MSP MESTRADO EM SAÚDE PÚBLICA

UNIVERSIDADE DO PORTO
FACULDADE DE MEDICINA
INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

Diana Manuela Fernandes Moreira

Effect of breastfeeding on child and maternal adiposity 4-5 years after birth: results from the Portuguese birth cohort

Porto, 2012

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EFFECT OF BREASTFEEDING ON CHILD AND MATERNAL ADIPOSITY 4-5 YEARS AFTER BIRTH: RESULTS FROM THE PORTUGUESE BIRTH COHORT

Dissertação de candidatura ao grau de Mestre em Saúde Pública apresentada à Faculdade de Medicina e ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, sob orientação da Professora Doutora Ana Cristina Santos.

Porto, 2012

Este trabalho foi efetuado no âmbito da coorte Geração XXI, projeto desenvolvido pelo Departamento de Epidemiologia Clínica, Medicina Preditiva e Saúde Pública da Faculdade de Medicina da Universidade do Porto e pelo Instituto de Saúde Pública da Universidade do Porto.

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AAP	American Academy of Pediatrics
ADA	American Dietetic Association
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
cm	Centimeter
CI	Confidence Interval
FM	Fat Mass
HDL	High Density Lipoproteins
IBM Corp.	International Business Machines Corporation
IOM	Institute of Medicine
IOTF	International Obesity Task Force
Kg	Kilograms
LDL	Low Density Lipoproteins
m^2	Square Meter
P25	Percentile 25
P75	Percentile 75
SES	Socioeconomic Status
SPSS	Statistical Package for Social Sciences
US	United States
WC	Waist Circumference
WHO	World Health Organization
WHtR	Waist-to-Height Ratio

RESUMO

Contextualização e objetivos

O excesso de peso e a obesidade, bem como as comorbilidades associadas, são importantes problemas de saúde pública à escala mundial, com inerentes consequências biológicas, sociais e económicas. A sua prevalência, tanto em crianças como em adultos, tem vindo a aumentar de forma consistente ao longo das últimas décadas, quer em países desenvolvidos quer em países em desenvolvimento.

Tem sido descrito o papel protetor que a amamentação poderá desempenhar no desenvolvimento do excesso de peso e obesidade, quer durante a infância, quer durante a vida adulta. Adicionalmente, a amamentação tem também sido inversamente associada à adiposidade materna no período pós-parto.

Esta tese tem como objetivo geral estudar o efeito da amamentação na adiposidade, tanto em crianças como em mulheres, nos primeiros anos após o nascimento/parto. Para responder a esta questão geral, foram definidos dois objetivos específicos:

1. Avaliar a associação entre a duração e o tipo de amamentação e a adiposidade da criança aos 4-5 anos de idade;

2. Estudar o efeito da duração e do tipo de amamentação na adiposidade da mulher, 4-5 anos após um parto.

Métodos

Este estudo teve por base a coorte de nascimentos portuguesa, Geração XXI, recrutada entre 2005 e 2006, nos 5 hospitais públicos com maternidade da área metropolitana do Porto, Portugal. Após o parto, as mães foram convidadas a participar, e 91,4% das mães convidadas aceitaram participar. Um total de 8647 crianças e suas respetivas mães (n=8495) foram incluídos na avaliação inicial da coorte.

Entre 2009 e 2011, aos 4-5 anos de idade da criança, todas as crianças e suas mães foram convidados a participar numa reavaliação, constituída por uma entrevista presencial e avaliação física. Caso recusassem a participação na avaliação presencial, as mães eram convidadas a responder a uma entrevista telefónica. Do total de participantes da coorte, 86,2% das crianças e 84,2% das mães foram reavaliadas. Inquiridores treinados foram responsáveis pela aplicação de dois questionários estruturados. O questionário sobre a mãe

permitiu a recolha de informação sobre características demográficas, história pessoal e familiar de doença, história ginecológica e obstétrica, e estilos de vida. O questionário da criança permitiu a obtenção de informações relativas a características socioeconómicas, e sobre o desenvolvimento, saúde e hábitos da criança. Realizou-se um exame físico às mães e às crianças, incluindo uma avaliação antropométrica constituída por avaliação do peso, estatura, perímetros corporais e, particularmente nas crianças, uma avaliação da composição corporal através de bioimpedância elétrica. Na entrevista por telefone foram, também, recolhidas informações relativas à saúde da mãe e da criança, ainda que com um número mais reduzido de questões.

Para a análise do artigo I foram considerados os dados de 5450 crianças, após terem sido excluídos os gémeos, questionários que não foram respondidos pela mãe da criança, crianças para as quais não havia informação relativa à amamentação e crianças para as quais não existia informação sobre as medições antropométricas. No artigo II foram excluídas as gravidezes múltiplas, os questionários que não foram respondidos pelas mães, mulheres que estavam grávidas no momento da reavaliação, mulheres que tiveram um parto após o nascimento da criança que integra a coorte, bem como mulheres sem informação relativa à amamentação e à avaliação antropométrica, tendo assim sido incluídas no estudo 4381 mulheres.

As mães foram questionadas se a criança alguma vez havia sido amamentada. Em caso de resposta positiva, foi-lhes perguntado por quanto tempo a criança foi amamentada de forma exclusiva e, também, por quanto tempo a criança foi amamentada no total (amamentação juntamente com fórmula láctea ou outros alimentos).

A duração da amamentação foi registada em semanas e, para responder aos objetivos do estudo, foi categorizada em meses.

A amamentação exclusiva foi definida quando a criança apenas foi alimentada com leite materno, e foi considerada até ao momento em que fórmula láctea ou outros alimentos foram introduzidos e a amamentação parou. A amamentação mista foi considerada quando a criança foi amamentada e alimentada com fórmula láctea em simultâneo desde o nascimento, mas também, quando a criança foi amamentada exclusivamente durante um determinado período de tempo, a partir do qual foi introduzida fórmula ou outros tipos de alimentos para complementar a amamentação.

Para estimar o efeito do tipo de amamentação na adiposidade da criança e da mãe, restringiu-se a análise estatística à mediana da duração da amamentação exclusiva, que foram as 10 semanas, e categorizou-se o tipo de amamentação em três classes (não amamentada, amamentação exclusiva e amamentação mista).

No artigo I, para estimar o efeito da duração e do tipo de amamentação no índice de massa corporal (IMC), na massa gorda (MG) medida por bioimpedância elétrica, no perímetro da cintura (PC) e na razão PC/altura (PC/Alt) da criança, foram calculados coeficientes de regressão (β) e respetivos intervalos de confiança (IC) a 95%, usando modelos lineares generalizados. Para responder ao objetivo do estudo, o IMC, a MG, o PC e a razão PC/Alt foram transformados em z-scores e incluídos nos modelos de regressão múltipla como variáveis contínuas. A análise foi estratificada de acordo com o sexo da criança.

No artigo II, o efeito da duração e do tipo de amamentação sobre o IMC, o PC e a razão PC/Alt da mãe foram estimados através de modelos lineares generalizados, calculando-se coeficientes de regressão (β) e respetivos intervalos de confiança (IC) a 95%. Para responder ao objetivo do estudo, o IMC, o PC e a razão PC/Alt foram incluídos nos modelos de regressão múltipla como variáveis contínuas.

Resultados

Artigo I

Nos rapazes, não foi observada uma associação entre a duração da amamentação e os z-scores de IMC, MG, PC e de razão PC/Alt. Por sua vez, as raparigas que foram amamentadas entre 4,1 e 6 meses e entre os 6,1 a 12 meses de idade, quando comparadas com as que não foram amamentadas, apresentaram reduções de 0,204 e 0,234 no z-score de IMC, respetivamente. Esta associação foi independente do peso ao nascimento, do tipo de parto e consumo de tabaco durante a gravidez. Quando o IMC materno anterior à gravidez, a idade e o nível de escolaridade materno foram incluídos no modelo, a associação deixou de ser estatisticamente significativa. As raparigas que foram amamentadas entre 6,1 a 12 meses apresentaram uma diminuição de 0,243 no z-score de MG, independentemente do peso ao nascimento, do tipo de parto e consumo de tabaco durante a gravidez. As raparigas que foram amamentadas dos 4,1 aos 6 meses e dos 6,1 aos 12 meses de idade tiveram um decréscimo de 0,197 e 0,195 no z-score de PC, respetivamente. No entanto, após ajuste para o IMC anterior à gravidez, idade e nível de escolaridade materno, estas associações deixaram de ser estatisticamente significativas. Não foi observada uma associação entre a duração da amamentação e o z-score de razão PC/Alt, nas raparigas.

Quer nos rapazes, quer nas raparigas, o tipo de amamentação não se associou a nenhuma das medidas de adiposidade avaliadas.

Artigo II

Nas mulheres que amamentaram durante um mês, comparativamente às que não amamentaram, verificou-se um decréscimo de 0.63kg/m^2 no IMC, de 1,27cm no PC e de 0,01 na razão PC/Alt, após ajuste para a paridade, o tipo de parto, o IMC anterior à gravidez, consumo de tabaco durante a gravidez, idade e nível de escolaridade. A amamentação até aos 6 meses associou-se a uma redução de 0.96kg/m^2 no IMC, a um decréscimo de 2,43cm no PC, e de 0,02 na razão PC/Alt. As mulheres que amamentaram durante mais de 6 meses apresentaram também reduções significativas no IMC, PC e razão PC/Alt, embora menores do que as acima referidas.

Independentemente da paridade, do tipo de parto, do IMC anterior à gravidez, do consumo de tabaco durante a gravidez, da idade e do nível de escolaridade, a amamentação, tanto exclusiva como mista, associou-se a uma diminuição de 0,86 e 0.78kg/m^2 no IMC, respetivamente. Nas mulheres que amamentaram de forma exclusiva verificou-se uma redução de 1,60cm no PC, e as que amamentaram de forma mista tiveram uma redução de 1,65cm no PC, comparativamente às mães que não amamentam. A amamentação, tanto a exclusiva como a mista, associou-se a uma diminuição de 0,01 na razão PC/Alt.

Conclusões

Não foi observada uma associação entre a duração eo tipo de amamentação e a adiposidade, em crianças 4-5 anos após o nascimento.

Por outro lado, a duração da amamentação foi inversamente associada com a adiposidade materna, 4-5 anos após o parto. O efeito protetor da amamentação em relação à adiposidade materna não parece estar relacionado com os diferentes tipos de amamentação, uma vez que não foram observadas diferenças neste efeito entre as mulheres que amamentaram de forma exclusiva ou mista.

Palavras-chave: amamentação, gravidez, obesidade, excesso de peso, crianças em idade pré-escolar, mulheres.

ABSTRACT

Background and aims

Overweight, obesity and related comorbidities are important public health problems worldwide. They account for important biological, social and economic burden, and the future perspective is disturbing due to their persistently rise throughout the years, in children and adults, both in developed and developing countries.

It has been suggested that breastfeeding may have a protective role in the development of overweight and obesity in childhood as well as later in life. Also, breastfeeding has been considered as a protective factor for the postpartum maternal adiposity.

This thesis aims to study the effect of breastfeeding on child and maternal adiposity in the first years after birth/delivery. To answer this main question, two specific objectives, which resulted in two papers, were defined:

1. To evaluate the association between duration and type of breastfeeding and child adiposity at 4-5 years of age;

2. To study the effect of duration and type of breastfeeding on maternal adiposity, 4-5 years after delivery.

Methods

This study was based in the Portuguese birth cohort, Generation XXI, which was assembled between 2005 and 2006 at all 5 public maternity units covering the metropolitan area of Porto, Portugal. Mothers were invited to participate, after delivery, and 91.4% of the invited mothers accepted participation. A total of 8647 infants, corresponding to 8495 mothers, were enrolled in the cohort.

At 4-5 years after birth, all children and their mothers were invited to attend the re-evaluation that occurred between 2009 and 2011. This revaluation comprised an interview and physical examination. If the participant refused to attend the face-to-face interview she was invited to answer a telephone interview. Overall, 86.2% of the children and 84.2% of the mothers were re-evaluated. In the face-to-face interview, information was collected by trained interviewers, using two structured questionnaires. Mothers' questionnaire comprised data on demographic and socioeconomic characteristics, lifestyles, obstetric and medical history. Children's questionnaire included data on demographic and socioeconomic characteristics,

children's lifestyle and medical history. A physical examination was also performed to all mothers and children, including anthropometric evaluation, which comprised weight, height, body circumferences and, in children, body composition evaluation through bioelectrical impedance. During the telephone interview, data concerning mother and the child's health were collected, although with a restricted number of questions.

After excluding twins, children whose questionnaires were not answered by their mothers, children with no information on breastfeeding and duration of breastfeeding and with no anthropometric measurements, 5450 children remained for the final analysis in paper I. Regarding paper II, multiple pregnancies, questionnaires that were not answered by the mothers, women who were pregnant at the time of the follow up evaluation, women that had a live born child since the delivery of the index child, and women with no information on breastfeeding and on anthropometric measurements were excluded, and 4381 mothers remained for the final analysis.

Mothers were asked if the child was ever breastfed. If the answer was yes then they were asked for how long the child was exclusively breastfed and also for how long the child was breastfed in total (breastfeeding along with formula feeding or other type of food).

Duration of breastfeeding was registered in weeks, and categorized in months.

Exclusive breastfeeding was defined when the child was only fed with breast milk, and it was considered until the time when formula or other food were introduced and breastfeeding stopped. Mixed breastfeeding was defined when the child was breast and formula fed, concurrently, since birth, and also when the child was exclusively breastfed during a period of time and then formula or other type of food was introduced to supplement breastfeeding. To estimate the effect of type of breastfeeding on child and maternal adiposity, the analysis was restricted to the median duration of exclusive breastfeeding, which was 10 weeks, and type of breastfeeding was categorized in classes (not breastfed, exclusively breastfed and mixed breastfed).

In paper I, regression coefficients (β) and their 95% confidence intervals (CIs) were computed using generalized linear models to estimate the effect of duration and type of breastfeeding on body mass index (BMI),fat mass (FM) measured by bioelectrical impedance, waist circumference (WC), and waist-to-height ratio (WHtR). For the purpose of the study, BMI, WC, FM and WHtR were z-scores transformed and included in the multiple regression models as continuous variables. A stratified analysis was performed according to child's gender. Two models were computed both for boys and girls.

In paper II, regression coefficients (β) and their 95% confidence intervals (CIs) were computed using generalized linear models to estimate the effect of duration and type of

breastfeeding on women's BMI, WC and WHtR. For the purpose of the study, maternal BMI, WC and WHtR were used in the multiple regression models as continuous variables.

Results

Paper I

No significant associations were observed in boys, regarding duration of breastfeeding and BMI, FM, WC and WHtR z-scores.

