evaluated the potential long-term benefits of dietary counseling on infant feeding during the first year of life among adolescents, particularly in developing countries.

Therefore, we assessed if the effects of a home-based infant nutrition intervention were still seen after the study population had reached the age of 12 years. We analyzed selected anthropometric variables, lipid and glucose profile, PCR and cortisol concentrations and ultra-processed products consumption. Furthermore, we aimed to verify whether the intervention influenced boys and girls differently, considering biological differences in relation to the outcomes and the evidence that there are different responses to dietary interventions between genders (Niinikoski, 2007).

METHODS

This randomized field trial was conducted from the maternity wards of a hospital in a low-income population setting in the city of São Leopoldo, Brazil. Mothers of healthy, singleton, full-term (> 37 weeks) and normal birth weight (≥ 2500 g) babies were invited to participate. We excluded women with gestational diabetes, HIV-positive mothers, infants with congenital malformations or admitted to neonatal intensive care unit, and individuals with breast-feeding impediments. The study was conducted according to the Helsinki guidelines and all procedures involving human subjects were approved by the Ethics Committee of the Universidade Federal de Ciências da Saúde de Porto Alegre. All parents provided written informed consent. We followed the Consolidated Standards for Reporting Trials (CONSORT) guidelines to report on this randomized field study (Schulz et al, 2010). The study has been registered at www.clinicaltrials.gov (Identifier NCT00629629).

A total of five hundred mother-child pairs were recruited by fieldworkers at the maternity, representing 89.5% of all invited mothers. To guarantee blinding of the intervention assignment, an investigator not involved in the recruitment conducted the randomization procedure during the trial. Block randomization was used to avoid imbalances during the randomization process. Mothers who agreed to participate were sequentially listed based on their time of delivery, grouped in blocks of 5, and their names separated in opaque, sealed envelopes. Two mothers from each block were randomly assigned to the intervention group and the remaining three mothers were allocated to the control group. At the end of the randomization, 200 children were allocated to the intervention group, and 300 to the control group. We included more mother-child pairs in the control group as we expected greater losses to follow-up in controls because of a lower frequency of follow-up home visits.

For the original trial, we calculated that a sample of 363 infants would be required to detect a 65% increase in the frequency of exclusive breastfeeding up to 4 months of age in the intervention group (with 80% power and alpha=5%), assuming a 21.5% frequency of exclusive breastfeeding in the control group. For the current follow-up at age 12 years, 128 children would be required to detect a difference in HDL levels between groups of 0.15 mmol/L (effect size of 0.50), considering a power of 80% and statistical significance of 5%.

Intervention

The intervention consisted of dietary advice about breastfeeding and complementary feeding based on the "Ten steps for the healthy feeding for Brazilian children from birth to two years of age" (Ministry of Health, 2002). It was carried out between October 2001 and June 2002 by home visits within 10 days of the child's birth, on a monthly basis up to 6 months old and at 8, 10, and 12 months old. The main purpose of the program was to promote exclusive breastfeeding for 6 months followed by healthy complementary foods. During each home visit mothers received dietary advice in accordance with the baby's age. Mothers were advised against the addition of sugars (cane sugar, honey) to fruit, porridge, juices, milk, or other liquids. They were encouraged to avoid fried food, soft drinks, sweets, and salty snacks and to use salt in moderation. Advice on hygiene practices in food preparation and handling was provided. A simple colored leaflet with food pictures composing a healthy meal was used to guide the dietary advice and was given to the mother as a reminder. The writing material was simplified to take into consideration the mothers' level of education. During each visit of about 40 to 60 minutes, the fieldworkers clarified and reinforced recommendations while respecting the mother's level of cognition as well as cultural and economic background. The dietary intervention summary and the main counseling strategies applied during each home visit have been described elsewhere in detail (Louzada et al, 2012). The counseling was carried out by paired undergraduate students in nutrition science. The fieldworkers who carried out the dietary advice received 8 hours of theoretical training. During the intervention program, quality control was ensured by weekly scheduled meetings with all fieldworkers and the coordinator of the program to discuss all dietary advice provided to mothers. Mothers were encouraged to report any adverse events that occurred with children during the intervention. After the first year of age, no subsequent intervention was performed.

Control Group

Mothers in the control group were interviewed twice during the first year after childbirth (at 6 and 12 months of age), for data collection only. All mothers were encouraged to maintain normal pediatric visits for their babies during the study period. After the first year, children in intervention and control groups were followed up during childhood and adolescence and new data were collected at 4, 8 and 12 years old.

Data Collection

For the present study, were analyzed anthropometric, metabolic and dietary data collected at 12 years old. Trained fieldworkers, not involved in the intervention and unaware of group allocation, collected the data. The adolescents' pubertal development was assessed by using a self-questionnaire with illustrations of the 5 pubertal stages, according to the criteria described by Marshall e Tanner for girls (1969) and boys (1970).

Anthropometric Measurements

At the age of 12 years, children were weighed to the nearest 0.1 kg in light clothing without shoes on a digital scale (Techline, São Paulo, Brazil), and standing height was measured to the nearest 0.1 cm using a stadiometer (SECA, Hamburg, Germany). All measures were converted into z-scores of height-for-age or BMI-for-age based on World

Health Organization Growth Standards (WHO, 2007). Tricipital and subscapular skinfold thickness were measured in triplicate with a Lange caliper to the nearest 0.5 mm. Intraobserver measurement error and the mean average bias of the observer were within the limits suggested by the World Health Organization in the Multicenter Growth Reference Study for all measurements (WHO, 2006). The sum of the two individual skinfolds was computed. Percentage of body fat, fat mass and fat-free mass were used as indicators of body composition and were determined via bioelectrical impedance (Biodynamics BIA310e, Seattle, WA). Measurements were conducted on the dominant side of the body (in most cases the right side), in the morning, in supine position.

Metabolic Measurements

At 12 years of age, venous blood samples were obtained from the right arm after an overnight fast for biochemical analyses. Analyses were performed at the laboratory of Cardiology Institute of Rio Grande do Sul by technicians who were unaware of study assignments. Total cholesterol (TC), high-density lipoprotein (HDL), and triglyceride concentrations were measured with an automatic analyzer (Cobas Integra, São Paulo, Brazil). Low-density lipoprotein (LDL) was calculated according to Friedewald's formula (all triglyceride concentrations were <4.52 mmol/L) (Friedewald, 1972). Non-high-density lipoprotein (nHDL) was calculated by subtracting HDL-cholesterol from total cholesterol. Total cholesterol - HDL cholesterol ratio (TC/HDL) was calculated by dividing total cholesterol per HDL-cholesterol. Glucose and insulin were estimated using an automatic analyzer (Cobas Integra®, Roche, São Paulo, Brazil). HOMA-IR was calculated as (insulin µU/ml x glucose mmol/l) / 22.5 (Matthews, 1985). Nephelometry was used for the dosage of plasma levels of ultrasensitive C-reactive protein. Plasma cortisol levels were estimated using a semi-automated enzymatic method.