Girls that were breastfed for 4.1 to 6 months and for 6.1 to 12 months of age had BMI z-score reductions of 0.204 and 0.234, respectively, when compared to girls that were not breastfed, independently of birth weight, mode of delivery and maternal smoking status during pregnancy. After including pre-pregnancy BMI, maternal age and educational level in the model, these associations were no longer statistically significant. Girls who were breastfed for 6.1 to 12 months had a FM z-score decrease of 0.243, independently of birth weight, mode of delivery and smoking during pregnancy, and girls who were breastfed for 4.1 to 6 months and for 6.1 to 12 months of age had a WC z-score decrease of 0.197 and 0.195, respectively. These associations were not statistically significant after adjustment for pre-pregnancy BMI, maternal age and educational level. No significant results were observed regarding duration of breastfeeding and WHtR z-score in girls.

The type of breastfeeding was not associated with any of the adiposity measures evaluated both in boys and girls.

Paper II

Mothers who breastfed for a month, compared to those who did not breastfeed, had a decrease of 0.63kg/m^2 in BMI, of 1.27cm in WC and of 0.01 in WHtR, after adjustment for parity, mode of delivery, pre-pregnancy BMI, smoking during pregnancy, age and educational level. Breastfeeding up to 6 months was associated with a reduction in maternal BMI of 0.96kg/m^2, a decrease of 2.43cm in WC, and of 0.02 in WHtR. Women who breastfed during more than 6 months experienced significant reductions in BMI, WC and WHtR, although smaller than those referred above.

Independently of parity, mode of delivery, pre-pregnancy BMI, smoking during pregnancy, age and educational level, breastfeeding, both exclusive and mixed, were associated with a decrease of 0.86 and 0.78kg/m^2 in BMI, respectively, comparing to not breastfeeding. Women who breastfed exclusively had a WC reduction of 1.60cm, and those who mixed breastfed had a WC reduction of 1.65cm, compared to mothers who did not

breastfeed. Exclusive and mixed breastfeeding were associated with a decrease of 0.01 in WHtR.

Conclusions

The duration and type of breastfeeding were not associated with a decreased adiposity in pre-school children.

On the other hand, duration of breastfeeding was inversely associated with maternal adiposity, 4-5 years after delivery, while no difference in the protective effect of breastfeeding was observed between women that exclusively or mixed breastfed.

Keywords: breastfeeding, pregnancy, adiposity, obesity, overweight, pre-school children, maternal, women.

INTRODUCTION

Obesity epidemic

Overweight and obesity are important clinical and public health problems worldwide (1), not only due to their important biological, social and economic burden but also, because they have been persistently rising throughout the years: the mean BMI has globally increased in the last decades among men and women, both in developed and developing countries (2).

According to the World Health Organization (WHO), obesity is one of the most serious public health challenges of the 21st century, which is replacing other health problems such as undernutrition or infectious diseases as the most important cause of disease and death (3). Overweight is responsible for a large proportion of the total burden of disease in the WHO European Region, accounting for more than 1 million deaths every year (4, 5). This relates to the association between overweight and obesity and many adverse health consequences such as hypertension, type 2 diabetes, dyslipidemia, coronary heart disease and cancer (6-8), as well as, their association with an increased risk of overall mortality (9, 10). Besides, obesity is also associated with pronounced economic consequences, including direct costs of health care services, indirect costs due to economic productivity losses, and individual costs, such as the acquisition of medications and treatments to obesity and/or related co-morbidities (5).

The prevalence of overweight in the European Region ranges from 31.9% to 79.3% among men, and from 27.8% to 77.8% among women, and the prevalence of obesity ranges from 5.4% to 22.8% in men, and from 7.1% to 35.6% in women (5).

In Portugal, the increase in obesity prevalence is also disturbing (11, 12). Data from a national health survey conducted in 2005-2006 reported that more than half of the Portuguese adult population was overweight or obese (63%). Regarding women aged between 25 and 35 years of age, the prevalence of combined overweight and obesity reached the 30% (13). Also, in an urban sample of Portuguese adults, the prevalence of obesity in women was about 26% (14), and similar trends have been reported in other low-middle and high income countries (15).

Similarly, the worldwide rise in childhood overweight and obesity over the past decades has been dramatic: in the period of 1990 to 2010 there was a relative increase of 21% (in the first decade) and 31% (in the second decade), respectively, in the prevalence of childhood overweight and obesity and it is expected to increase around 36% in the current decade. In fact, although the prevalence of childhood overweight and obesity is higher in developed countries than in developing countries (11.7% *vs* 6.1%), the relative increase in

the past two decades has been higher in developing countries (+65%) than in developed ones (+48%) (16).

The prevalence of overweight and obesity among Portuguese children and adolescents is also alarming (17). Padez et al. (18) described a very high prevalence of combined overweight and obesity (31.6%) among Portuguese children and adolescents, when compared to other European countries (5). As metabolically adverse profiles tend to track from childhood into adult life, it is also considered as a predictor of adult obesity (5, 19). Moreover, overweight and obesity are associated with worse risk parameters for cardiovascular disease in children (20), also contributing for the earlier onset of overall morbidity and mortality in adulthood (21, 22).

The primary purposes for defining overweight and obesity are to predict health risks and to compare populations, and for practical reasons the definitions usually have been based on anthropometry, with waist circumference (WC) and BMI being the most often used indicators, both clinically and in population studies (23).

In adults, adiposity is commonly assessed using BMI, calculated as weight (kg) divided by height squared (m^2), (24, 25), which is widely used to monitor overweight and obesity trends, defined as BMI between 25.0 and 29.9 kg/m^2 and as a BMI ≥30 kg/m^2, respectively (4). BMI is easy to measure and considered as a good method for screening the disease, being commonly used to identify individuals and populations at risk of future cardiovascular disease and type 2 diabetes (26-29).

Similarly, in childhood and adolescence, BMI is significantly associated with relative fatness, and is an appropriate method to measure relative adiposity in these populations. BMI varies with age and gender, increasing during the first months of life, falling after the first year and increasing again around the sixth year of life, thus a given value of BMI needs to be evaluated against age- and gender-specific reference values (23).

A wide variety of obesity definitions for children have been used and no commonly accepted standard has been established (30).

According to the Centers for Disease Control and Prevention (CDC) Growth Charts, which were based in nationally representative data from United States (US), the 85^{th} and 95^{th} percentiles of BMI for sex and age were recommended as cut-off points to identify childhood overweight and obesity (31, 32). In 2000, Cole et al (30) published a method to define overweight and obesity in children, based in pooled international data for BMI and linked to the widely accepted adult cut-off points of BMI of 25 and 30 kg/m^2: the reference population was obtained by averaging across surveys from six different countries, and the cut-off points were defined based on adult BMI units and extrapolated to childhood. The International

Obesity Task Force (IOTF) has recommended that the later age and gender specific cut-off points should be used in order to provide international comparisons of trends in childhood obesity (30).

More recently, the WHO published the *Child Growth Standards*, which provided BMI-for-age that can be used to estimate overweight and obesity in children, also based in 85th and 95th percentiles, respectively. They were derived from children who were raised under optimal environmental conditions and can be used to assess children around the world, regardless of ethnicity, socioeconomic status and type of feeding (33).

One of the main pointed advantage of using BMI-for-age charts is that a child can be described as being above or below certain centile lines, which can be useful in clinical practice.

However, data are derived from a reference population and, classifying an individual as overweight or obese assumes that the individual is comparable to that reference population. Also, these centiles may be misinterpreted as representing an ideal population, when the figures may in fact come from a population with high prevalence of obesity, such as the US CDC Growth Charts data (23).

In general, the IOTF method appears to give a more conservative view of the extent of overweight and obesity among paediatric populations. Differences between methods are related with differences in data sets, smoothing methods and theoretical approaches (Table 1) (23, 34).

	Age 6–8 years		Age 12–14 years	
	Boys	Girls	Boys	Girls
Overweight				
NHANES/WHO >85th	25%	31%	30%	30%
CDC >85th	23%	23%	29%	31%
IOTF >BMI 25 equivalent	18%	23%	29%	31%
Obese				
NHANES/WHO >95th	13%	17%	11%	12%
CDC >95th	11%	11%	12%	12%
IOTF >BMI 30 equivalent	8%	8%	9%	10%

Table 1. Comparison of prevalence rates of overweight and obesity using different criteria (23, 34).

Thus, some uncertainty remains regarding the optimal approach to measure adiposity, and the utility of BMI has been questioned (25). It provides only a crude measure

of adiposity, and the diagnostic performance of BMI is limited since it does not distinguishes weight related with fat mass from weight related with lean mass (26, 35, 36). Furthermore, BMI seems to have a low sensitivity for detecting body fatness, which may underestimate the true prevalence of obesity (35). Individuals with similar BMI can vary considerably in their abdominal fat mass, thus a measure of obesity that takes into account the increased risk of disease related to the accumulation of abdominal fat is needed (36).

Measures of central obesity have been shown to better discriminate obesity related cardiovascular disease risk factors compared with BMI (37, 38). One of such measures is WC, which is a simple method that might be used to assess nutritional status both in children and adults (23). It is related both with body weight and abdominal fat distribution, and is a strong indicator of obesity (39, 40). According to WHO, the recommended sex-specific cut-off points regarding WC are 94cm for men, and 80cm for women, for increased risk of metabolic complications related with obesity, and 102cm in men and 88cm in women for substantially increased risk (41). Although, appropriate cut-off points for defining high or low health risk have not been identified for paediatric populations (23).

More recently, several studies have recognized that the ratio between WC and height (WHtR) can be used as an easily measurable anthropometric index for detection of central obesity, being even better than WC or BMI in predicting obesity related cardiometabolic problems for both children and adults (37, 42-44). In fact, WHtR has been demonstrated to have more than 90% accuracy in defining overweight and obesity, combining the advantages of both BMI and WC, by taking in account height and abdominal adiposity (42). Additionally, it seems less correlated with age than BMI, which makes it possible to propose a non-age-dependent cut-off point (42). A recent meta-analysis showed that WHtR was a good predictor for diabetes, dyslipidemia, hypertension and cardiovascular disease risk in both sexes, in populations of various nationalities and ethnic groups (42, 43). The traditional cut-off points for BMI may underestimate the cardiometabolic risk in normal weight individuals and overestimate the same in overweight/obese individuals, while WHtR is more sensitive in identifying people at risk: normal weight individuals with central obesity have adverse levels of cardiovascular risk factors, such as blood pressure, cholesterol, triglycerides, glucose and insulin, compared to those without central obesity, and the overweight/obese without central obesity have lower levels in relation to those with central obesity (44).

Bioelectrical impedance analysis (BIA) has also been proposed as a good method to measure adiposity. It is based in predictive equations that estimate the fat-free mass from the measured impedance and, by subtraction, the fat mass (23). These equations have been validated in children, adolescents, adults, elderly, white populations, Asian and black populations (23, 45). BIA is a quick, non-invasive method that has high inter- and intra-

observer reliability, although requires equations specific to the instrument used and for the population under investigation, and the measurement may be influenced by hydration status and ethnic status (23, 46). With proper standardization of methods, instruments, and subject preparation, this body composition assessment approach provides accurate and reliable estimates of free-fat mass and total body water that can be used to calculate absolute and relative adiposity (45).

Obesity is defined as an excess on body adiposity and has complex aetiology (47). Although genetics plays an important role (48), the explanation for the rapid advance in worldwide obesity rates observed over the last decades has been offered in terms of the body's inability to adequately compensate for modern lifestyle choices with its excessive calories and inadequate physical activity, thus highlighting the role of environmental influences (19). It has been commonly accepted that obesity is the result of complex gene-environment and gene-behaviour interaction effects that make some individuals more susceptible to weight and fat gain when exposed to an obesogenic environment (47, 49, 50). Nonetheless, individual and family level risk factors, such as birth weight, maternal smoking during pregnancy, ethnicity, breastfeeding and timing of introduction of solid foods, parental nutritional status and socioeconomic position, were found to be more influential in childhood obesity prevention, than community level factors, such as neighbourhood conditions and access to food shops or places to play and/or exercise safely (18, 51-54).

More recently, a life-course approach to public health intervention regarding obesity has been proposed, taking into account the interaction of a constellation of risk factors, including genetic, environmental, metabolic and social features. This life-course approach considers the opportunities to improve individual's health at each stage in life, through nutrition in infancy, including breastfeeding and weaning, childhood and current diet and physical activity, and parental and social influences, that will have a positive impact on later childhood and adult life (Figure 1) (5, 51).

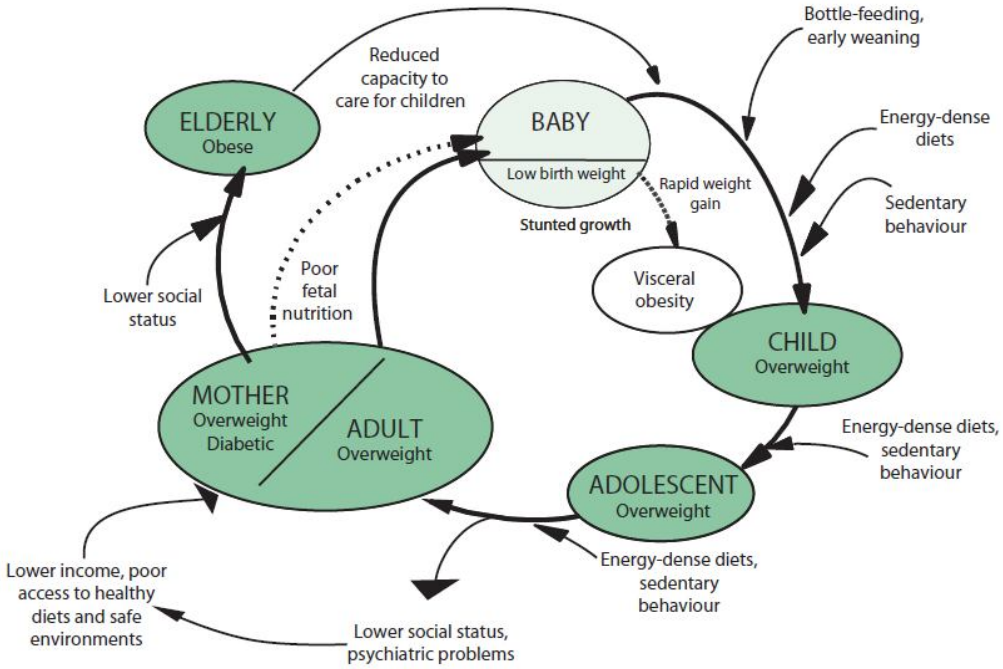

Figure 1. Risk factors for obesity (5).

Breastfeeding

Breastfeeding is the normative standard for infant feeding and nutrition (55), involving primary and secondary prevention of acute and chronic diseases (56).