Dietary data

Ultra-processed products consumption at 12 years old was assessed through self-reported dietary calls. Two 24-hour dietary recalls were collected on two nonconsecutive days, and the mean values were used in the analyses. To quantify food portion size, pictures were used to illustrate standard household measurements. The Nutrition Support software (Nutwin 1.5 [Federal University of São Paulo, São Paulo/Brazil]) was used to convert average sizes in grams to different measurements and to estimate energy intake from the selected foods. The consumption of processed products by adolescents was assessed using the food classification system proposed by Monteiro and colleagues (Monteiro et al, 2012). This system gives primary importance to the nature, extent and purpose of food processing and is based on three groups: unprocessed and minimally processed foods; processed culinary ingredients; and processed and ultra-processed products. For the purposes of this study, only the ultra-processed products were analyzed. The ultra-processed products are mostly made from substances extracted from foods or obtained with the further processing of constituents of foods or through chemical synthesis. The usual dietary intake of total energy and ultra-processed products were estimated by the Multiple Source Method (MSM) https://msm.dife.de/ (Harttig et al, 2011). The MSM calculates dietary intake for individuals and then constructs the population distribution based this data. This method was used to correct dietary data for intra- and inter-personal variability. A probability value of 0.5 (50%) was used to assign habitual ultra-processed products consumer status, assuming that there is a certain percentage (50%) of real habitual consumers among the individuals who did not consume ultra-processed products during to the two dietary recall periods. Therefore, 50% of those who did not consume ultra-processed products in the 24-hour dietary recall period were randomly assigned habitual consumer status. An intake estimate was calculated for those who were selected in this manner. After the MSM was applied, the grams and the calories of intake of ultra-processed products and the percentage of energy from ultra-processed products based on total energy intake on the day were analyzed as continuous variables.

Statistical Analyses

Analyses were performed by intention to treat and by gender. Student's t test was used to evaluate the effect of the intervention on independent continuous variables. Non-normally distributed variables were log-transformed. If the distribution remained non-normal after the transformation, Mann-Whitney tests were used with the non-transformed values. Despite the use of log-transformed variables in the analyses, untransformed values were presented in tables for clinical interpretation. The result was expressed through mean differences and 95% confidence intervals (95% CIs). All statistical analysis were performed using SPSS 16.0 (SPSS IBM Inc., Chicago, IL USA) and statistical significance was set at $p<0.05$, two-sided.

RESULTS

Among the 500 children initially recruited, 397 underwent the 1 year, 345 the 4 year, 315 the 8 year and 214 the 12 years assessment (Fig 1). No adverse events were reported during the intervention. No differences were found between subjects who were lost to follow-up and those who remained on study at 12 years of age in terms of pre-pregnancy BMI (p=0.145), gestational weight gain (p=0.700), weight at birth (p=0.270), maternal age at child's birth (p=0.112) and family income (p=0.648). There were no differences between intervention and control groups on selected baseline characteristics (Table 1).

The proportion of overweight adolescents was 39.4% (n=50) among boys, and 42.5% (n=37) among girls. Regarding results of pubertal development, most of boys were in Tanner stage II (33.9%, n=43) or III (50.4%, n=64), while most of girls were in Tanner stage III (31.0%, n=27) or IV (35.6%, n=31) at 12 years old. A girls proportion of 65.1% (n=56) reported experiencing the menarche; for whom, the mean age at menarche was 11.9±0.78 years. The proportion of overweight and the percentage distribution of Tanner stage among boys and girls were not different between intervention and control group. The percentage of energy provided by consuming ultra-processed products was 57.5±10.6 (1034.3±326.7 kcal) among boys, and 58.1±8.9 (998.9±308.1 kcal) among girls, on average.

There were no differences between intervention and control groups on height-for-age *z*-score, BMI-for-age *z*-score, sum of triceps and subscapular skinfolds, percentage of body fat, fat mass and fat-free mass measurements, for both genders (Table 2). Similarly, serum lipid profile, glucose, insulin, PCR and cortisol concentrations and HOMA-IR values did not differ significantly between groups (Table 3). Finally, there were no significant differences between intervention and control groups comparing grams and calories of intake of ultra-processed products and the percentage of energy from ultra-processed products based on total energy intake on the day among 12 year-old adolescents, for both genders (Table 4). We analyzed the data regarding the whole sample, and similar findings were found.

DISCUSSION

To the best of our knowledge, this is the first randomized trial to examine the potential long-term effects of dietary counseling accomplished during the first year of life on anthropometric, metabolic and dietary parametric at 12 years old. Our findings did not show any difference in the studied measurements at this age, regarding intervention or control groups.

We considered two possibilities that may explain the lack of effectiveness of the trial among adolescents. First, the improvements in dietary practices observed in this population early in life (Vitolo et al, 2005; Vitolo et al, 2010; Vitolo et al, 2012; Louzada et al, 2012) might not track until adolescence. This could be related to the major autonomy of older children and adolescents regarding their food choices, including economic autonomy and easily access to unhealthy foods and beverages in their social (and, in general, obesogenic) environment (Leite et al, 2012; Virtanen et al, 2015; Larson et al, 2016; Azeredo et al, 2016; Watson et al, 2016). Eating patterns may change gradually during childhood and adolescence (Mannino et al, 2004) and children's diets tend to decline in quality, as they got move from middle childhood into adolescence (Lytle et al, 2000; Demory-Luce et al, 2004). Furthermore, as many others middle and low-income countries, Brazil had underwent swift nutritional and economic transitions, exposing individuals to environmental conditions that promote unhealthy feeding patterns (Vandevijvere et al, 2013). Increased trends in the last ten years were observed for energy-dense snacks, calorie-sweeteners, and fast foods consumption (Larson et al, 2016), concomitant with low intakes of fruit, vegetables, and whole grains in all ages, including children and adolescents (Levy-Costa, 2005; Popkin et al, 2012). In line with this findings, we observed a significantly increase in ultra-processed consumption in our sample from 33.9% at preschool age, to 37.9% at school age (Rauber et al, 2015), reaching 57.8% among adolescents.

Second, the lack of effectiveness of the trial could be related to weight gain changes between the ages 1 and 12 years. When the "Ten steps for the healthy feeding for Brazilian children from birth to two years of age" were drawn up, and the study's design was planned (15 years ago) intervention plan was mainly focused on dietary issues, and no special emphasis was placed on the total energy intake or obesity prevention. Population-based studies in Brazil highlight an alarming increase in overweight rates in adolescence in the last twenty years (Veiga, 2004; Rivera, 2014). In corroboration, in this sample, almost fifty percent of the adolescents were overweight. Therefore, it is reasonable that the long-term benefits caused by this dietary intervention on metabolic parameters at school age (Louzada et al, 2012) cannot be maintained in the current context of increasing prevalence of excess weight gain among adolescents. It is important to note results from recent meta-analysis which showed that interventions programs for obesity prevention among children and adolescents, beyond improvement on dietary patterns, should include recommendations regarding sedentary behavior and sleep ad screen time duration (Waters et al, 2011).

It is already established that the process of food preference learning begins in pregnancy and in the first two years of life (Birch and Doub, 2014). Although food preferences are persistent from infancy to childhood, and then to adolescence (Emmett et al, 2015), they are often modified in response to changes in the environment (Bojorquez et al, 2014). The achievement of adequate public policies for dietary quality improvement should be therefore highly dependent on supporting an environment that encourages healthy food preference learning early in life (Birch and Doub, 2014), and also that is continued during childhood and adolescence (Mallarino et al, 2013; Scherr et al, 2014). Moreover, previous studies have shown that dietary habits acquired during adolescence are likely to persist throughout adulthood (WHO, 2003). In this line, programs for adolescents have the potential to consolidate, among adults, the health dietary habits that were learned in early and middle childhood (Diers, 2013). In summary, if we protect and stimulate infancy health food learning process and create a healthful environment during childhood and adolescence, we are also safeguarding future adults (and hence new generations) from obesity and other nutrition-related non-communicable diseases.

Regarding our analysis for lipid profile, especial attention is necessary. We have previously showed that this randomized trial significantly improved the serum HDL and TG concentrations among girls at 8 years old (Louzada et al, 2012). This effect was not observed at the present analysis, when children reached 12 years old. Nevertheless, even if not statistically significant, girls in the intervention group presented lower serum triglyceride levels and higher HDL values than girls in the control group at 12 years of. These findings are in line with the (not-statistically significant) lower BMI z-score value among intervention girls, comparing to the control girls. We choose to verify whether the intervention influenced boys and girls differently regarding biological differences in relation to the outcomes and the evidence that there are different responses to dietary interventions between genders. However, we did not reached the sufficient sample size for girls, considering a power of 80% and a of 0.05. So, beyond the assumptions discussed above for the lack of the intervention among adolescents, it is possible that a type II b error have prevented us from finding a statistically significant result for lipid profile among 12 year-old girls.

This study has others limitations. Despite exhaustive attempts to locate all families and adolescents enrolled in the trial, there was a significant rate of loss to follow-up, especially due to adolescents who refused to participate. However, while we experienced typical rates of losses considering the duration of the trial (Kristman et al, 2004; Fewtrell et al, 2015), we found no differences between the baseline characteristics of the children who remained in the study and those who were lost. A limitation with respect to generalizability of the study findings is the choice of the study population, including only children between birth and 12 years of age from a low-income population. In response, we note that this target population is of specific interest for the introduction and evaluation of large-scale dietary interventions to improve population health in regional or national health programs. Major strengths of the study are the long follow-up period and the use of well-established methods.

CONCLUSION

We demonstrate that the favorable effect of this dietary counseling intervention accomplished during the first year of life among infants and children may not be maintained until adolescence, in a low-income community in Brazil. Nevertheless, even if this intervention is not associated with anthropometric and metabolic measurements or dietary pattern improvement later in adolescence, other benefits of prolonged breastfeeding still stand at ages 1, 4 and 8 years. Intervention programs to promote healthy eating could focus on early modifiable risk factors, including maternal pre-pregnancy weight, gestational weight gain and breastfeeding practices, but they should continue in school contexts (from pre-school age until adolescence). Health dietary behaviors established during adolescence continue into adulthood. Consequently, it is an important life phase for prevention of obesity and non-communicable diseases. Our findings suggest the importance of supporting a dietary safe environment throughout adolescence as a priority in public health strategies.

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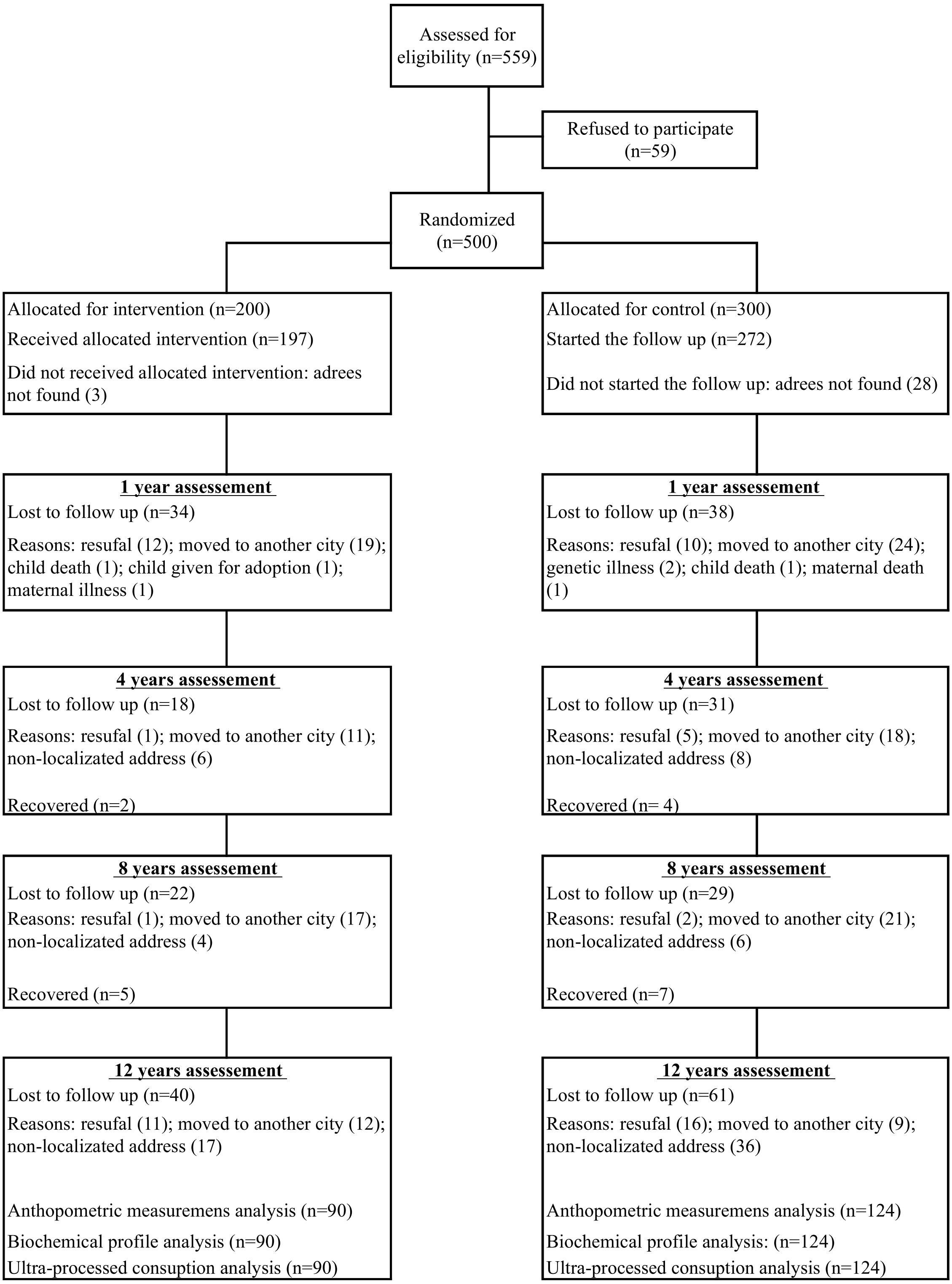

Figure 1: Trial profile of participation in the randomized controlled trial from recruitment of mother-child pairs through the assessment at 12 years of age.

Table 1: Characteristics of children and their households at baseline according to the group, at age 12 years.

Characteristics	*n* [a]	Intervention	Control	P[b]
Child				
Boys, n (%)	214	53 (58.9)	74 (59.7)	0.908
Skin color (white), n (%)	198	33 (38.8)	50 (44.2)	0.444
Birth weight (g), mean (SD)	211	3396.00 (479.66)	3383.02 (444.89)	0.840
Birth length (cm), mean (SD)	211	48.79 (1.86)	48.93 (2.12)	0.606
Health care follow-up at first year after birth, n (%)	209	50 (55.6)	51 (42.9)	0.069
Households at baseline				
Maternal pre-gestational BMI, mean (SD)	195	25.08 (5.69)	24.19 (4.26)	0.231
Gestational weight gain, mean (SD)	199	12.44 (6.20)	13.52 (6.36)	0.235
Gestational age, mean (SD)	202	39.35 (1.27)	39.36 (1.23)	0.941
Maternal age at child's birth <20 years, n (%)	214	15 (16.7)	21 (16.9)	0.959
Mother's education <8 years, n (%)	213	50 (56.2)	60 (48.4)	0.262
Father's education <8 years, n (%)	196	45 (54.2)	55 (48.7)	0.443
Mother's employment, n (%)	208	29 (32.2)	40 (33.9)	0.799
Father's employment, n (%)	191	71 (86.6)	101 (92.7)	0.165
Annual household income £ U$3000, n (%)	208	68 (75.6)	77 (65.3)	0.109

BMI: body mass index; g: grams; cm: centimeters. [a] n indicates the number of responses recorded for each characteristic. [b] Student's t test or x were used (depending on categorical or continuous variable).