The WHO states that breastfeeding is the perfect way of providing ideal food for a healthy infant growth and development (57), and has proposed a number of infant feeding categories regarding breastfeeding (Table 2). The WHO and the American Academy of Pediatrics (AAP) recommend that infants should be exclusively breastfed from birth to 6 months of age, continuing for up to two years of age or longer with other complementary foods, to achieve optimal growth, development and health (55, 57). The American Dietetic Association (ADA) supports that breastfeeding is a public health strategy for improving infant and child health, as well as, preventing maternal morbidity, controlling health care costs and conserving natural resources (56).

Category of infant feeding	Requires that the infant receives	Allows the infant to receive	Does not allow the infant to receive
Breastfeeding	Breast milk (direct from the breast or expressed)	-	-
Exclusive breastfeeding	Breast milk (direct from the breast or expressed)	Drops, syrup (vitamins, minerals, medicines)	Anything else
Predominant breastfeeding	Breast milk (direct from the breast or expressed) as the predominant source of nourishment	Liquids (water, water based drinks, fruit juice, tea, oral rehydratation salts), drops, syrup (vitamins, minerals, medicines)	Anything else (particularly non-human milk, food-based fluids)
Complementary feeding	Breast milk and solid or semi-solid foods	Any food or liquid including non-human milk	-
Bottle-feeding	Any liquid or semi-solid food from a bottle with nipple/teat	Breast milk by bottle	-

Table 2. Criteria for inclusion in infant feeding categories, according to WHO (58, 59).

In Portugal, approximately 90% of the mothers start to breastfeed their infants, and continue to do it for a medium duration of 3 months (60). However, the breastfeeding rates decrease to 60% and 35% at 3 and 6 months, respectively (60, 61). Also, the duration of breastfeeding has been positively associated with a higher maternal educational level, good past experiences with breastfeeding, practice of physical activity and with non-smoking habits in Portuguese mothers (60).

In fact, a wide range of variables may influence women decision and ability to breastfeed (62). Mothers who choose to breastfeed are more likely to be aware and practice other health promoter behaviours: breastfeeding intention and initiation is highly influenced by maternal socio-demographic characteristics, such as maternal age and educational level (60, 63, 64). Also, it has been proposed that overweight and obese women are less likely to breastfeed than normal weight women (65), as obesity seems to be associated with a delay in lactogenesis. Other factors may influence overweight/obese women decision on breastfeeding, as overweight/obese women may feel uncomfortable with the idea of breastfeeding in public, have lower self-esteem and are more likely to have postpartum depression, all of which are inversely related to the duration of breastfeeding (62). Maternal smoking was also associated with a shorter duration of breastfeeding (60, 66, 67): it has been described that smoking mothers have lower milk production (68). The suppressive effect of nicotine on prolactin production and the subsequent reduction of milk supply has

been proposed as one possible mechanism (69), but also, psychosocial factors may be responsible for the lower rates of breastfeeding among smoking women (70). Mother's perception of father's negative attitude towards breastfeeding, as well as mother's uncertainty regarding the amount of milk the infant receives have been suggested has possible reasons for initiating bottle-feeding over breastfeeding (71). Return to work may also be an important reason for mothers not to breastfeed and/or to the early abandon of breastfeeding (60, 71).

Breastfeeding provides economic benefits to the family and society. It allows the family to save money that would be necessary to buy infant formula or other milk substitutes, and feeding equipment (56). In another economic perspective, a cost analysis on breastfeeding in the US showed that the gap between recommendations on infant feeding and current US rates (12.3% of exclusive breastfeeding at 6 months, and 21.5% of any breastfeeding at 12 months), has substantial economic impact, basically through direct effects on children's health and mortality. It was estimated that the US incurs 13 billion dollars in excess costs per year, and suffers 911 preventable deaths per year, due to breastfeeding rates being far below the recommendations (72). Also, it has been described that exclusive use of formula is associated with substantial cost to the health care system, as children that are feed through formula tend to have higher number of hospitalizations and prescriptions when compared to those that are breastfed. Therefore, considerable savings could be expected through increasing the number of mothers who breastfeed according to the recommendations (55, 73).

Along with the economic advantages, breastfeeding has also many health benefits. In developing countries it is essential for child survival, but in developed countries breastfeeding is more important for the general well-being and health of child and mother (58).

Breastfeeding and children health

The short-term health benefits of breastfeeding in children are well established, leading to improved health outcomes both in developing and industrialized countries (55).

Breast milk is specifically tailored to meet all the infant's nutritional needs. It has a complex and rich composition, including carbohydrates, essential fatty-acids, long-chain polyunsaturated fatty-acids, anti-microbial and anti-inflammatory factors, immunoglobulins, enzymes and hormones that have been accounted for reducing morbidity and mortality during the first years of life (56).

Also, it has been established that it influence the immune status protecting infants from gastrointestinal and respiratory infections (74, 75), and against the development of allergic and atopic diseases, mainly through the effect on gut permeability by controlling the growth of intestinal microbiota, through the contact with antigens present in breast milk. Also, the immunosuppressive activity of breast milk components, down regulates inflammation, thus preventing the development of allergies and asthma (76-81).

Furthermore, it has been reported that breastfeeding improves children's performance on cognitive development tests, although it remains unclear whether the cognitive benefits of breastfeeding are due to constituents of breast milk or are related to the physical and social interactions inherent to breastfeeding (82-84). The physical and emotional act of breastfeeding might lead to physiologic changes that accelerate neurocognitive development, and the maternal-infant contact implicit in breastfeeding seems to increase verbal interaction between mother and son also stimulating cognitive development (83).

Breast milk intake, particularly when exclusive, has been also associated with increased levels of total cholesterol during the first months of life, mainly through the improvement of the HDL-cholesterol/LDL-cholesterol ratio, and with lower mean concentration of total cholesterol in adulthood (85-87). Breast milk has a higher cholesterol content (142 ± 33mg/l) compared to standard infant formula milk (23 ± 11mg/l), and it is possible that early exposure to high cholesterol content of breast milk down-regulates the endogenous synthesis of cholesterol in children and adults (87, 88).

Breastfeeding has also been associated with lower blood pressure levels in adulthood (1.4 and 0.5mmHg reductions in systolic and diastolic blood pressure, respectively), possibly by reducing sodium intake in infancy, increasing the intake of long-chain polyunsaturated fatty acids, which are important structural components of vascular endothelium, and by protecting against hyperinsulinemia and insulin resistance in infancy and adulthood that may in turn raise the blood pressure (89).

Furthermore breastfeeding has been associated with a reduced risk of type 2 diabetes, mainly explained by the lower energy intake in breastfed infants, as well as, by the breast milk content in fat, amino acids, proteins and hormones, that affect insulin response (90, 91).

Also, it has been suggested that prolonged breastfeeding may be a protective factor for overweight during childhood (54, 92-94) and in later life (95, 96). Some explaining mechanisms for this association have been reported. Breastfed infants develop better self-regulation of milk intake than bottle-fed infants, as babies that are fed from the breast can control milk intake, deciding when to start and stop sucking (97, 98). Besides, mothers who breastfeed seem to develop lower controlling feeding style, exerting less pressure on their children to eat without hunger (94). This allows the infant to maintain the natural ability to

self-regulate the energy intake and to balance his energy needs, which may partially mediate a positive relationship between breastfeeding and childhood obesity (94, 97).

In addition, it has been suggested that breastfed infants have considerably lower protein intake due to the increased efficiency of nitrogen utilization, which depends on protein intake, compared with formula fed infants. Breastfed infants conserve more nitrogen by decreasing protein turnover, and increasing conservation of endogenous amino acids (99). Higher protein intake in infancy has been suggested to promote later obesity by stimulating insulin release and programming of higher long term insulin concentrations (98). Also, higher plasma concentrations of insulin have been observed in infants who were formula fed, leading to the stimulation of fat deposition and early development of adipocytes (100, 101).

Breastfeeding has been related to slower pattern of growth, (98, 102). This growth pattern seems to be associated with a reduced risk of later obesity (98, 103). In fact, faster growth is related to an up-regulation of appetite that may be valuable in the short term, driving child's development and improving the likelihood of survival until reproductive age; but, in the long term, this programmed increase in appetite can contribute to obesity (98). Concordantly, breastfeeding has been associated with a down-regulation of appetite related to breast milk content in leptin, ghrelin and adiponectin, which have a positive effect on the early control of satiety in infants and may influence the energy balance regulation process in childhood and adulthood, thereby protecting against later obesity (98, 104-106). Leptin is an adipokine that regulates food intake, appetite and energy metabolism, and its presence in breast milk, but not in formulas, leads to higher levels of leptin levels in children that were breastfed (107), having a positive impact on body fat distribution (107, 108). Furthermore, it was established that adiponectin levels are inversely related to weight gain during the first months of life, and with adiposity during childhood, and positively associated with insulin concentrations and sensitivity (105, 109).

Thus, the potential mechanisms behind the relationship between breastfeeding and later childhood obesity can be categorized as those that influence behaviour, and those related to the exclusive nutritional composition of human milk(98)

Pregnancy, breastfeeding and maternal health

Pregnancy has been identified as a critical period of increased risk in the development of overweight and obesity in young aged women (110-112). The Institute of Medicine (IOM) recommends that women with a normal pre-pregnancy BMI should aim for a weight gain during pregnancy between 11.5kg and 16kg (Table 3) (113).

Pre-pregnancy BMI category (kg/m^2)	Recommended total weight gain during pregnancy (kg)
Low (<19.8)	12.5 – 18
Normal (19.8 – 26.0)	11.5 – 16
High (>26.0 to 29.0)	7 – 11.5

Table 3. Recommended total weight gain range for pregnant women, according to the pre-pregnancy body mass index (113).

A wide variation weight gain has been observed in women giving birth single, full term infants, ranging from the 15th and the 85th percentiles of weight gain, which corresponds to 7kg to 18kg, respectively, for normal weight women. This variation seems to be attributable to several physiologic and environmental factors, such as hormonal changes that affect the use of energy sources and thus the amount of weight gained, but also, maternal characteristics such as pre-pregnancy BMI, age, parity, and socioeconomic status, that are important predictors of gestational weight gain (113).

The excessive weight gain during pregnancy, as well as retaining this weight, are associated with a higher probability of overweight and obesity later in life (114, 115). In fact, gestational weight gain has been described has one of the most consistent and strongest predictors of postpartum weight changes (116). Moreover, pregnancy is related with an adverse adiposity distribution that may persist throughout the years (117, 118), as the adipose tissue volume that is retained 1 year after delivery, tends to be located centrally (111, 112, 119).

In previous studies, it has been described that about 20% of women retain at least 5kg in the 6 to 18 months postpartum, suggesting that pregnancy may increase postpartum body weight, especially for subgroups of women, such as those with high gestational weight gain (110, 120, 121). One of the pointed explanations for this inappropriate weight gain is that pregnancy may absolve women from the responsibility for their weight, leading to lifestyle changes that may include wrong eating habits, increased food intake and physical inactivity (122). Factors such as depression, lack of social support, body image concerns, and parity were also suggested to be associated with weight retention after pregnancy, in pre-pregnancy normal weight women (122). Additionally, heritable predisposition to weight gain and body size perception may also play independent roles on maternal body weight after pregnancy, as mothers who were more dissatisfied with their bodies after delivery may have significantly greater weight gain than those mothers that showed no increased dissatisfaction with their bodies (123).

During pregnancy, many metabolic changes occur to support fetal growth and milk synthesis (124, 125). The hormonal adjustments of pregnancy, such as increased levels of cortisol and progesterone, contribute to an increase in maternal insulin resistance and the hepatic conversion of glucose to triglycerides that promote energy storage as fat mass (113). Also, these changes lead to increased blood pressure and, by the end of the pregnancy, LDL cholesterol and triglycerides levels might be two to three times higher than the pre-pregnancy levels (126).

Women health can also be affected by other pregnancy related event that occur after the pregnancy itself. Lactation plays an important role in mediating the maternal metabolic status after gestation, with positive effects on adiposity, lipid and glucose homeostasis, being inversely associated with metabolic syndrome, hyperlipidemia and risk of myocardial infarction (124), thus having a considerable protective effect on women's risk of cardiovascular disease (126-129). Lactation is partially supported by mobilization of tissue stores, mainly by using body fat (116). Also, lactating women appear to have a faster return to pre-pregnancy levels of blood lipids, and hormonal effects inherent to breastfeeding may affect maternal blood pressure (126, 127). Breastfeeding mothers experience a decrease in blood pressure in response to oxytocin release, that is stimulated by infant's milk sucking (130). Also, breastfeeding was found to be inversely associated with risk of type 2 diabetes, independent of other diabetes risk factors, such as BMI, diet, exercise and smoking status (131, 132), possibly through the higher carbohydrate use and the total energy expenditure observed in lactating women (125). Additionally, hormonal changes in pituitary function that occur during lactation may affect the risk of diabetes in women who breastfed, although further studies are needed to clarify the relation between lactation and pituitary function (131). These favourable metabolic effects of lactation, contribute to the return to pre-pregnancy metabolism, and seem to persist in the long term, not being limited to the period of lactation (126).

Breastfeeding has been also related to greater postpartum weight loss and less postpartum weight retention (115, 133), and smaller amounts of metabolically active visceral fat were found in women that breastfed for more than 3 months (117, 134). One possible mechanism explaining these results relates to the increased energy expenditure involved in lactation, by the energy cost of milk production itself and the higher utilization of carbohydrates by the mammary glands (125). Besides, the neuroendocrine changes that occur during lactation seem to be promoters of lipolysis, as prolactin acts at the mammary gland level by stimulating the synthesis and secretion of milk components, but it also depresses lipogenesis in the liver and adipose tissue and increases the delivery of glucose

and lactate, which are lipogenic precursors. Additionally, insulin sensitivity is enhanced in the mammary gland and lowered in muscle and adipose tissue, reducing lipogenesis in the periphery (116).

Breastfeeding was also associated with other health benefits to the mother, including reduced postpartum blood loss, which preserves iron stores, and faster involution of the uterus, and decreased risk of breast and ovarian cancer (55, 135, 136).

Thus, breastfeeding is positively associated with health, social and economic advantages, both for children and women. The short term health benefits of breastfeeding are widely acknowledged and it has been also recommended for obesity prevention. Nevertheless, the impact of duration and type of breastfeeding on child and maternal adiposity in the medium-long term remains poorly studied.

AIMS

This study aims to evaluate the effect of breastfeeding on child and maternal adiposity in the first years after birth/delivery. To answer this main objective, data was used based on the Portuguese birth cohort (Generation XXI), and the following specific objectives, which resulted in two papers, were defined:

1. To evaluate the association between duration and type of breastfeeding and child adiposity, measured by body mass index, waist circumference, fat mass and waist-to-height ratio, at 4-5 years of age;

2. To study the effect of duration and type of breastfeeding on maternal adiposity, measured by body mass index, waist circumference and waist-to-height ratio, 4-5 years after delivery.