Table 2: Intervention effect on anthropometric measurements at 12 years old.

	Intervention		Control		Difference (95% CI)	p
	n	Mean±SD	n	Mean±SD		
Boys (n=127)						
Height-for-age z-score[a]	53	0.28±1.21	74	0.27±0.99	-0.00 (-0.38 to 0.39)	0.990
BMI-for-age z-score[a]	53	0.92±1.40	74	0.47±1.25	-0.45 (-0.91 to 0.02)	0.060
Skinfold sum (mm)[a,b,c]	53	24.67±2.81	74	21.45±1.99[d]	0.04 (-0.91 to 1.34)	0.140
Body fat (%)[a]	44	25.41±7.36	65	23.02±6.95	-2.38 (-5.14 to 0.37)	0.089
Fat mass (kg)[a]	44	13.21±6.52	65	11.23±5.86	-1.99 (-4.35 to 0.39)	0.101
Fat-free mass (kg)[a]	44	36.28±8.14	65	35.53±6.88	-0.75 (-3.12 to 2.12)	0.606
Girls (n=87)						
Height-for-age z-score[a]	37	0.03±0.93	50	0.36±0.90	0.32 (-0.07 to 0.72)	0.105
BMI-for-age z-score[a]	37	0.49±1.36	50	0.80±1.25	0.30 (-0.26 to 0.86)	0.284
Skinfold sum (mm)[a,b,c]	37	25.63±2.04	50	28.54±2.04	1.11 (-0.91 to 1.34)	0.269
Body fat (%)[a]	33	26.98±6.66	42	28.17±7.02	1.18 (-1.99 to 4.36)	0.461
Fat mass (kg)[a]	33	13.87±6.12	42	15.66±7.14	1.79 (-1.31 to 4.90)	0.254
Fat-free mass (kg)[a]	33	35.43±6.67	42	37.37±7.93	1.93 (-1.49 to 5.36)	0.265

BMI: body mass index; [a]Student't test was used; [b]Sum of the subscapular and triceps skinfolds, [c]This variable was log-transformated for the analyses and was presented as geometric mean±IR.

Table 3: Intervention effect on clinic and laboratorial measurements at 12 years old.

	Intervention		Control		Difference (95% CI)	p
	n	Mean±SD	n	Mean±SD		
Boys (n=127)						
TC, mg/dL[a]	53	153.39±31.78	74	151.62±26.62	-1.77 (-12.06 to 8.51)	0.733
LDL, mg/dL[a]	53	92.29±25.86	74	89.47±21.13	-2.81 (-11.08 to 5.45)	0.502
HDL, mg/dL[a]	53	46.15±9.41	74	48.58±10.81	2.43 (-1.22 to 6.08)	0.190
n-HDL[a]	53	107.24±28.83	74	103.04±22.75	-4.20 (-13.27 to 4.86)	0.360
TC/HDL[a,b]	53	3.14±1.29	74	3.32±1.31	1.20 (-0.97 to 1.47)	0.130
Triglycerides, mg/dL[a,b]	53	64.75±2.09	74	60.09±1.99	1.14 (-0.94 to 1.37)	0.408
Glucose mg/dl[a]	53	82.85±6.81	74	82.72±7.27	-0.13 (-2.65 to 2.39)	0.917
Insulin uU/ml[a,b]	53	10.18±2.40	74	9.07±1.95	-0.89 (-0.72 to 1.09)	0.272
HOMA-IR[a,b]	53	2.07±2.69	74	1.84±2.14	-0.89 (-0.71 to 1.11)	0.297
PCR, mg/L[a,b]	53	2.56±3.47	74	2.59±3.47	1.01 (-0.67 to 1.53)	0.950
Cortisol, ug/dl[a]	53	8.35±3.85	74	8.56±3.81	0.2 (-1.15 to 1.57)	0.760
Girls (n=87)						
TC, mg/dL[a]	37	154.16±26.72	50	152.44±26.95	-1.72 (-13.30 to 9.85)	0.768
LDL, mg/dL[a]	37	92.07±20.28	50	90.53±24.69	-1.53 (-11.42 to 8.34)	0.758
HDL, mg/dL[a]	37	47.16±10.83	50	44.40±7.65	-2.76 (-6.70 to 1.17)	0.167
n-HDL[a]	37	107.00±22.91	50	108.04±27.38	1.04 (-9.99 to 12.07)	0.852
TC/HDL[a,b]	37	3.43±1.31	50	3.29±1.25	1.20 (-0.98 to 1.48)	0.392
Triglycerides, mg/dL[a,b]	37	69.07±1.82	50	78.84±1.82	1.14 (-0.94 to 1.37)	0.156
Glucose mg/dl[a]	37	82.85±6.81	50	82.77±7.36	-0.63 (-3.41 to 2.13)	0.648
Insulin uU/ml[a,b]	37	12.28±1.90	50	13.39±1.73	1.09 (-0.85 to 1.39)	0.487
HOMA-IR[a,b]	37	2.45±2.09	50	2.65±1.77	1.08 (-0.83 to 1.40)	0.548
PCR, mg/L[a,b]	37	2.79±3.38	50	1.95±2.69	-0.70 (-0.44 to 1.10)	0.121
Cortisol, ug/dl[a]	37	9.92±4.33	49	8.79±3.83	-1.13 (-2.89 to 0.62)	0.203

[a]Student't test was used; [b] These variables were log-transformed for the analysis and were presented as geometric mean±IR.

Table 4: Intervention effect on ultra-processed consumption at 12 years old.

	Intervention		Control		Difference (95% CI)	p
	n	Mean±SD	n	Mean±SD		
Boys (n=127)						
Grams[b,c]	53	557.96±256.91	74	593.64±236.39	35.68 (-51.62 to 122.98)	0.420
Calories[b,d]	53	1024.23±329.34	74	1041.52±326.89	17.28 (-99.49 to 134.07)	0.770
Percentage[b,e]	53	57.05±10.05	74	57.87±11.08	0.82 (-2.97 to 4.62)	0.668
Girls (n=87)						
Grams[b,c]	37	511.03±198.31	50	534.99±215.43	23.96 (-65.87 to 113.80)	0.597
Calories[b,d]	37	964.94±248.93	50	1023.98±345.88	59.03 (-67.76 to 185.84)	0.357
Percentage[b,e]	37	58.37±9.30	50	57.81±8.52	0.56 (-3.31 to 4.43)	0.773

[a]Ultra-processed products: bread, savory and chips, biscuits (crackers, cookies), sweets (candy, chocolate and ice cream), sugared drinks (soda, sweetened juice, sugared milk beverages), processed meat, powdered chocolate and breakfast cereal and others (mayonnaise, dressing, instant noodle, dehydrated soup). [b]Student't test was used; [c]grams of ultra-processed products; [d]calories from ultra-processed products; [e]percentage of total energy intake by ultra-processed products.

5. CONCLUSÕES E CONSIDERAÇÕES FINAIS

O presente estudo adiciona subsídios para compreensão da epidemia das alterações metabólicas em crianças na medida em que mostrou que o ganho de peso acelerado desde o nascimento é preditor de alterações precoces na resistência insulínica em idade bastante precoce. Por outro lado, observamos que o efeito favorável da intervenção de aconselhamento dietético baseada nos “Dez passos para alimentação saudável para crianças menores de dois anos” realizado durante o primeiro ano de vida em lactentes não foi suficiente para melhorar valores de glicose, insulina e HOMA-IR em escolares ou parâmetros antropométricos, bioquímicos e dietéticos em adolescentes, em uma comunidade de baixa renda no sul do Brasil. De qualquer forma, há evidências quanto ao efeito favorável desta intervenção na promoção da amamentação prolongada e melhora do padrão alimentar na infância e em idade pré-escolar.

Evidências atuais sugerem fortemente que programas de intervenção para divulgação de conhecimento e informações adequadas quanto ao aleitamento materno e alimentação complementar são fundamentais para mudança de comportamento e melhora da alimentação no início da vida. A promoção de hábitos alimentares saudáveis deve iniciar precocemente, considerando seu potencial efeito protetor a curto e a longo prazo. No entanto, os resultados desta tese indicam que tais ações, sem seguimento, não são suficientes para que os efeitos positivos sejam mantidos em idade avançada. A análise dos resultados da presente tese evidenciam a importância de apoiar um ambiente saudável durante toda a infância, até adolescência como uma prioridade nas estratégias de saúde pública. Urge a necessidade de planejemento e viabilização de políticas nacionais capazes de promover, apoiar e proteger os sistemas alimentares tradicionais e os padrões alimentares saudáveis, além do monitoramento da velocidade de ganho de peso, desde o nascimento até a adolescência.

6. ANEXOS

ANEXO A Termos de consentimento livre e esclarecido

CARTA DE CONSENTIMENTO

Eu..aceito fazer parte do programa de acompanhamento nutricional em que receberei visitas domiciliares para orientação quanto à práticas alimentares de meu filho e que o mesmo será pesado e medido. Confirmo que fui informada de todos os procedimento que serão tomados, inclusive quanto aos exames de fezes e sangue e que os dados obtidos serão utilizados em benefício da saúde do meu filho e da população.

Data ________/______/____

Assinatura_________________________

ESCLARECIMENTO GRUPO CONTROLE

Está sendo realizado um estudo sobre a importância da alimentação na saúde geral da criança. Para isso é necessário coletarmos dados de sua família referentes à moradia, à escolaridade, à renda e às práticas alimentares de seu filho. Serão feitas duas, uma quando o filho menor tiver seis meses e outra quando tiver um ano. Os seus filhos serão pesados e medidos hoje e daqui a seis meses. A Sra receberá a avaliação do peso e da estatura deles no mesmo momento. Na segunda visita, na qual o seu filho menor terá um ano, para avaliar a anemia, e exames das fezes. Os resultados desses exames serão devolvidos à senhora, acompanhados de orientação, se necessário.

Caso a Sra ou sua família necessite entrar em contato conosco é só telefonar para os números 590 3333 ramal 1203 ou 1240 e procurar professora Márcia Vitolo.

CONSENTIMENTO GRUPO CONTROLE

Eu ______________________________ aceito participar do estudo acima esclarecido e estou ciente que poderei deixar de fazê-lo em qualquer momento que quiser.

Assinatura ____ Data ___/___/___

TERMO DE CONSENTIMENTO INFORMADO

O presente estudo “Investigação dos Fatores de Risco para Obesidade Precoce e Anemia em uma Coorte de Crianças Submetidas a um Programa de Intervenção Nutricional no Primeiro Ano de Vida” pretende dar continuidade ao trabalho realizado no 1º ano de vida de seu filho, visando acompanhar as condições de crescimento e desenvolvimento por meio das medidas de peso, altura, quantidade de gordura corporal, as quais não conferem riscos nem dor para seu filho. Utilizaremos um 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. Em data marcada com o pesquisador, será verificada a pressão arterial e será realizada coleta de sangue por profissional treinado com agulhas descartáveis, sem risco de contaminação, para análise dos níveis de colesterol, LDL, triglicerídeos, proteína-C reativa e glicemia. A criança sentirá um breve desconforto, porém não haverá riscos a sua saúde. Essas informações serão transformadas em números e a identidade da sua família não será divulgada em nenhum momento. Este estudo é importante para se conhecer os fatores que são responsáveis pela obesidade e anemia na infância e dessa forma intervir de forma mais ampla na população. 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.

São Leopoldo, ____ de ___________________ de 200___.

Nome____

Assinatura____

Tel Prof. Márcia Regina Vitolo – tel 81629929 – 32248822 (ramal 153)

TERMO DE CONSENTIMENTO INFORMADO

O presente estudo IMPACTO DE UM PROGAMA DE INTERVENÇÃO NUTRICIONAL NO PRIMEIRO ANO DE VIDA EM CRIANÇAS COM IDADE ESCOLAR pretende dar continuidade ao trabalho realizado no 1º ano de vida e aos 3 e 4 anos de vida do seu filho, visando acompanhar as condições de crescimento e desenvolvimento por meio das medidas de peso, altura, quantidade de gordura corporal, as quais não conferem riscos nem dor para seu filho. Utilizaremos um questionário para fazer-lhe perguntas sobre sua família, o qual conterá: condições de vida, práticas alimentares de seu filho, atividades diárias e presença de doenças. Em data marcada com o pesquisador, será verificada a pressão arterial e será realizada coleta de sangue por profissional treinado com agulhas descartáveis, sem risco de contaminação, para análise dos níveis de hemoglobina, ferritina, colesterol, LDL, triglicerídeos, proteína-C reativa, insulina e glicemia. A criança sentirá um breve desconforto, porém não haverá riscos a sua saúde. Essas informações serão transformadas em números e a identidade da sua família não será divulgada em nenhum momento. Este estudo é importante para se conhecer os fatores que são responsáveis pela obesidade e anemia na idade escolar e dessa forma intervir de forma mais ampla na população. 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.

Eu, ...(responsável pela criança) fui informado dos objetivos da pesquisa acima de maneira clara e detalhada. Recebi informação a respeito do projeto de pesquisa e esclareci minhas dúvidas. Sei que em qualquer momento poderei solicitar novas informações e modificar minha decisão se assim eu o desejar. A Dra. Márcia Regina Vitolo certificou-me 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. Declaro ainda, que fui informado que caso existam danos à minha saúde, causados diretamente pela pesquisa, terei direito a tratamento médico e indenização conforme estabelece a lei. Também sei que caso existam gastos adicionais, estes serão absorvidos pelo orçamento da pesquisa.

Caso tiver novas perguntas sobre este estudo, posso chamar a Dra Márcia Regina Vitolo no telefone 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) 3303-9000 .

Declaro que recebi cópia do presente Termo de Consentimento.

São Leopoldo, ____ de ___________________ de 200___.