PAPER I

EFFECT OF DURATION AND TYPE OF BREASTFEEDING ON CHILD ADIPOSITY AT 4-5 YEARS OF AGE: RESULTS FROM THE PORTUGUESE BIRTH COHORT

ABSTRACT

Objective: To evaluate the association between duration and type of breastfeeding and child adiposity, measured by body mass index (BMI), waist circumference (WC), fat mass (FM) and waist-to-height ratio (WHtR), at 4-5 years of age.

Methods: This study was based in the Portuguese birth cohort, Generation XXI (n=8647). At 4-5 years of age, all children were invited for a follow up evaluation, and 7458 children participated. After excluding twins, children whose questionnaires were not answered by their mothers, and children with no information on breastfeeding and duration of breastfeeding and with no anthropometric measurements, 5450 children remained for the final analysis.
Trained interviewers administered a structured questionnaire on demographic and socioeconomic characteristics, children lifestyles and medical history, and performed anthropometric measurements, according to standard procedures. Duration of breastfeeding was registered in weeks, and categorized in months. To estimate the effect of type of breastfeeding on adiposity, the analysis was restricted to the median duration of exclusive breastfeeding, which was 10 weeks, both for children who were exclusively breastfed and for those who were mixed breastfed, thus 1639 children were evaluated. Type of breastfeeding was categorized in three classes: not breastfed, exclusively breastfed and mixed breastfed. Stratified analysis was performed according to child's gender. Regression coefficients (β) and their 95% confidence intervals were computed using generalized linear models to estimate the association of duration and type of breastfeeding in child BMI, WC, FM and WHtR z-scores.

Results: Not having been breastfed was reported in 8.4% of children. The median duration of exclusive breastfeeding was 10 weeks and the median duration of overall breastfeeding was 22 weeks. Overweight/obesity was observed in 20.7% of children.
No significant associations were observed in boys, regarding duration of breastfeeding and BMI, FM, WC and WHtR z-scores. Girls that were breastfed for 4.1 to 6 months and for 6.1 to 12 months of age had BMI z-score reductions of 0.204 and 0.234, respectively, when compared to girls that were not breastfed, independently of birth weight, mode of delivery and maternal smoking status during pregnancy. After including pre-pregnancy BMI, maternal age and maternal educational level in the model, these associations were no longer statistically significant. Girls who were breastfed for 6.1 to 12 months had a FM z-score decrease of 0.243, independently of child birth weight, mode of delivery and maternal smoking status during pregnancy, and girls who were breastfed for 4.1 to 6 months and for 6.1 to 12 months of age had a WC z-score decrease of 0.197 and 0.195, respectively. These

associations were not statistically significant after adjustment for pre-pregnancy BMI, maternal age and maternal educational level. No significant results were observed regarding duration of breastfeeding and WHtR z-score in girls.
The type of breastfeeding was not associated with any of the adiposity measures evaluated both in boys and girls.

Conclusion: The duration and type of breastfeeding were not associated with a decreased adiposity in this sample of pre-school children. Thus, further studies are needed to clarify this relationship, accounting for a longer follow up period, which may help to elucidate the role of breastfeeding on overweight/obesity development through the child life-course.

Keywords: breastfeeding, adiposity, overweight, obesity, pre-school children.

Introduction

Worldwide, the prevalence of childhood overweight and obesity have been increasing dramatically over the past decades and is expected to increase around 36% in this decade (1). In Portugal, we also have experienced an increase in the prevalence of overweight and obesity among children (2). When compared with the European Region, where about 20% of children and adolescents are overweight and a third of these are obese, Portuguese children and adolescents present a higher prevalence of overweight/obesity (31.5%) (3-5).

Overweight and obesity are associated with adverse effects on child's social well-being and with low self-esteem (6, 7). Moreover, they are associated with adverse risk parameters for cardiovascular disease in children (8), also contributing for the earlier onset of overall morbidity and mortality in adulthood (9, 10).

Obesity results from the interaction of a constellation of risk factors, including genetic, environmental, metabolic and social features (5). Parental overweight (3, 11-13), lower socioeconomic status (SES) (12, 13), high birth weight (13), maternal smoking during pregnancy (12-14), early introduction to solid foods, shorter duration of breastfeeding or not having been breastfed al all (3, 13-15), sedentary lifestyle and physical inactivity (16, 17) have been described as the most important risk factors for childhood obesity.

In children, the short-term health benefits of breastfeeding leading to improved health outcomes both in developing and industrialized countries are well established, (18). According to World Health Organization (WHO) breastfeeding is the ideal way of providing food for a healthy infant growth and development. The WHO and the American Academy of Pediatrics (AAP) recommend that infants should be exclusively breastfed from birth to 6 months of age, continuing for up to one year of age or longer, with other complementary foods (18, 19).

Prolonged breastfeeding has been suggested to be a protective factor for overweight during childhood (15, 20-22), as well as later in life (23). Breastfed infants develop better self-regulation of milk intake (24), have considerably lower energy and protein intake (25), and experience slower weight gain (26), which are associated with a reduced risk of later obesity (27).

Thus, this study aimed to evaluate the association between duration and type of breastfeeding and child adiposity at 4-5 years of age.

Methods

Study design and subjects

This study was based in the Portuguese birth cohort, Generation XXI. A total of 8647 newborns were enrolled in this cohort between April 2005 and August 2006 in five public maternity units of the metropolitan area of Porto, Portugal. Mothers were consecutively invited to participate after delivery, and 91.4% of the invited mothers accepted to participate.

Four to five years after birth, the total cohort was invited to a reevaluation that occurred between April 2009 and July 2011. All the children and their mothers were invited to participate through a telephone contact. Trained interviewers applied two structured questionnaires focused on child and mother´s health, in a face-to-face interview. The questionnaire concerning children, collected information on demographic and socioeconomic characteristics, caregiver's information, child's lifestyle (such as physical activity, screen-based media use and current diet), sleeping habits, health care utilization and medical history. The questionnaire concerning the mother collected demographic data, personal clinical history, obstetric history, and lifestyle information (including physical activity, current diet, alcohol intake and smoking habits).

A shorter version of the questionnaires used in the face-to-face interview, was created to be administered by telephone interview to participants that were unable to attend the face-to-face interview.

Overall, 86.2% of the entire cohort was assessed, corresponding to 7458 children evaluated, 5986 children attended the face-to-face interview, (69.2% of the entire cohort) and 1472 provided data by the telephone interview (17.0% of the entire cohort).

In this study, only children who attended the face-to-face interview and with data on anthropometric measurements were included. We excluded twins (n=215), children whose questionnaires were not answered by their mothers (n=121), and children with no information on breastfeeding and duration of breastfeeding (n=200), remaining for the final analysis, 5450 children.

The local institutional ethics committee and the Portuguese Data Protection Authority approved the study and all the mothers provided written informed consent.

The comparisons between the participants and non-participants regarding of maternal socio-demographic, obstetric and perinatal characteristics at birth are presented in table 1. There were no differences between the two groups concerning the mean maternal pre-pregnancy body mass index (BMI), the mode of delivery and the child's gender. The

participation in this study was associated with higher maternal age, educational level and household income.

Measurements

Breastfeeding

Mothers were asked if the child was ever breastfed. If the answer was yes then they were asked for how long the child was exclusively breastfed and also for how long the child was breastfed in total (breastfeeding along with formula feeding or other type of food).

Duration of breastfeeding was registered in weeks, and then categorized in months, creating six groups: those who were not breastfed, those that were breastfed during ≤ 1 month, breastfed between 1.1 and 4 months, breastfed between 4.1 and 6 months, breastfed between 6.1 and 12 months, and those who were breastfed for more than 12 months. These cut-off points were based on WHO and AAP recommendations regarding the duration of breastfeeding mentioned above (18, 19). The cut-off point of 4 months was also considered because, according to the Portuguese law, this is the length of the full paid maternity leave.

Exclusive breastfeeding was defined when the child was only fed with breast milk, and it was considered until the time when formula or other food were introduced and breastfeeding stopped. Mixed breastfeeding was defined when the child was breast and formula fed, concurrently, since birth, and also when the child was exclusively breastfed during a period of time and then formula or other type of food was introduced to supplement breastfeeding.

To estimate the effect of type of breastfeeding in child adiposity, we restricted the analysis to the median duration of exclusive breastfeeding, which was 10 weeks after birth, both for children who were exclusively breastfed and for those who were mixed breastfed. Thus, considering this restriction, 1639 children were evaluated, and categorized in three classes, those who were not breastfed, those who were exclusively breastfed and those who were mixed breastfed since birth.

Anthropometry

Anthropometric measurements were performed by trained interviewers, according to standard procedures.

Height was measured using a portable stadiometer to the nearest 0.1cm, and weight was measured with a digital scale to the nearest 0.1kg, while the child was barefooted and in

underwear. Child BMI was calculated as weight in kg/height in m^2. Specific age and gender cut off values for overweight/obesity were established according to the International Obesity Task Force (IOTF) (28). Waist circumference (WC) measurements were taken to the nearest 0.1cm, using a non-flexible tape at the umbilicus level, with the child in a standing position, with abdomen relaxed, arms at the sides and feet positioned together. The body composition was measured by a bioelectrical impedance analysis (BIA) generator, with four surface electrodes placed on the right wrist and ankle with the child lying horizontally, after the skin cleaning with 78 vol% alcohol. All BIA measurements were performed at least 30 minutes after the last meal. Total body fat mass (FM) (kg), adjusted for sex, age, weight and height was used to estimate the effect of breastfeeding on body composition. Waist to height ratio (WHtR) was obtained as WC in cm/height in cm.

Covariates

Mode of delivery (vaginal or Caesarian section), child birth weight, maternal smoking during pregnancy (smoker, stopped smoking during pregnancy, ex-smoker, never smoked), maternal pre-pregnancy BMI, maternal age at the time of follow-up and maternal educational level (in years of schooling) at the time of follow-up were also considered. Pre-pregnancy BMI was calculated as pre-pregnancy weight in kg/height in m^2 and categorized as underweight, normal weight, overweight and obesity, according to the WHO cut-off points (29). Pre-pregnancy weight was self-reported to the nearest 0.1kg and height was measured by the interviewers to the nearest 0.1cm. When this measurement was not performed, maternal height was abstracted from the mothers' national identity card (for 30.8% of women height data was obtained this way).

Statistical analysis

Sample characteristics are presented as proportions for categorical variables and comparison between groups was performed using a chi-square test. Group comparisons for continuous variables were performed using the ANOVA (normally distributed variables) and Kruskal-Wallis test (non-normally distributed variables).

Regression coefficients (β) and their 95% confidence intervals (CIs) were computed using generalized linear models to estimate the effect of duration and type of breastfeeding on BMI, WC, FM and WHtR. For the purpose of the study, BMI, WC, FM and WHtR were transformed in z-scores and included in the multiple regression models as continuous variables.

A stratified analysis was performed according to child's gender. Two models were computed both for boys and girls. The first model (Model 1) was adjusted for child birth

weight (continuous variable), mode of delivery (categorical variable) and maternal smoking status during pregnancy (categorical variable), and the second model (Model 2) was additionally adjusted for pre-pregnancy BMI (continuous variable), maternal age at follow-up (continuous variable), and maternal educational level at follow-up (continuous variable).

All the statistical analyses were performed using Statistical Package for Social Sciences (SPSS) for Windows, version 20.0 (IBM Corp., New York, USA).

Results

In this sample, 8.4% of children were not breastfed, 12.1% were breastfed to1 month of age, 26.5% to 4 months, 15.2% to 6 months, 17.0% were breastfed to 12 months of age and 20.8% were breastfed for more than 12 months. The overall breastfeeding median (P25-P75) duration was 22 weeks (10-36).

The characteristics of the children and their mothers enrolled in our study, according to duration and type of breastfeeding, are presented in tables 2 and 3, respectively.

Children who were breastfed for a longer duration had older ($p<0.001$), higher educated mothers ($p<0.001$), and had mothers that reported higher household income ($p<0.001$). On the other hand, mothers of children that were not breastfed or were breastfed for less than a month reported higher pre-pregnancy BMI ($p<0.001$) and more frequently smoked during pregnancy ($p<0.001$). Also, children that were breastfed for less than a month were more frequently overweight/obese ($p<0.001$). Regarding the other child adiposity measures, no differences were observed as regards to the duration of breastfeeding (Table 2).

Concerning type of breastfeeding, mothers of children that were exclusively breastfed were significantly younger ($p=0.016$) and less educated ($p<0.001$). Conversely, children that experienced mixed breastfeeding were from households with higher incomes, and more frequently were born by a Caesarian section. No differences regarding children anthropometrics were observed according to type of breastfeeding (Table 3).

The association between duration and type of breastfeeding and adiposity measures (z-scores of BMI, WC, FM and WHtR) in boys and girls are presented in tables 4, 5, 6 and 7, respectively.

Boys that were breastfed between 4.1-6 months had a BMI z-score reduction of 0.029, and those breastfed during 6.1-12 months had a reduction in the BMI z-score of 0.009, compared to boys that were not breastfed. However, after adjustment for potential confounding factors, this inverse association did not remain statistically significant (Model 1 and Model 2). Also, in boys, no significant associations were observed regarding duration of breastfeeding and FM, WC and WHtR z-scores.

Among girls, being breastfed for 4.1 to 6 months and for 6.1 to 12 months was associated with a 0.204 and 0.234 in reduction BMI z-score, respectively, when compared to girls that were not breastfed. These results were independent of the child birth weight, mode of delivery and maternal smoking status during pregnancy (Model 1). However, after including pre-pregnancy BMI, maternal age and maternal educational level in the model these associations were no longer statistically significant (Model 2).

Also, girls who were breastfed for 6.1 to 12 months had a FM z-score decrease of 0.243, independently of child birth weight, mode of delivery and maternal smoking status during pregnancy (Model 1). Girls who were breastfed for 4.1 to 6 months and for 6.1 to 12 months experienced a WC z-score decrease of 0.197 and 0.195, respectively (Model 1). However, these associations were not statistically significant after adjustment for pre-pregnancy BMI, maternal age and maternal educational level (Model 2). No significant associations were also observed regarding duration of breastfeeding and WHtR z-score in girls.

The type of breastfeeding was not associated with any of the adiposity measures evaluated, both in boys and girls, 4-5 years after birth.

The interaction between duration and type of breastfeeding and adiposity measures was also tested, but no modifying effect was observed.