Nome____

Assinatura____

Tel Prof. Márcia Regina Vitolo – tel 81629929 – 32248822 (ramal 153)

ANEXO B Pareceres do Comitê de Ética em Pesquisa

PRÓ-REITORIA DE PESQUISA PROPESQ

COMITÊ DE ÉTICA EM PESQUISA

RESOLUÇÃO

O Comitê de Ética em Pesquisa da Universidade Federal do Rio Grande do Sul analisou o projeto:

Número:200245

Título: Implementação e Avaliação do Impacto do Programa de Promoção para a Alimentação Saudável para crianças menores de dois anos

Investigador principal: Márcia Regina Vitolo(UNISINOS)

- O mesmo foi aprovado em reunião realizada dia 11.04.2002, por estar adequado ética e metodologicamente e de acordo com a Resolução 196/96 do Conselho Nacional de Saúde. **O investigador deverá encaminhar relatórios semestrais sobre o andamento do Projeto.**

Porto Alegre, 12 de abril de 2002.

Prof. Luiz Osvaldo Leite
Coordenador CEP/UFRGS

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
PRÓ-REITORIA DE PESQUISA
Av. Paulo da Gama, 110 - 7º andar - Porto Alegre - RS - CEP: 90046-900
Fone: 0xx(51) 316 3629 Fax: 0xx(51) 316 4085 E-mail: pro-reitoria@propesq.ufrgs.br

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.tche.br

Of. 160/06-CEP

Porto Alegre, 12 de janeiro de 2006.

Ilma. Sra.
Profa. Márcia Regina Vitolo
Nesta Faculdade

Prezada Senhora

Informamos que seu projeto "Investigação dos fatores de risco para obesidade precoce e anemia em uma coorte de crianças submetidas a um programa de intervenção nutricional no primeiro ano de vida.", Processo nº 061/05, foi avaliado pelo Comitê de Ética em Pesquisa, na reunião de 12 de janeiro de 2006, sendo o projeto aprovado, conforme parecer consubstanciado nº 122-06, em anexo.

Outrossim informamos que de acordo com o Art. 4º, letra c, do Regulamento do CEP, V. Sa. deverá nos encaminhar relatórios semestrais do desenvolvimento do projeto.

Atenciosamente,

p.p. Prof. José Geraldo Vernet Taborda
Coordenador do CEP/FFFCMPA

Katya Vianna Rigatto
Adjunto do Depto. de Ciências
...ológicas - FFFCMPA

COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

COMITÊ DE ÉTICA EM PESQUISA - CEP
UFCSPA

O Comitê de Ética em Pesquisa da UFCSPA, registrado na Comissão Nacional de Ética em Pesquisa (CONEP) sob o nº 075/05 em 23/07/04, analisou o Projeto:

Projeto: 09-479 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
MÁRCIA REGINA VITOLO
CÍNTIA MENDES GAMA
PAULA DAL BÓ CAMPAGNOLO

Título: IMPACTO DE UM PROGRAMA DE INTERVENÇÃO NUTRICIONAL NO PRIMEIRO ANO DE VIDA EM CRIANÇAS COM IDADE ESCOLAR.

Esse projeto foi aprovado em seus aspectos éticos e metodológicos conforme as Resoluções 196/09 e demais Resoluções complementares. Toda e qualquer alteração do projeto, assim como eventos adversos graves, deverão ser comunicados a este CEP. Os TCLE, quando necessários, somente poderão ser utilizados após prévia e explícita aprovação (carimbo) de sua redação por este CEP".

Porto Alegre, 19 de junho de 2009.

José Geraldo Vernet Taborda
Coordenador

ANEXO C Instrução aos autores: Normas do periódico *British Journal of Nutrition*.

Instructions for Contributors

British Journal of Nutrition (BJN) is an international peer-reviewed journal that publishes original papers and review articles in all branches of nutritional science. The underlying aim of all work should be to develop nutritional concepts.

SUBMISSION

This journal uses ScholarOne Manuscripts for online submission and peer review. Complete guidelines for preparing and submitting your manuscript to this journal are provided below.

SCOPE

BJN encompasses the full spectrum of nutritional science and reports of studies in the following areas will be considered for publication: Epidemiology, dietary surveys, nutritional requirements and behaviour, metabolic studies, body composition, energetics, appetite, obesity, ageing, endocrinology, immunology, neuroscience, microbiology, genetics, and molecular and cell biology. The focus of all manuscripts submitted to the journal must be to increase knowledge in nutritional science.

The journal does NOT publish papers on the following topics: Case studies; papers on food technology, food science or food chemistry; studies of primarily local interest; studies on herbs, spices or other flavouring agents, pharmaceutical agents or that compare the effects of nutrients to those of medicines, complementary medicines or other substances that are considered to be primarily medicinal agents; studies in which a nutrient or extract is not administered by the oral route (unless the specific aim of the study is to investigate parenteral nutrition); studies using non-physiological amounts of nutrients (unless the specific aim of the study is to investigate toxic effects); food contaminants.

In vivo and in vitro models

Studies involving animal models of human nutrition and health or disease will only be considered for publication if the amount of a nutrient or combination of nutrients used could reasonably be expected to be achieved in the human population.
Studies involving in vitro models will only be considered for publication if the amount of a nutrient or combination of nutrients is demonstrated to be within the range that could reasonably be expected to be encountered in vivo, and that the molecular form of the nutrient or nutrients is the same as that which the cell type used in the model would encounter in vivo.

Extracts

Studies involving extracts will only be considered for publication if the source of starting material is readily accessible to other researchers and that there are appropriate measures for quality control, that the method of extraction is described in sufficient detail with appropriate quality control measures, that the nutrient composition of the extract is characterised in detail and that there are measures to control the quality of the composition of the extract between preparations, and that the amount of extract used could reasonably be expected to be achieved in in the human population (or in animals if they are the specific target of an intervention).

Studies involving extracts in in vitro models will only be considered for publication if the above guidelines for studies involving extracts are followed, and that the amount and molecular form of the extract is the same as that which would be encountered by the cell type used in the model in vivo.

Probiotics

Studies involving probiotics may be considered provided that the primary focus of the study/review is the effects on nutrient absorption and/or metabolism. Studies/reviews that focus primarily on probiotics per se will not be considered.

Manuscripts submitted to BJN that are outside of the journal's scope or do not meet the above requirements will be rejected immediately.

REVIEW PROCESS

BJN uses a single blind review process. As part of the online submission process, authors are asked to affirm that the submission represents original work that has not been published previously, and that it is not currently being considered by another journal. Authors must also confirm that each author has seen and approved the contents of the submitted manuscript. Finally, authors should confirm that permission for all appropriate uses has been obtained from the copyright holder for any figures or other material not in his/her copyright, and that the appropriate acknowledgement has been made to the original source.

At submission, authors are asked to nominate at least four potential referees who may then be asked by the Editorial Board to help review the work. Manuscripts are normally reviewed by two external peer reviewers and a member of the Editorial Board.

When substantial revisions are required to manuscripts after review, authors are normally given the opportunity to do this once only; the need for any further changes should at most reflect only minor issues. If a paper requiring revision is not resubmitted within 2 months, it may, on resubmission, be deemed a new paper and the date of receipt altered accordingly.

PUBLISHING ETHICS

BJN considers all manuscripts on the strict condition that: the manuscript is your own original work, and does not duplicate any other previously published work; the manuscript has been submitted only to the journal - it is not under consideration or peer review or accepted for publication or in press or published elsewhere; all listed authors know of and agree to the manuscript being submitted to the journal; and the manuscript contains nothing that is abusive, defamatory, fraudulent, illegal, libellous, or obscene.

The Journal adheres to the Committee on Publication Ethics (COPE) guidelines on research and publications ethics. Text taken directly or closely paraphrased from earlier published work that has not been acknowledged or referenced will be considered plagiarism. Submitted manuscripts in which such text is identified will be withdrawn from the editorial process. If a concern is raised about possible plagiarism in an article submitted to or published in BJN, this will be investigated fully and dealt with in accordance with the COPE guidelines.

ARTICLE TYPES

BJN publishes the following: Research Articles, Review Articles, Systematic Reviews, Horizons in Nutritional Science, Workshop Reports, Invited Commentaries, Letters to the Editor, Obituaries, and Editorials.

Research Articles, Reviews, Systematic Reviews, Horizons Articles, Letters to the Editor and Workshop Reports should be submitted to http://mc.manuscriptcentral.com/bjn. Please contact the Editorial Office on bjn.edoffice@cambridge.org regarding any other types of article.

Review Articles

BJN is willing to accept critical reviews that are designed to advance knowledge, policy and practice in nutritional science. Current knowledge should be appropriately contextualised and presented such that knowledge gaps and research needs can be characterised and prioritised, or so that changes in policy and practice can be proposed along with suggestions as to how any changes can be monitored. The purpose or objective of a review should be clearly expressed, perhaps as question in the Introduction, and the review's conclusions should be congruent with the initial objective or question. Reviews will be handled by specialist Reviews Editors. Please contact the Editorial Office with any queries regarding the submission of potential review articles. All reviews, including systematic reviews and meta-analyses, should present the uncertainties and variabilities associated with the papers and data being reviewed; in particular BJN cautions against uncritical acceptance of definitions and non-specific global terminology, the advice of advisory bodies, and reference ranges for example.

- Reviews: These articles are written in a narrative style, and aim to critically evaluate a specific topic in nutritional science.
- Horizons in Nutritional Science: These are shorter than Review articles and aim to critically evaluate recent novel developments that are likely to produce substantial advances in nutritional science. These articles should be thought-provoking and possibly controversial.
- Systematic Reviews and meta-analyses: A systematic review or meta-analysis of randomised trials and other evaluation studies must be accompanied by a completed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement checklist, a guideline to help authors report a systematic review and meta-analysis (see British Medical Journal (2009) 339, b2535). Meta-analysis of observational studies must be accompanied by a completed Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting checklist, indicating the page where each item is included (see JAMA (2000) 283, 2008-2012). Manuscripts in these areas of review will not be sent for peer review unless accompanied by the relevant completed checklist.

Letters to the Editor

Letters are invited that discuss, criticise or develop themes put forward in papers published in BJN. They should not, however, be used as a means of publishing new work. Acceptance will be at the discretion of the Editorial Board, and editorial changes may be required. Wherever possible, letters from responding authors will be included in the same issue as the original article.

DETAILED MANUSCRIPT PREPARATION INSTRUCTIONS

Language

Papers submitted for publication must be written in English and should be as concise as possible. We recommend that authors have their manuscript checked by someone whose first language is English before submission, to ensure that submissions are judged at peer review exclusively on academic merit. We list a number of third-party services specialising in language editing and / or translation, and suggest that authors contact as appropriate. Use of any of these services is voluntary, and at the author's own expense.

Spelling should generally be that of the Concise Oxford Dictionary (1995), 9th ed. Oxford: Clarendon Press. Authors are advised to consult a current issue in order to make themselves familiar with BJN as to typographical and other conventions, layout of tables etc. Sufficient information should be given to permit repetition of the published work by any competent reader of BJN.

Published examples of BJN article types can be found below: Research Article, Review Article, Horizons Article, Letter to the Editor.

Authorship

The Journal conforms to the International Committee of Medical Journal Editors (ICMJE) definition of authorship, as described by P.C. Calder (Br J Nutr (2009) 101, 775). The contribution of individuals who were involved in the study but do not meet these criteria should be described in the Acknowledgments section.

Ethical standards

The required standards for reporting studies involving humans and experimental animals are detailed in an Editorial by G.C. Burdge (Br J Nutr (2014) 112).

Experiments involving human subjects

The notice of contributors is drawn to the guidelines in the World Medical Association (2000) Declaration of Helsinki: ethical principles for medical research involving human subjects, with notes of clarification of 2002 and 2004 (http://www.wma.net/en/30publications/10policies/b3/), the Guidelines on the Practice of Ethics Committees Involved in Medical Research Involving Human Subjects (3rd ed., 1996; London: The Royal College of Physicians) and the Guidelines for the ethical conduct of medical research involving children, revised in 2000 by the Royal College of Paediatrics and Child Health: Ethics Advisory Committee (Arch Dis Child (2000) 82, 177–182). Articles reporting randomised trials must conform to the standards set by the Consolidated Standards of Reporting Trials (CONSORT) consortium.

Required disclosures: A paper describing any experimental work on human subjects must include the following statement in the Experimental Methods section: “This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the [insert name of the ethics committee; a specific ethics number may be inserted if you wish]. Written [or Verbal] informed consent was obtained from all subjects/patients.

[Where verbal consent was obtained this must be followed by a statement such as: Verbal consent was witnessed and formally recorded].” For clinical trials, the trial registry name, registration identification number, and the URL for the registry should be included.

PLEASE NOTE: From 1 October 2014, as a condition for publication, all randomised controlled trials that involve human subjects submitted to BJN for review must be registered in a public trials registry. A clinical trial is defined by the ICMJE (in accordance with the definition of the World Health Organisation) as any research project that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes. Registration information must be provided at the time of submission, including the trial registry name, registration identification number, and the URL for the registry.

Experiments involving the use of other vertebrate animals

Papers that report studies involving vertebrate animals must conform to the ‘ARRIVE Guidelines for Reporting Animal Research’ detailed in Kilkenny et al. (J Pharmacol Pharmacother (2010) 1, 94-99) and summarised at www.nc3rs.org.uk. Authors must ensure that their manuscript conforms to the checklist that is available from the nc3Rs website. The attention of authors is drawn particularly to the ARRIVE guidelines point 3b (‘Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study’s relevance to human biology’, point 9c (‘Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment’) and point 17a (‘Give details of all important adverse events in each experimental group’). The Editors will not accept papers reporting work carried out involving procedures that cause or are considered likely to cause distress or suffering which would confound the outcomes of the experiments, or experiments that have not been reviewed and approved by an animal experimentation ethics committee or regulatory organisation.

Required disclosures: Where a paper reports studies involving vertebrate animals, authors must state in the Experimental Methods section the institutional and national guidelines for the care and use of animals that were followed and that all experimental procedures involving animals were approved by the [insert name of the ethics committee or other approving body; wherever possible authors should also insert a specific ethics/approval number].

Manuscript Format

The requirements of BJN are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the ICMJE.

Typescripts should be prepared with 1.5 line spacing and wide margins (2 cm), the preferred font being Times New Roman size 12. At the ends of lines, words should not be hyphenated unless hyphens are to be printed. Line numbering and page numbering are required.

Manuscripts should be organised as follows:

- Cover letter

Papers should be accompanied by a cover letter including a brief summary of the work and a short explanation of how it advances nutritional science. The text for the cover letter should be entered in the appropriate box as part of the online submission process.

- Title Page
The title page should include:

1. The title of the article;
2. Authors' names;
3. Name and address of department(s) and institution(s) to which the work should be attributed for each author;
4. Name, mailing address, email address, telephone and fax numbers of the author responsible for correspondence about the manuscript;
5. A shortened version of the title, not exceeding 45 characters (including letters and spaces) in length;
6. At least four keywords or phrases (each containing up to three words).

Authors' names should be given without titles or degrees and one forename may be given in full. Identify each author's institution by a superscript number (e.g. A.B. Smith[1]) and list the institutions underneath and after the final author.