Discussion

In Portugal, the breastfeeding rate at birth is about 90% (30-32), similarly to what we found in our study, as 8.4% of our children were not breastfed. However, it was previously described that in our country, breastfeeding rates decrease to 60% and 35% at 3 and 6 months, respectively (33, 34). The median duration of exclusive breastfeeding in our sample was 10 weeks and the median duration of overall breastfeeding was 22 weeks, which are far below the current recommendations of the WHO and AAP, that support exclusive breastfeeding up to 6 months of age and supplemented breastfeeding for at least 12 months, to achieve optimal child growth and development (18, 19).

Overweight and obesity are quite common conditions in Portuguese children. A study carried out between 2002-2003, in a national representative sample of Portuguese children, reported that the prevalence of combined overweight and obesity in children aged 7 years old was 29.5% (4). Among Portuguese pre-school children, the prevalence of overweight/obesity is 20.1% in boys and 27.3% in girls (2, 35). In our study, according to age and gender adjusted BMI cut-off points (28), we also found a high prevalence of overweight/obesity (20.7%), which was also higher in girls (25.7%) than in boys (16.0%).

It has been proposed that breastfeeding may have a protective effect regarding childhood obesity (36-38). Nonetheless, in our study, no statistically significant association was observed between duration or type of breastfeeding and adiposity among boys at 4-5 years of age. In fact, we observed a decreased adiposity, measured as BMI, WC and FM z-scores, in girls who were breastfed between 4.1 and 12 months, comparing with girls who were not breastfed. These results were independent of child birth weight, mode of delivery and maternal smoking during pregnancy. However, after adjustment for maternal characteristics such as pre-pregnancy BMI, age and educational level, this protective effect was no longer present. Also, no association was observed between type of breastfeeding (exclusive or mixed) and child's adiposity, both for boys and girls.

Our results are similar to what was observed in a sample of Swedish children aged 5 years old, where short-term exclusive breastfeeding was not significantly associated with obesity (39). Burdette et al (40) also reported that neither duration and type of breastfeeding nor the timing of the introduction of complementary foods were associated with adiposity, in a sample of 313 children at 5 years of age. In a sample of Anglo- and Mexican-American 4 years old children, no association between infant feeding practices and children adiposity, measured by BMI and skin fold thickness, were also observed (41).

The lack of association observed in boys and also in girls (Model 2), may reflect the longer term effect of breastfeeding in adiposity. Thus, the expected protective effect could

only manifest later in the child life-course. Also, the indication of an inverse association between duration of breastfeeding and adiposity in girls (Model 1 in table 5) might be a result of the higher adiposity that has been described in females compared to males, which may have enhanced the protective effect of breastfeeding among girls (42-45).

Besides, similarly to what happened in our analysis, the association previously described between breastfeeding and later adiposity was weakened after adjustment for some important confounding factors such as SES, parental age and education (46). In fact, marked differences in the socioeconomic patterning of breastfeeding have been observed, Brion et al (47) found that breastfeeding was more common among women from higher SES in a high-income country, whereas in a low- middle-income country, breastfeeding was not strongly associated with SES. Also, it has been described that lower maternal social class and maternal obesity are related to a higher probability of formula feeding and higher risk of obesity among the offspring (21, 23), suggesting that healthier family lifestyles rather than infant feeding, may explain a lower overweight/adiposity risk.

In this study, the information on breastfeeding was self-reported, and therefore recall bias may have weaken the relationship between breastfeeding and child adiposity. Although, Tomeo et al (48) suggest that maternal long-term recall of pregnancy related events, including breastfeeding history, is accurate and reproducible.

Finally, the extrapolation of our findings to the general population might be limited because the participation in this study, as usually happens, was associated with higher maternal age, educational level and household income.

This is the first study estimating the effect of duration and type of breastfeeding on adiposity in a large sample of Portuguese pre-school aged children. Often, one pointed limitation to studies on obesity is that the prevalence of the disease may not be correctly estimated when only information on BMI is used as an adiposity indicator, because the imprecise classification of obese children (37, 49), as this index does not distinguishes weight related to fat from weight related to muscle. The association between breastfeeding and later adiposity may differ when different measures of adiposity are used (40). In our study, trying to obviate this limitation, WC, FM estimated by bioelectrical impedance and WHtR, which were previously described good predictors of obesity and other cardiovascular disease risk factors in children (50-52), were used to assess the relationship between breastfeeding and child adiposity, and the results were consistent regarding all these adiposity measures.

Conclusion

A protective effect of duration of breastfeeding between 4.1 and 12 months in girl's BMI, WC and FM z-scores, at 4-5 years of age was observed. This association was independent of mode of delivery, child birth weight and smoking status during pregnancy. After adjustment for pre-pregnancy BMI, maternal age and educational level, the significant protective effect of breastfeeding disappeared, although remained in the same direction. The type of breastfeeding was not associated with a decreased adiposity in this sample of pre-school children.

The expected protective effect of breastfeeding in child adiposity was not observed in this sample of pre-school aged children. Thus, further studies with longer follow-up periods are needed to clarify this relationship and to elucidate on the role of breastfeeding in the development of obesity in the child life-course.

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Table 1: Comparison between non participants and participants regarding children and maternal characteristics at birth.

	Non participants (n=3197) n (%)	**Participants (n=5450)** n (%)	p
Maternal age at birth (years)			
<25	911 (28.6)	966 (17.8)	<0.001
25-29	934 (29.4)	1635 (30.1)	
30-34	883 (27.8)	1871 (34.4)	
≥35	452 (14.2)	964 (17.7)	
Mean (SD)	28.1 (5.8)	29.6 (5.4)	<0.001
Maternal educational level at birth (years)			
≤6	931 (29.1)	1183 (21.7)	<0.001
7-9	902 (28.2)	1284 (23.6)	
10-12	803 (25.1)	1492 (27.4)	
>12	561 (17.5)	1491 (27.4)	
Median (P25-P75)	9 (6-12)	11 (7-15)	<0.001
Household income at birth (€/month)			
≤1000	1272 (39.8)	1752 (32.1)	<0.001
1001-1500	669 (20.9)	1436 (26.3)	
>1500	737 (23.1)	1570 (28.8)	
Does not know/ Prefers not to answer	376 (11.8)	448 (8.2)	
Maternal pre-pregnancy BMI (kg/m^2)[a]			
Underweight	132 (4.6)	186 (3.7)	0.036
Normal weight	1872 (65.9)	3258 (65.2)	
Overweight	576 (20.3)	1121 (22.4)	
Obese	262 (9.2)	432 (8.6)	
Mean (SD)	23.8 (4.3)	23.9 (4.2)	0.140
Mode of delivery			
Vaginal	1975 (62.7)	3403 (63.1)	0.746
Caesarian	1173 (37.3)	1991 (36.9)	
Gender			
Girls	1570 (49.1)	2672 (49.0)	0.942
Boys	1627 (50.9)	2778 (51.0)	
Gestational age (weeks)			
Mean (SD)	38.2 (2.3)	38.6 (1.7)	<0.001
Child birth weight (g)			
≤2500	431 (13.5)	412 (7.6)	<0.001
2501-4000	2650 (83.1)	4841 (88.9)	
>4000	108 (3.4)	193 (3.5)	
Mean (SD)	3077.8 (647.0)	3188.4 (490.3)	<0.001
Child birth length (cm)			
Mean (SD)	48.4 (2.7)	48.8 (2.2)	<0.001

[a]according to WHO cut off points for BMI (29).

Note: in each variable, differences in total number are due to missing data.

Table 2: Participants' characteristics according to duration of breastfeeding.

		Breastfeeding duration						
	Total (n=5450) n (%)[a]	**Not breastfed (n=457)** n (%)[a]	**≤ 1 month (n=659)** n (%)[a]	**1.1-4 months (n=1446)** n (%)[a]	**4.1-6 months (n=829)** n (%)[a]	**6.1-12 months (n=927)** n (%)[a]	**> 12 months (n=1132)** n (%)[a]	p
Maternal ageat follow up (years)								
<25	249 (4.6)	37 (8.1)	55 (8.4)	73 (5.1)	24 (2.9)	15 (1.6)	45 (4.0)	<0.001
25-29	847 (15.6)	90 (19.7)	126 (19.2)	241 (16.7)	112 (13.5)	108 (11.7)	170 (15.1)	
30-34	1792 (33.3)	137 (30.0)	214 (32.6)	483 (33.5)	295 (35.7)	319 (34.5)	344 (30.5)	
≥35	2547 (46.9)	193 (42.2)	261 (39.8)	646 (44.8)	396 (47.9)	483 (52.2)	568 (50.4)	
Mean (SD)	33.9 (5.3)	33.3 (6.2)	32.8 (5.6)	33.6 (5.4)	34.2 (5.0)	34.6 (4.6)	34.3 (5.4)	<0.001
Maternal educational level at follow up (years)								
≤6	1041 (19.1)	141 (30.9)	164 (24.9)	261 (18.0)	126 (15.2)	133 (14.3)	216 (19.1)	<0.001
7-9	1373 (25.2)	134 (29.3)	186 (28.2)	369 (25.5)	185 (22.3)	184 (19.8)	315 (27.9)	
10-12	1524 (28.0)	95 (20.8)	170 (25.8)	422 (29.2)	252 (30.4)	270 (29.1)	315 (27.9)	
>12	1510 (27.7)	87 (19.0)	139 (21.1)	394 (27.2)	266 (32.1)	340 (36.7)	284 (25.1)	
Median (P25-P75)	12 (8-15)	9 (6-12)	9 (7-12)	12 (8-15)	12 (9-16)	12 (9-16)	11 (8-13)	<0.001
Household income at follow up (€/month)								
≤1000	1508 (27.7)	169 (37.0)	224 (34.0)	403 (27.9)	186 (22.5)	192 (20.8)	334 (29.5)	<0.001
1001-1500	1534 (28.2)	123 (26.9)	187 (28.4)	417 (28.9)	228 (27.5)	251 (27.1)	328 (29.0)	
>1500	2311 (42.5)	156 (34.1)	238 (36.1)	602 (41.7)	395 (47.7)	471 (50.9)	449 (39.7)	
Does not know/Prefers not to answer	91 (1.7)	9 (2.0)	10 (1.5)	22 (1.5)	19 (2.3)	11 (1.2)	20 (1.8)	
Maternal pre-pregnancy BMI (kg/m^2)[b]								
Underweight	186 (3.7)	20 (4.9)	19 (3.1)	58 (4.4)	23 (3.0)	40 (4.7)	26 (2.5)	<0.001
Normal weight	3258 (65.2)	245 (59.5)	360 (59.6)	860 (65.3)	528 (68.7)	580 (68.2)	685 (65.6)	
Overweight	1121 (22.4)	95 (23.1)	155 (25.7)	286 (21.7)	161 (20.9)	178 (20.9)	246 (23.6)	
Obese	432 (8.6)	52 (12.6)	70 (11.6)	113 (8.6)	57 (7.4)	53 (6.2)	87 (8.3)	
Mean (SD)	23.9 (4.2)	24.7 (5.1)	24.4 (4.5)	23.9 (4.3)	23.6 (4.1)	23.5 (3.8)	23.9 (4.0)	<0.001
Mode of delivery								
Vaginal	3403 (63.1)	266 (58.8)	415 (63.4)	910 (63.8)	509 (62.0)	591 (64.4)	712 (63.5)	0.415
Caesarian	1991 (36.9)	186 (41.2)	240 (36.6)	517 (36.2)	312 (38.0)	326 (35.6)	410 (36.5)	
Gender								
Girls	2672 (49.0)	219 (47.9)	305 (46.3)	729 (50.4)	418 (50.4)	445 (48.0)	556 (49.1)	0.499
Boys	2778 (51.0)	238 (52.1)	354 (53.7)	717 (49.6)	411 (49.6)	482 (52.0)	576 (50.9)	

Maternal smoking during pregnancy								
Smoker	711 (13.2)	92 (20.5)	111 (17.0)	214 (14.9)	93 (11.3)	82 (8.9)	119 (10.6)	<0.001
Stopped smoking	812 (15.0)	61 (13.6)	107 (16.4)	242 (16.9)	128 (15.6)	120 (13.1)	154 (13.7)	
Ex-smoker	676 (12.5)	46 (10.2)	76 (11.7)	174 (12.1)	113 (13.7)	120 (13.1)	147 (13.1)	
Never smoked	3200 (59.3)	250 (55.7)	358 (54.9)	805 (56.1)	489 (59.4)	597 (65.0)	701 (62.5)	
Child weight status (kg/m^2)[c]								
Underweight	230 (4.2)	29 (6.3)	37 (5.6)	61 (4.2)	38 (4.2)	31 (3.3)	34 (3.0)	<0.001
Normal weight	4092 (75.1)	317 (69.4)	462 (70.1)	1091 (75.4)	630 (76.0)	733 (79.1)	859 (75.9)	
Overweight/Obese	1128 (20.7)	111 (24.3)	160 (24.3)	294 (20.3)	161 (19.4)	163 (17.6)	239 (21.1)	
Mean (SD)	16.3 (1.8)	16.4 (2.0)	16.5 (2.1)	16.3 (1.7)	16.2 (1.7)	16.2 (1.6)	16.3 (1.7)	0.008
Waist circumference (cm) Mean (SD)	52.8 (4.5)	53.0 (5.0)	53.1 (5.1)	52.9 (4.4)	52.6 (4.5)	52.7 (4.1)	52.9 (4.3)	0.228
Fat mass (kg) Mean (SD)	3.2 (2.0)	3.3 (2.2)	3.3 (2.3)	3.2 (2.0)	3.2 (1.9)	3.1 (1.8)	3.2 (1.9)	0.166
Waist-to-height ratio Mean (SD)	0.50 (0.04)	0.50 (0.05)	0.50 (0.05)	0.50 (0.04)	0.50 (0.04)	0.50 (0.04)	0.50 (0.04)	0.429

BMI, body mass index; SD, standard deviation; P25, percentile 25; P75, percentile 75.

[a]except if otherwise specified.

[b]according to WHO cut off points for BMI (29).

[c]according to age and gender specific international cut off points for BMI (28).

Note: in each variable, differences in total number are due to missing data.

Table 3: Participants' characteristics according to type of breastfeeding.