- Abstract
Each paper must open with an unstructured abstract of not more than 250 words. The abstract should be a single paragraph of continuous text without subheadings outlining the aims of the work, the experimental approach taken, the principal results (including effect size and the results of statistical analysis) and the conclusions and their relevance to nutritional science.

- Introduction
It is not necessary to introduce a paper with a full account of the relevant literature, but the introduction should indicate briefly the nature of the question asked and the reasons for asking it. It should be no longer than two manuscript pages.

- Experimental methods
The methods section must include a subsection that describes the methods used for statistical analysis (see the section on statistical analysis in the Appendix) and the sample size must be justified by the results of appropriate calculations and related to the study outcomes.

For studies involving humans subjects or experimental animals, the Methods section must include a subsection that reports the appropriate ethical approvals for the study (see Ethical Standards above).
All analytical procedures must be accompanied by a statement of within and between assay precision.

Diets: The nutrient composition of diets used in studies published in BJN must be described in detail, preferably in a table(s). Experimentally relevant differences in composition between diets are essential. For instance, studies of fat nutrition should always include fatty acid compositions of all diets.

PCR analysis: Where experiments involve measurement of mRNA including microarray analysis, for analysis of individual genes, mRNA should be measured by quantitative RTPCR. A statement about the quality and integrity of the RNA must be provided together with the results of eletrophoretic analysis of the purity of the PCR products. Unless published elsewhere, full details of the oligonuceoltide primers and of the PCR protocol must be stated either in the text or in Supplementary Material. The stability of reference genes used for normalisation of PCR data must be reported for the experimental conditions described. Where possible, analysis of mRNA levels should be accompanied by assessment of either protein levels or activities.

Microarray analysis: Studies involving microarray analysis of mRNA must conform to the “Minimum Information about a Microarray Experiment” (MIAME) guidelines including deposition of the raw data in an appropriate repository (the Access Code must be state din the Methods). All microarray experiments must be accompanied by appropriate validation by quantitative RTPCR.

- Results

These should be given as concisely as possible, using figures or tables as appropriate. Data must not be duplicated in tables and figures.

- Discussion

While it is generally desirable that the presentation of the results and the discussion of their significance should be presented separately, there may be occasions when combining these sections may be beneficial. Authors may also find that additional or alternative sections such as ‘conclusions’ may be useful. The discussion should be no longer than five manuscript pages.

- Acknowledgments

Here you may acknowledge individuals or organizations that provided advice and/or support (non-financial). Formal financial support and funding should be listed in the following section.

- Financial Support

Please provide details of the sources of financial support for all authors, including grant numbers. For example, “This work was supported by the Medical research Council (grant number XXXXXXX)”. Multiple grant numbers should be separated by a comma and space, and where research was funded by more than one agency the different agencies should be separated by a semi-colon, with “and” before the final funder. Grants held by different authors should be identified as belonging to individual authors by the authors’ initials. For example, “This work was supported by the Wellcome Trust (A.B., grant numbers XXXX, YYYY), (C.D., grant number ZZZZ); the Natural Environment Research Council (E.F., grant number FFFF); and the National Institutes of Health (A.B., grant number GGGG), (E.F., grant number HHHH)”.

This disclosure is particularly important in the case of research that is supported by industry. Support from industry not only includes direct financial support for the study but also support in kind such as provision of medications, equipment, kits or reagents without charge or at reduced cost and provision of services such as statistical analysis; all such support must be disclosed here and if no such support was received this must be stated. Where no specific funding has been provided for research, please provide the following statement: “This research received no specific grant from any funding agency, commercial or not-for-profit sectors.”

In addition to the source of financial support, please state whether the funder contributed to the study design, conduct of the study, analysis of samples or data, interpretation of findings or the preparation of the manuscript. If the funder made no such contribution, please provide the following statement: "[Funder's name] had no role in the design, analysis or writing of this article."

- Conflict of Interest
Please provide details of all known financial, professional and personal relationships with the potential to bias the work. Where no known conflicts of interest exist, please include the following statement: "None." For more information on what constitutes a conflict of interest, please see the International Committee of Medical Journal Editors (ICMJE) guidelines.

- Authorship
Please provide a very brief description of the contribution of each author to the research. Their roles in formulating the research question(s), designing the study, carrying it out, analysing the data and writing the article should be made plain.

- References
References should be numbered consecutively in the order in which they first appear in the text using superscript Arabic numerals in parentheses, e.g. 'The conceptual difficulty of this approach has recently been highlighted(1,2)'. If a reference is cited more than once, the same number should be used each time. References cited only in tables and figure legends should be numbered in sequence from the last number used in the text and in the order of mention of the individual tables and figures in the text.

Names and initials of authors of unpublished work should be given in the text as 'unpublished results' and not included in the References. References that have been published online only but not yet in an issue should include the online publication date and the Digital Object Identifier (doi) reference, as per the example below.

At the end of the paper, on a page(s) separate from the text, references should be listed in numerical order using the Vancouver system. When an article has more than three authors only the names of the first three authors should be given followed by 'et al.' The issue number should be omitted if there is continuous pagination throughout a volume. Titles of journals should appear in their abbreviated form using the NCBI LinkOut page. References to books and monographs should include the town of publication and the number of the edition to which reference is made. References to material available on websites should follow a similar style, with the full URL included at the end of the reference, as well as the date of the version cited and the date of access.

Examples of correct forms of references are given below.

Journal articles

Rebello SA, Koh H, Chen C et al. (2014) Amount, type, and sources of carbohydrates in relation to ischemic heart disease mortality in a Chinese population: a prospective cohort study. Am J Clin Nutr 100, 53-64.

Villar J, Ismail LC, Victora CG et al. (2014) International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. Lancet 384, 857-868.

Alonso VR & Guarner F (2013) Linking the gut microbiota to human health. Br J Nutr 109, Suppl. 2, S21–S26.

Bauserman M, Lokangaka A, Gado J et al. A cluster-randomized trial determining the efficacy of caterpillar cereal as a locally available and sustainable complementary food to prevent stunting and anaemia. Public Health Nutr. Published online: 29 January 2015. doi: 10.1017/S1368980014003334.

Books and monographs

Bradbury J (2002) Dietary intervention in edentulous patients. PhD Thesis, University of Newcastle.

Ailhaud G & Hauner H (2004) Development of white adipose tissue. In Handbook of Obesity. Etiology and Pathophysiology, 2nd ed., pp. 481–514 [GA Bray and C Bouchard, editors]. New York: Marcel Dekker.

Bruinsma J (editor) (2003) World Agriculture towards 2015/2030: An FAO Perspective. London: Earthscan Publications.

World Health Organization (2003) Diet, Nutrition and the Prevention of Chronic Diseases. Joint WHO/FAO Expert Consultation. WHO Technical Report Series no. 916. Geneva: WHO.

Keiding L (1997) Astma, Allergi og Anden Overfølsomhed i Danmark – Og Udviklingen 1987–199I (Asthma, Allergy and Other Hypersensitivities in Denmark, 1987–1991). Copenhagen, Denmark: Dansk Institut for Klinisk Epidemiologi.

Sources from the internet

Nationmaster (2005) HIV AIDS – Adult prevalence rate. http://www.nationmaster.com/graph-T/hea_hiv_aid_adu_pre_rat (accessed June 2013).

- Figures

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ANEXO E Instrução aos autores: Normas do periódico *Preventive Medicine*.

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