		Breastfeeding			
	Total (n=1639)	**Never** (n=457)	**Exclusive** (n=579)	**Mixed** (n=603)	p
	n (%)a	n (%)a	n (%)a	n (%)a	
Maternal age at follow up (years)					
<25	121 (7.4)	37 (8.1)	53 (9.2)	31 (5.2)	0.004
25-29	306 (18.7)	90 (19.7)	124 (21.5)	92 (15.3)	
30-34	538 (32.9)	137 (30.0)	180 (31.1)	221 (36.8)	
≥35	671 (41.0)	193 (42.2)	221 (38.2)	257 (42.8)	
Mean (SD)	33.1 (5.7)	33.3 (6.2)	32.6 (5.8)	33.5 (5.2)	0.016
Maternal educational level at follow up (years)					
≤6	394 (24.0)	141 (30.9)	156 (26.9)	97 (16.1)	<0.001
7-9	467 (28.5)	134 (29.3)	173 (29.9)	160 (26.5)	
10-12	423 (25.8)	95 (20.8)	150 (25.9)	178 (29.5)	
>12	355 (21.7)	87 (19.0)	100 (17.3)	168 (27.9)	
Median (P25-P75)	12 (8-15)	9 (6-12)	9 (6-12)	12 (9-15)	<0.001
Household income at follow up (€/month)					
≤1000	533 (32.5)	169 (37.0)	216 (37.3)	148 (24.5)	<0.001
1001-1500	466 (28.4)	123 (26.9)	169 (29.2)	174 (28.9)	
>1500	611 (37.3)	156 (34.1)	183 (31.6)	272 (45.1)	
Does not know/ Prefers not to answer	29 (1.8)	9 (2.0)	11 (1.9)	9 (1.5)	
Maternal pre-pregnancy BMI (kg/m^2)[b]					
Underweight	63 (4.2)	20 (4.9)	23 (4.3)	20 (3.6)	0.769
Normal weight	908 (60.8)	245 (59.5)	321 (60.2)	342 (62.3)	
Overweight	361 (24.2)	95 (23.1)	133 (25.0)	133 (24.2)	
Obese	162 (10.8)	52 (12.6)	56 (10.5)	54 (9.8)	
Mean (SD)	24.3 (4.6)	24.7 (5.1)	24.2 (4.4)	24.2 (4.5)	0.185
Mode of delivery					
Vaginal	1007 (62.0)	266 (58.8)	396 (69.0)	345 (57.8)	<0.001
Caesarian	616 (38.0)	186 (41.2)	178 (31.0)	252 (42.2)	
Gender					
Girls	786 (48.0)	219 (47.9)	281 (48.5)	286 (47.4)	0.930
Boys	853 (52.0)	238 (52.1)	298 (51.5)	317 (52.6)	
Maternal smoking during pregnancy					
Smoker	295 (18.2)	92 (20.5)	108 (18.9)	95 (15.8)	0.110
Stopped smoking	257 (15.8)	61 (13.6)	104 (18.2)	92 (15.3)	
Ex-smoker	183 (11.3)	46 (10.2)	69 (12.1)	68 (11.3)	
Never smoked	887 (54.7)	250 (55.7)	290 (50.8)	347 (57.6)	
Child weight status (kg/m^2)[c]					
Underweight	92 (5.6)	29 (6.3)	25 (4.3)	38 (6.3)	0.504
Normal weight	1164 (71.0)	317 (69.4)	421 (72.7)	426 (70.6)	
Overweight/Obese	383 (23.4)	111 (24.2)	133 (23.0)	139 (23.1)	
Mean (SD)	16.4 (2.0)	16.4 (2.0)	16.4 (2.0)	16.4 (1.9)	0.886
Waist circumference (cm)					
Mean (SD)	53.0 (4.9)	53.0 (5.0)	53.0 (5.1)	52.9 (4.7)	0.895
Fat mass (kg)					
Mean (SD)	3.3 (2.2)	3.3 (2.2)	3.3 (2.3)	3.2 (2.0)	0.430
Waist-to-height ratio					
Mean (SD)	0.50 (0.05)	0.50 (0.05)	0.50 (0.05)	0.50 (0.04)	0.788

BMI, body mass index; SD, standard deviation; P25, percentile 25; P75, percentile 75.

[a]except if otherwise specified.

[b]according to WHO cut off points for BMI (29).

[c]according to age and gender specific international cut off points for BMI (28).

Note: in each variable, differences in total number are due to missing data.

Table 4: Associations between duration of breastfeeding and body mass index, fat mass, waist circumference and waist-to-height ratio z-scores, among 4-5 years old boys.

	BMI z-score β (95%CI)	FM z-score β (95%CI)	WC z-score β (95%CI)	WHtR z-score β (95%CI)
Breastfeeding duration				
Crude model				
Not breastfed	0[b]	0[b]	0[b]	0[b]
≤ 1 month	0.079 (-0.070 to 0.229)	-0.024 (-0.187 to 0.139)	0.007 (-0.146 to 0.159)	0.032 (-0.130 to 0.194)
1.1-4 months	-0.050 (-0.183 to 0.083)	-0.099 (-0.245 to 0.048)	-0.017 (-0.153 to 0.120)	0.019 (-0.126 to 0.164)
4.1-6 months	-0.029 (-0.174 to -0.116)	-0.058 (-0.218 to 0.101)	-0.015 (-0.163 to 0.134)	0.036 (-0.122 to 0.194)
6.1-12 months	-0.009 (-0.150 to -0.132)	-0.060 (-0.214 to 0.095)	-0.011 (-0.155 to 0.133)	-0.030 (-0.184 to 0.123)
> 12 months	0.025 (-0.112 to 0.163)	-0.042 (-0.194 to 0.109)	0.029 (-0.112 to 0.169)	0.022 (-0.128 to 0.171)
Model 1[a]				
Not breastfed	0[b]	0[b]	0[b]	0[b]
≤ 1 month	0.077 (-0.072 to 0.226)	-0.024 (-0.187 to 0.139)	0.014 (-0.137 to 0.166)	0.041 (-0.124 to 0.205)
1.1-4 months	-0.073 (-0.206 to 0.060)	-0.125 (-0.272 to 0.022)	-0.034 (-0.170 to 0.102)	0.027 (-0.121 to 0.175)
4.1-6 months	-0.061 (-0.206 to 0.085)	-0.095 (-0.256 to 0.067)	-0.044 (-0.192 to 0.105)	0.045 (-0.117 to 0.207)
6.1-12 months	-0.026 (-0.168 to 0.116)	-0.085 (-0.241 to 0.071)	-0.024 (-0.168 to 0.121)	-0.015 (-0.172 to 0.143)
> 12 months	-0.014 (-0.152 to 0.124)	-0.084 (-0.237 to 0.068)	-0.008 (-0.149 to 0.133)	0.033 (-0.120 to 0.186)
Model 2[a]				
Not breastfed	0[b]	0[b]	0[b]	0[b]
≤ 1 month	0.065 (-0.088 to 0.217)	0.015 (-0.153 to 0.184)	0.028 (-0.127 to 0.184)	0.070 (-0.097 to 0.236)
1.1-4 months	-0.041 (-0.177 to 0.096)	-0.075 (-0.227 to 0.078)	-0.004 (-0.144 to 0.135)	0.044 (-0.106 to 0.193)
4.1-6 months	-0.020 (-0.169 to 0.129)	-0.030 (-0.197 to 0.137)	0.007 (-0.146 to 0.159)	0.075 (-0.089 to 0.238)
6.1-12 months	0.008 (-0.138 to 0.154)	-0.036 (-0.199 to 0.126)	0.008 (-0.141 to 0.157)	0.023 (-0.137 to 0.182)
> 12 months	0.018 (-0.124 to 0.160)	-0.017 (-0.175 to 0.142)	0.037 (-0.108 to 0.182)	0.070 (-0.085 to 0.225)

β, regression coefficient; 95%CI, 95% confidence interval; BMI, body mass index; FM, fat mass; WC, waist circumference; WHtR, waist-to-height ratio.

[a] model adjusted for child birth weight (continuous variable), mode of delivery (categorical variable), and maternal smoking status during pregnancy (categorical variable).

[b] model adjusted for child birth weight (continuous variable), mode of delivery (categorical variable), maternal smoking status during pregnancy (categorical variable), maternal pre-pregnancy BMI (continuous variable), maternal age (continuous variable) and maternal educational level in years of schooling (continuous variable).

[c]reference class.

Table 5: Associations between duration of breastfeeding and body mass index, fat mass, waist circumference and waist-to-height ratio z-scores,among 4-5 years old girls.

	BMI z-score	FM z-score	WC z-score	WHtR z-score
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
Breastfeeding duration				
Crude model				
Not breastfed	0[b]	0[b]	0[b]	0[b]
≤ 1 month	0.055 (-0.134 to 0.244)	0.039 (-0.164 to 0.243)	0.021 (-0.167 to 0.209)	0.026 (-0.152 to 0.204)
1.1-4 months	-0.081 (-0.245 to 0.084)	-0.046 (-0.223 to 0.131)	-0.059 (-0.223 to 0.105)	-0.058 (-0.213 to 0.097)
4.1-6 months	-0.195 (-0.373 to -0.017)	-0.077 (-0.269 to 0.115)	-0.187 (-0.364 to -0.010)	-0.138 (-0.306 to 0.030)
6.1-12 months	-0.209 (-0.385 to -0.032)	-0.224 (-0.414 to -0.034)	-0.162 (-0.338 to 0.013)	-0.119 (-0.285 to 0.047)
> 12 months	-0.093 (-0.263 to 0.078)	-0.125 (-0.308 to 0.058)	-0.105 (-0.275 to 0.064)	-0.123 (-0.283 to 0.038)
Model 1[a]				
Not breastfed	0[b]	0[b]	0[b]	0[b]
≤ 1 month	0.051 (-0.138 to 0.239)	0.056 (-0.148 to 0.260)	0.013 (-0.175 to 0.201)	0.023 (-0.157 to 0.203)
1.1-4 months	-0.117 (-0.281 to 0.047)	-0.066 (-0.244 to 0.111)	-0.093 (-0.258 to 0.071)	-0.079 (-0.237 to 0.078)
4.1-6 months	-0.204 (-0.382 to -0.027)	-0.084 (-0.276 to 0.107)	-0.197 (-0.374 to -0.020)	-0.137 (-0.307 to 0.033)
6.1-12 months	-0.234 (-0.410 to -0.057)	-0.243 (-0.433 to -0.053)	-0.195 (-0.372 to -0.019)	-0.131 (-0.300 to 0.038)
> 12 months	-0.153 (-0.323 to 0.018)	-0.167 (-0.351 to 0.017)	-0.165 (-0.335 to 0.006)	-0.146 (-0.309 to 0.017)
Model 2[a]				
Not breastfed	0[b]	0[b]	0[b]	0[b]
≤ 1 month	0.145 (-0.046 to 0.337)	0.130 (-0.083 to 0.342)	0.090 (-0.103 to 0.283)	0.070 (-0.115 to 0.256)
1.1-4 months	-0.006 (-0.174 to 0.162)	0.017 (-0.170 to 0.203)	-0.018 (-0.188 to 0.151)	-0.034 (-0.197 to 0.130)
4.1-6 months	-0.061 (-0.243 to 0.120)	0.020 (-0.181 to 0.220)	-0.109 (-0.292 to 0.074)	-0.075 (-0.251 to 0.101)
6.1-12 months	-0.061 (-0.243 to 0.121)	-0.127 (-0.328 to 0.074)	-0.061 (-0.244 to 0.123)	-0.044 (-0.221 to 0.133)
> 12 months	-0.028 (-0.202 to 0.146)	-0.093 (-0.286 to 0.100)	-0.086 (-0.261 to 0.090)	-0.096 (-0.265 to 0.073)

β, regression coefficient; 95%CI, 95% confidence interval; BMI, body mass index; FM, fat mass; WC, waist circumference; WHtR, waist-to-height ratio.

[a] model adjusted for child birth weight (continuous variable), mode of delivery (categorical variable), and maternal smoking status during pregnancy (categorical variable).

[b] model adjusted for child birth weight (continuous variable), mode of delivery (categorical variable), maternal smoking status during pregnancy (categorical variable), maternal pre-pregnancy BMI (continuous variable), maternal age (continuous variable) and maternal educational level in years of schooling (continuous variable).

[c]reference class.

Table 6: Associations between type of breastfeeding and body mass index, fat mass, waist circumference and waist-to-height ratio z-scores, among 4-5 years old boys.

	BMI z-score	FM z-score	WC z-score	WHtR z-score
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
Breastfeeding				
Crude model				
Not breastfed	0[c]	0[c]	0[c]	0[c]
Exclusive	0.058 (-0.117 to 0.233)	-0.004 (-0.187 to 0.180)	0.040 (-0.136 to 0.216)	-0.001 (-0.185 to 0.182)
Mixed	0.008 (-0.165 to 0.180)	-0.099 (-0.280 to 0.082)	-0.052 (-0.227 to 0.122)	0.064 (-0.117 to 0.246)
Model 1[a]				
Not breastfed	0[c]	0[c]	0[c]	0[c]
Exclusive	0.052 (-0.124 to 0.229)	0.002 (-0.183 to 0.187)	0.041 (-0.138 to 0.220)	0.031 (-0.157 to 0.219)
Mixed	-0.007 (-0.179 to 0.165)	-0.121 (-0.303 to 0.061)	-0.052 (-0.227 to 0.123)	0.077 (-0.107 to 0.261)
Model 2[b]				
Not breastfed	0[c]	0[c]	0[c]	0[c]
Exclusive	0.046 (-0.132 to 0.224)	0.035 (-0.153 to 0.223)	0.054 (-0.125 to 0.234)	0.068 (-0.118 to 0.253)
Mixed	-0.004 (-0.178 to 0.170)	-0.072 (-0.257 to 0.113)	-0.043 (-0.219 to 0.133)	0.065 (-0.117 to 0.247)

β, regression coefficient; 95%CI, 95% confidence interval; BMI, body mass index; FM, fat mass; WC, waist circumference; WHtR, waist-to-height ratio.

[a] model adjusted for child birth weight (continuous variable), mode of delivery (categorical variable), and maternal smoking status during pregnancy (categorical variable).

[b] model adjusted for child birth weight (continuous variable), mode of delivery (categorical variable), maternal smoking status during pregnancy (categorical variable), maternal pre-pregnancy BMI (continuous variable), maternal age (continuous variable) and maternal educational level in years of schooling (continuous variable).

[c]reference class.

Table 7: Associations between type of breastfeeding and body mass index, fat mass, waist circumference and waist-to-height ratio z-scores, among 4-5 years old girls.

	BMI z-score β (95%CI)	FM z-score β (95%CI)	WC z-score β (95%CI)	WHtR z-score β (95%CI)
Breastfeeding				
Crude model				
Not breastfed	0[c]	0[c]	0[c]	0[c]
Exclusive	-0.030 (-0.242 to 0.181)	-0.000 (-0.225 to 0.225)	-0.047 (-0.254 to 0.161)	-0.056 (-0.262 to 0.150)
Mixed	-0.037 (-0.248 to 0.174)	-0.050 (-0.277 to 0.177)	0.002 (-0.206 to 0.209)	-0.027 (-0.233 to 0.179)
Model 1[a]				
Not breastfed	0[c]	0[c]	0[c]	0[c]
Exclusive	-0.047 (-0.261 to 0.166)	0.033 (-0.193 to 0.259)	-0.056 (-0.264 to 0.153)	-0.062 (-0.272 to 0.149)
Mixed	-0.054 (-0.265 to 0.158)	-0.054 (-0.281 to 0.172)	-0.022 (-0.229 to -0.186)	-0.052 (-0.261 to 0.157)
Model 2[b]				
Not breastfed	0[c]	0[c]	0[c]	0[c]
Exclusive	0.058 (-0.161 to 0.277)	0.103 (-0.136 to 0.341)	0.014 (-0.203 to 0.231)	-0.006 (-0.225 to 0.213)
Mixed	0.090 (-0.133 to 0.313)	0.058 (-0.187 to 0.302)	0.087 (-0.134 to 0.307)	0.016 (-0.207 to 0.238)

β, regression coefficient; 95%CI, 95% confidence interval; BMI, body mass index; FM, fat mass; WC, waist circumference; WHtR, waist-to-height ratio.

[a] model adjusted for child birth weight (continuous variable), mode of delivery (categorical variable), and maternal smoking status during pregnancy (categorical variable).

[b] model adjusted for child birth weight (continuous variable), mode of delivery (categorical variable), maternal smoking status during pregnancy (categorical variable), maternal pre-pregnancy BMI (continuous variable), maternal age (continuous variable) and maternal educational level in years of schooling (continuous variable).

[c]reference class.

PAPER II

EFFECT OF DURATION AND TYPE OF BREASTFEEDING ON MATERNAL ADIPOSITY 4-5 YEARS AFTER DELIVERY: RESULTS FROM THE PORTUGUESE BIRTH COHORT

ABSTRACT

Objective: To study the effect of duration and type of breastfeeding on maternal adiposity, measured by body mass index (BMI), waist circumference (WC) and waist-to-height ratio (WHtR), 4-5 years after delivery.

Methods: This study was based in the Portuguese birth cohort, Generation XXI (n=8495 mothers, corresponding to 8647 newborns). All the mothers were invited to the children's 4-5 years of age follow up evaluation, and 7157 mothers participated. Trained interviewers administered a structured questionnaire on demographic and socioeconomic characteristics, lifestyles, obstetric and medical history, and performed anthropometric measurements, according to standard procedures.

After excluding multiple pregnancies, questionnaires that were not answered by the mothers, women who were pregnant at the time of the follow up evaluation, women that had a live born child since the delivery of the index child, and women with no information on breastfeeding and on anthropometric measurements, 4381 mothers were enrolled in this analysis.

Duration of breastfeeding was registered in weeks, and categorized in months. To estimate the effect of type of breastfeeding on adiposity, the analysis was restricted to the median duration of exclusive breastfeeding, which was 10 weeks, both for mothers who were exclusively breastfed and for those who mixed breastfed, thus 1335 women were evaluated. Type of breastfeeding was categorized in three classes: did not breastfeed, exclusively breastfed and mixed breastfed. Regression coefficients (β) and their 95% confidence intervals were computed using generalized linear models to estimate the effect of duration and type of breastfeeding on maternal BMI, WC and WHtR.

Results: About 8% of women did not breastfeed their infants. The median duration of exclusive breastfeeding was 10 weeks and the median duration of overall breastfeeding was 24 weeks. Overweight and obesity were observed in 31.8% and 21.4% of women, respectively.

When compared to women that did not breastfeed, those that did it for a month had a decrease of 0.63kg/m^2 in BMI, of 1.27cm in WC and of 0.01 in WHtR, after adjustment for parity, mode of delivery, pre-pregnancy BMI, smoking during pregnancy, age and educational level. Breastfeeding up to 6 months was associated with a maternal BMI reduction of 0.96kg/m^2, a decrease of 2.43cm in WC of, and of 0.02 in WHtR. Women who breastfed during more than 6 months experienced BMI, WC and WHtR significant reductions,

although smaller than those referred above. Independently of parity, mode of delivery, pre-pregnancy BMI, smoking during pregnancy, age and educational level, breastfeeding, both exclusive and mixed, were associated with a decrease of 0.86 and 0.78kg/m^2 in BMI, respectively, comparing to not breastfeeding. Women who breastfed exclusively had a WC reduction of 1.60cm, and those who mixed breastfed had a WC reduction of 1.65cm, compared to mothers who did not breastfeed. Exclusive and mixed breastfeeding were associated with a decrease of 0.01 in WHtR.

Conclusion: Duration of breastfeeding was inversely associated with maternal adiposity, assessed with BMI, WC and WHtR, 4-5 years after delivery. No difference in the protective effect of breastfeeding was observed between women that exclusively or mixed breastfed. This association was independent of parity, mode of delivery, pre-pregnancy BMI, smoking status during pregnancy, age and educational level. This study contributes to the understanding of the role played by breastfeeding in maternal adiposity in the first years after a delivery.

Keywords: breastfeeding, pregnancy, adiposity, obesity, overweight, maternal, women.

Introduction

Overweight, obesity and related comorbidities are important public health problems worldwide. They have inherent important biological, social and economic burden, and the future perspective is disturbing due to their persistently rise throughout the years, both in developed and developing countries (1).

In Portugal, the prevalence of overweight and obesity among women has been described as one of the highest of the European Region, and 30% of Portuguese women aged between 25 and 35 years old are overweight or obese (2, 3).

Pregnancy has been associated to an increased risk of overweight and obesity (4, 5). The excessive weight gain during this period and the retention of this weight afterward, contribute to a higher probability of being overweight and obese later in life (6, 7). In addition to the substantial weight gain, pregnant women also experience an increased accumulation of abdominal visceral fat (8, 9), which is associated with an increased metabolic risk (10), and this adverse adiposity distribution may persist through the years (11, 12).

Another pregnancy related event that may influence maternal weight status is breastfeeding, which has been considered as a protective factor for the postpartum maternal adiposity, helping the mother returning to a healthier physical shape (13-15). The main protective mechanisms described are the increased energy expenditure in lactating women (16), the energy cost of milk production itself and the higher carbohydrate utilization by the mammary glands along with some neuroendocrine changes that seem to be lipolysis promoting (17). Additionally, breastfeeding may have a considerable protective effect on women's risk of cardiovascular disease, independently of body mass index (BMI) (18-21), and increased duration of breastfeeding was also found to be protective against type 2 diabetes (22, 23).

In Portugal, approximately 90% of the mothers breastfeed their infants in the neonatal period (24). However, the breastfeeding rates decrease to 60% and 35% at 3 and 6 months, respectively (24, 25).

Trying to understand if breastfeeding offers protection against maternal adiposity in the medium/long term, we aimed to study the effect of duration and type of breastfeeding on maternal adiposity 4-5 years after delivery, in a cohort of Portuguese women.

Methods

Study design and subjects

This study was based in the Portuguese birth cohort, Generation XXI. A total of 8495 mothers, corresponding to 8647 newborns, were enrolled in this cohort, recruited between April 2005 and August 2006, in the five public maternity units of the metropolitan area of Porto, Portugal. Mothers were consecutively invited to participate after delivery, and 91.4% accepted participation.

Between April 2009 and July 2011, the 4-5 years of age follow up occurred. All children and their mothers were invited to participate in this re-evaluation, after a telephone contact. Trained interviewers applied two structured questionnaires focused on child and mother´s health, in a face-to-face interview. The questionnaire concerning children allowed collecting demographic and socioeconomic data, caregiver's information, child's lifestyle information (such as physical activity, screen-based media use and current diet), sleeping habits, health care utilization and medical history. The questionnaire regarding the mother, allowed collecting data on demographic, personal clinical history, obstetric history, and lifestyle information (including physical activity, current diet and alcohol intake and smoking history).

Shorter versions of the questionnaires used in the face-to-face interview were created in order to evaluate the families that were unable to attend the face-to-face interview. Trained interviewers were responsible for the assessment of these families by a telephone interview.

Overall, 84.2% of the mothers were re-evaluated, 5729 mothers attended to the face-to-face interview (67.4% of the entire cohort) and 1428 provided data by telephone interview (16.8%).

The present study was based in the face-to-face interview data, because only mothers with available anthropometric measurements were included. We excluded multiple pregnancies (n=105), questionnaires that were not answered by the mothers (n=121), women who were pregnant at the time of the follow up evaluation (n=179), as well as those that had a live born child since the delivery of the index child (n=832). Also, participants with no information on breastfeeding and on the duration of breastfeeding, and with no information on anthropometric measurements (n=111) were excluded, combining a total of 4381 mothers enrolled in this analysis.

The local institutional ethics committee and the Portuguese Data Protection Authority approved the study and all the mothers provided written informed consent.

Table 1 presents the comparison between the mothers who participated in this study and those that did not, regarding maternal socio-demographic, obstetric and perinatal characteristics at the time of delivery. No differences between the two groups were observed regarding the mode of delivery. When compared to non-participants, those included in this study were older, had a higher educational level and higher household income.

Measurements

Breastfeeding

Mothers were asked if the child was ever breastfed. If the answer was yes then they were asked for how long the child was exclusively breastfed and also for how long the child was breastfed in total (breastfeeding combined with formula feeding or with other type of food).

Duration of breastfeeding was registered in weeks, and to estimate its effect in maternal adiposity, we categorized it in months, creating six groups: those who did not breastfeed, breastfed during ≤ 1 month, breastfed between 1.1 and 4 months, between 4.1 and 6 months, between 6.1 and 12 months, and those who breastfed for more than 12 months. These cut-off points were chosen based on World Health Organization (WHO) and American Academy of Pediatrics (AAP) recommendations regarding duration of breastfeeding, supporting that exclusive breastfeeding should be performed during the first 6 months, continuing up to 12 months or longer, with other complementary foods (26, 27). The cut-off point of 4 months was also considered because, according to the Portuguese law, is the length of the full paid maternity leave.

Exclusive breastfeeding was defined when the child was only fed with breast milk, and it was considered until formula or other type of food were introduced and breastfeeding stopped. Mixed breastfeeding was defined when the child was breast and formula fed (or other type of food), concurrently, since birth, and also when the child was exclusively breastfed during a period of time and then formula or other type of food was introduced to supplement breastfeeding.

Additionally, to estimate the effect of type of breastfeeding in maternal adiposity we restricted the analysis to the median duration of exclusive breastfeeding, which was 10 weeks, both for women who exclusively breastfed and for women who mixed breastfed. Thus, in this analysis, 1335 women were included. Women were categorized in three groups according to type of breastfeeding: those who did not breastfeed at all, those who exclusively breastfed and those who mixed breastfed.

Anthropometry

Anthropometric measurements were performed by trained interviewers, according to standard procedures.

Height was measured by using a portable stadiometer to the nearest 0.1cm, and weight was measured with a digital scale to the nearest 0.1kg, while the mother was barefooted and in light clothing. Maternal BMI was calculated as weight in kg/height in m^2 and categorized as underweight, normal weight, overweight and obesity, according to the WHO cut-off points (28).

Waist circumference (WC) was measured to the nearest 0.1cm, using a non-flexible tape, at a parallel level to the floor, with the mother in a standing position, with arms at the sides, feet positioned close together, and with the body weight evenly distributed. WC was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest (29).

Waist-to-height ratio (WHtR) was calculated as WC in cm/height in cm.

Covariates

This study also included possibly associated characteristics, such as parity (continuous variable), mode of delivery (categorical variable), pre-pregnancy BMI (continuous variable), smoking status during pregnancy (categorical variable), maternal age (continuous variable) and educational level at the time of the follow-up evaluation (continuous variable). Pre-pregnancy BMI was calculated as pre-pregnancy weight in kg/height in m^2 and categorized as underweight, normal weight, overweight and obesity, according to the WHO cut-off points (28). Pre-pregnancy weight was self-reported to the nearest 0.1kg and height was measured by the interviewers to the nearest 0.1cm, at time of delivery. When measurement was not possible height was self-reported as registered in the mothers' national identity card (for 30.8% of women height data was obtained this way).

Statistical analysis

Sample characteristics are presented as proportions for categorical variables and comparison between groups was performed using a chi-square test. Group comparisons for continuous variables were performed using the ANOVA (normally distributed variables) and Kruskal-Wallis test (non-normally distributed variables).

Regression coefficients (β) and their 95% confidence intervals (CIs) were computed using generalized linear models to estimate the effect of duration and type of breastfeeding

on women's BMI, WC and WHtR. For the purpose of the study, maternal BMI, WC and WHtR were used in the multiple regression models as continuous variables. The final model was adjusted for parity (continuous variable), mode of delivery (categorical variable), pre-pregnancy BMI (continuous variable), smoking status during pregnancy (categorical variable), age at the time of the follow-up (continuous variable) and educational level at the time of the follow-up (continuous variable).

All the statistical analyses were performed using Statistical Package for Social Sciences (SPSS) for Windows, version 20.0 (IBM Corp., New York, USA).

Results

In this sample, 8.4% of women did not to breastfeed their child, 12.3% breastfed up to 1 month, 26.2% to 4 months, 15.6% to 6 months, 17.0% to 12 months, and 20.3% breastfed for more than 12 months. The overall breastfeeding median (P25-P75) duration was 24 weeks (10-40).

Tables 2 and 3 summarize the characteristics of the study participants, according to duration and type of breastfeeding, respectively.

The mean [standard deviation (SD)] age of mothers that participated in this study was 34.3 years (5.3), and older women ($p<0.001$) and those with higher household incomes ($p<0.001$) reported higher duration of breastfeeding. On the other hand, women that reported having breastfed their child for less than a month were less educated ($p<0.001$), more frequently obese before pregnancy ($p=0.009$), reported smoking during pregnancy ($p<0.001$), and four to five years after delivery were also more frequently obese, and had higher mean WC and WHtR ($p<0.001$) (Table 2).

Regarding type of breastfeeding, women that did not breastfed their infants, were older ($p=0.045$) and had higher mean BMI, WC and WHtR, four to five years after delivery ($p<0.001$). No differences regarding pre-pregnancy BMI and smoking during pregnancy were observed according to type of breastfeeding. In this sample, mixed breastfeeding was associated with higher maternal education ($p<0.001$), higher household income ($p=0.001$) and having a Caesarian section ($p=0.002$) (Table 3).

The association between duration and type of breastfeeding and maternal adiposity measures (BMI, WC and WHtR) are presented in tables 4 and 5, respectively.

As shown in table 4, duration of breastfeeding was inversely associated with adiposity four to five years after delivery, regardless the adiposity measure. After adjustment for parity, mode of delivery, pre-pregnancy BMI, smoking during pregnancy, age and educational level, mothers who breastfed up to 1 month experienced reductions of 0.63kg/m^2 in BMI, of 1.27cm in WC and of 0.01 in WHtR, compared to those who did not breastfeed. Breastfeeding up to 6 months was associated with a BMI reduction of 0.96kg/m^2 (p trend=0.009), a WC decrease of 2.43cm (p trend=0.007), and a 0.02 lower WHtR (p trend<0.001). Also, women who breastfed during more than 6 months experienced BMI, WC and WHtR significant reductions, although smaller than those referred above. Also, significant negative trends were observed between the duration of breastfeeding and the three adiposity measures evaluated.

Independently of parity, mode of delivery, pre-pregnancy BMI, smoking during pregnancy, age and educational level, breastfeeding, both exclusive and mixed, were associated with a decrease of 0.86 and 0.78kg/m^2 in BMI, respectively, comparing to not

breastfeeding. Women who breastfed exclusively had a WC reduction of 1.60cm, and those who mixed breastfed had a WC reduction of 1.65cm, compared to mothers who did not breastfeed. Similarly, exclusive and mixed breastfeeding were associated with a decrease of 0.01 in WHtR.

The interaction between duration and type of breastfeeding and adiposity measures was also tested, but no modifying effect was observed.

Discussion

In this sample of young Portuguese women, an impressive prevalence of overweight and obesity, 31.8% and 21.4%, respectively, was observed. These results are close to those reported in US, where the combined prevalence of overweight and obesity among women aged between 20 and 39 years was 60%. (30). In Portugal, data from a national health survey conducted in 2005-2006, showed that the prevalence of overweight and obesity among young women were 21.5% and 8%, respectively (2).

Our results suggest that this population might also be at increased risk of other obesity related comorbidities, such as coronary heart disease, hypertension, diabetes, dyslipidemia, and other metabolic complications (28, 31).

Pregnancy has been associated with overweight/obesity by the inherent weight gain during gestation, but also by the failure to lose this weight after delivery. Both these aspects have been pointed as important predictors of long-term weight changes and higher BMI, years after a pregnancy (4, 7). A positive relation between lactation, postpartum weight loss and body composition changes over time was described, as women who exclusively breastfed seemed to experience a greater postpartum weight loss than women who mixed breastfed (32), also longer duration of breastfeeding appeared to be associated with reduced visceral fat, as mothers who did not used the fat stores acquired during gestation to milk production, seemed to retain more visceral fat later in life (12).

In our study, the median duration of exclusive breastfeeding was 10 weeks, which is far below the international recommendations that support exclusive breastfeeding up to 6 months (26, 27). However, we observed that a longer duration of breastfeeding, regardless if it was exclusive or mixed, was associated with a lower BMI, WC and WHtR, 4-5 years after delivery, independently of parity, mode of delivery, pre-pregnancy BMI, smoking during pregnancy, age and educational level. Our findings are consistent with previous studies which indicated that breastfeeding is inversely associated with maternal adiposity, supporting the protective effect of breastfeeding in the postpartum weight loss both on the short and long term (12, 33-35).

In a similar study, Gigante et al (36) examined the effect of breastfeeding in maternal adiposity 5 years after delivery. They observed a BMI reduction of 0.63kg/m^2 and WC reduction of 1.83cm for women who breastfed during 6 to 11.9 months, while those who breastfed for less than 1 month had an increase of 1.01kg/m^2 in their BMI and a 0.95cm increase in WC, compared to mothers who breastfed during 12 months or longer. Later, Rooney and Schauberger (7), reported that women who breastfed their child for at least 3 months had a significant lower weight gain over an average of 8 years, than women who breastfed for less than 3 months or did not breastfed. They described that 10 years after

delivering, women who did not breastfed or quit by 2 weeks in postpartum, had an average BMI of 28.1kg/m^2, while women who breastfed during more than 12 weeks had an average BMI of 26.2kg/m^2.

Concerning the type of breastfeeding, we found that both exclusive and mixed breastfeeding had a significant protective effect on all maternal adiposity measures, compared to not breastfeeding, although no difference between these two types of breastfeeding was observed in our sample. These results are different from those previously reported, which described that women who breastfed mixed or not breastfed at all, had an increase of 3.30cm in their WC and a 1.18kg/m^2 higher BMI, compared to women who breastfed exclusively or predominantly (36). On the other hand, the results reported by Krause et al (15), are in accordance to ours, suggesting that both mixed and exclusive breastfeeding resulted in greater postpartum weight loss when compared to not breastfeeding, although they found that exclusive breastfeeding conferred greater protection (β=-1.38; 95%CI 0.89-1.87) than mixed breastfeeding (β=-0.84; 95%CI 0.39-1.29).

There are behavioral and genetic factors that influence weight and fat metabolism regulation during and after pregnancy. During pregnancy, women experience an increase in body fat that is greatest at central sites and least at peripheral sites. Lactation mobilizes this fat from the trunk and thighs, in response to the increased levels of estrogen (9). Besides, prolactin stimulates the synthesis and secretion of milk components, but also depresses lipogenesis in the liver and adipose tissue, and increases the delivery of glucose and lactate, lipogenic precursors, to the mammary gland (17). These are possible mechanisms related to breastfeeding that promote weight loss and body composition changes after pregnancy. Thus, we estimated the effect of breastfeeding on body fat, using BMI, but also on fat distribution, using WC and WHtR, since they are strong indicators of obesity and of obesity related comorbidities (37, 38).

We found significant inverse associations between all outcomes and duration of breastfeeding and, for all of them, the magnitude of the associations were higher for women who breastfed for 6 months. The three objective measures of adiposity used in our analysis, as well as the consistency and similarity of the associations between them, strengthen our results.

The retrospective nature of our study, as all information on breastfeeding was self-reported, indicates that our results could be affected by recall bias. However, in a Brazilian study, 70% of mothers correctly recalled breastfeeding duration when their children were 4

years old (39). Also, Tomeo et al (40) suggested that maternal long-term recall of pregnancy related events, including breastfeeding history, is accurate and reproducible.

As shown in table 1, women that participated in this study were older, had higher educational level and higher household income. Also, it is expected that women from higher socioeconomic status, are more likely to engage in other beneficial health behaviors that may also play a protective role on their adiposity profile. This possibly overestimates the real effect of breastfeeding on women's adiposity. We attenuated the potential influence of this limitation by adjusting for age and educational level, though some residual confounding may still persist; also, we cannot exclude the possible effect of other potential confounding factors that were not evaluated in our study.

Another limitation of our study is that we only used data on one breastfeeding event in women's life, because no information about women's breastfeeding history was collected. McClure et al (12) assessed women's breastfeeding history, which allowed the comparison between women who never breastfed, those who breastfed some of their children and women who breastfed all of their children. They found that women who breastfed some of their infants exhibited significantly greater amounts of adiposity (20% greater visceral adiposity and 3.38cm greater WC) when compared to mothers who breastfed all of their children for more than 3 months. Thus, our results may be underestimating the effect of breastfeeding, as one would expect greater magnitude of associations if the complete breastfeeding history was considered.

Conclusion

We found a significant inverse association between duration of breastfeeding and maternal adiposity, assessed with BMI, WC and WHtR, 4-5 years after delivery.

Also, in this Portuguese sample, no differences in the protective effect of breastfeeding were observed between women that exclusively or mixed breastfed their child. This association was independent of parity, mode of delivery, pre-pregnancy BMI, smoking status during pregnancy, age and educational level.

This study contributes to the understanding of the role of breastfeeding in maternal adiposity in the first years after a delivery. These results strengthen the recommendation of encouraging mothers to breastfeed their infants, at least during 6 months, irrespective if it is exclusive or mixed with formula or other type of food.

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Table 1: Comparison between participant and non-participant mothers, according to maternal socio-demographic, obstetric and perinatal characteristics, at the time of delivery.

	Non participants (n=4266) n (%)	**Participants** (n=4381) n (%)	p
Age (years)			
<25	1199 (28.2)	678 (15.5)	<0.001
25-29	1279 (30.1)	1290 (29.5)	
30-34	1233 (29.0)	1521 (34.8)	
≥35	535 (12.6)	881 (20.2)	
Mean (SD)	28.0 (5.7)	30.0 (5.3)	<0.001
Educational level (years)			
≤6	1110 (26.0)	1004 (22.9)	<0.001
7-9	1140 (26.7)	1046 (23.9)	
10-12	1065 (25.0)	1230 (28.1)	
>12	951 (22.3)	1101 (25.1)	
Median (P25-P75)	9 (6-12)	11 (7-13)	<0.001
Marital status			
Married/cohabiting	3931 (92.1)	4124 (94.1)	0.001
Single/divorced/widow	300 (7.0)	238 (5.4)	
Household income (€/month)			
≤1000	1569 (36.8)	1455 (33.2)	<0.001
1001-1500	924 (21.7)	1181 (27.0)	
>1500	1099 (25.8)	1208 (27.6)	
Does not know/Prefers not to answer	476 (11.2)	348 (7.9)	
Pre-pregnancy BMI (kg/m^2)[a]			
Underweight	169 (4.4)	149 (3.7)	0.001
Normal weight	2563 (67.1)	2567 (63.9)	
Overweight	760 (19.9)	937 (23.3)	
Obese	327 (8.6)	367 (9.1)	
Mean (SD)	23.7 (4.2)	24.0 (4.3)	<0.001
Mode of delivery			
Vaginal	2653 (63.1)	2725 (62.8)	0.825
Caesarian	1553 (36.9)	1611 (37.2)	
Gestational age (weeks)			
Mean (SD)	38.3 (2.2)	38.7 (1.7)	<0.001
Child birth weight (g)			
≤2500	528 (12.4)	315 (7.2)	<0.001
2501-4000	3586 (84.2)	3905 (89.2)	
>4000	143 (3.4)	158 (3.6)	
Mean (SD)	3103.0 (617.0)	3190.9 (485.0)	<0.001

[a]according to WHO cut off points for BMI (28).

Note: in each variable, differences in total number are due to missing data.

Table 2: Participants' characteristics according to duration of breastfeeding.

		Breastfeeding duration						
	Total (n=4381)	**Did not breastfeed (n=370)**	**≤ 1 month (n=541)**	**1.1-4 months (n=1150)**	**4.1-6 months (n=683)**	**6.1-12 months (n=746)**	**> 12 months (n=891)**	p
	n (%)[a]	n (%)[a]	n (%)[a]	n (%)[a]	n (%)[a]	n (%)[a]	n (%)[a]	
Age at follow-up (years)								
<25	166 (3.8)	21 (5.7)	38 (7.1)	50 (4.4)	18 (2.6)	12 (1.6)	27 (3.0)	<0.001
25-29	616 (14.1)	63 (17.0)	88 (16.3)	178 (15.5)	88 (12.9)	83 (11.2)	116 (13.1)	
30-34	1409 (32.2)	111 (30.0)	176 (32.7)	378 (32.9)	235 (34.5)	252 (33.9)	257 (29.0)	
≥35	2178 (49.9)	175 (47.3)	237 (44.0)	542 (47.2)	341 (50.0)	397 (53.4)	486 (54.9)	
Mean (SD)	34.3 (5.3)	34.1 (6.1)	33.3 (5.5)	34.0 (5.4)	34.5 (5.0)	34.8 (4.7)	34.9 (5.4)	<0.001
Educational level at follow-up (years)								
≤6	862 (19.7)	114 (30.8)	138 (25.5)	218 (19.0)	111 (16.3)	113 (15.1)	168 (18.9)	<0.001
7-9	1135 (25.9)	102 (27.6)	152 (28.1)	305 (26.5)	164 (24.0)	159 (21.3)	253 (28.5)	
10-12	1268 (29.0)	82 (22.2)	141 (26.1)	341 (29.7)	216 (31.6)	233 (31.2)	255 (28.7)	
>12	1114 (25.4)	72 (19.5)	110 (20.3)	286 (24.9)	192 (28.1)	241 (32.3)	213 (24.0)	
Median (P25-P75)	11 (8-13)	9 (6-12)	9 (6-12)	11 (8-12)	12 (9-15)	12 (9-16)	11 (8-12)	<0.001
Marital status at follow-up								
Married/cohabiting	3852 (88.0)	327 (88.4)	465 (86.0)	991 (86.2)	615 (90.2)	669 (89.8)	785 (88.3)	0.047
Single/divorced/widow	525 (12.0)	43 (11.6)	76 (14.0)	159 (13.8)	67 (9.8)	76 (10.2)	104 (11.7)	
Household income at follow-up (€/month)								
≤1000	1233 (28.2)	134 (36.2)	180 (33.3)	330 (28.7)	154 (22.6)	171 (23.0)	264 (29.7)	<0.001
1001-1500	1290 (29.5)	102 (27.6)	159 (29.4)	351 (30.6)	198 (29.0)	212 (28.5)	268 (30.1)	
>1500	1781 (40.7)	128 (34.6)	193 (35.7)	447 (38.9)	313 (45.9)	354 (47.5)	346 (38.9)	
Does not know/Prefers not to answer	72 (1.6)	6 (1.6)	9 (1.7)	20 (1.7)	17 (2.5)	8 (1.1)	12 (1.3)	
Pre-pregnancy BMI (kg/m^2)[b]								
Underweight	149 (3.7)	16 (4.8)	16 (3.2)	45 (4.3)	20 (3.1)	33 (4.8)	19 (2.3)	0.009
Normal weight	2567 (63.9)	199 (59.6)	299 (60.2)	660 (62.8)	422 (66.5)	460 (67.5)	527 (64.1)	
Overweight	937 (23.3)	76 (22.8)	125 (25.2)	248 (23.6)	142 (22.4)	142 (20.9)	204 (24.8)	
Obese	367 (9.1)	43 (12.9)	57 (11.5)	98 (9.3)	51 (8.0)	46 (6.8)	72 (8.8)	
Mean (SD)	24.0 (4.3)	24.7 (5.1)	24.3 (4.5)	24.1 (4.4)	23.8 (4.2)	23.6 (3.9)	24.0 (4.0)	0.001

Mode of delivery								
Vaginal	2725 (62.8)	222 (60.8)	337 (62.8)	718 (63.3)	416 (61.4)	481 (65.1)	551 (62.4)	0.706
Caesarian	1611 (37.2)	143 (39.2)	200 (37.2)	417 (36.7)	261 (38.6)	258 (34.9)	332 (37.6)	
Smoking during pregnancy								
Smoker	584 (13.3)	71 (19.2)	92 (17.0)	176 (15.3)	77 (11.3)	71 (9.5)	97 (10.9)	<0.001
Stopped smoking	659 (15.0)	50 (13.5)	85 (15.7)	192 (16.7)	109 (16.0)	95 (12.7)	128 (14.4)	
Ex-smoker	553 (12.6)	40 (10.8)	63 (11.6)	140 (12.2)	98 (14.3)	98 (13.1)	114 (12.8)	
Never smoked	2585 (59.0)	209 (56.5)	301 (55.6)	642 (55.8)	399 (58.4)	482 (64.6)	552 (62.0)	
BMI (kg/m^2)[b]								
Underweight	47 (1.1)	4 (1.1)	3 (0.6)	17 (1.5)	8 (1.2)	5 (0.7)	10 (1.1)	<0.001
Normal weight	2007 (45.8)	135 (36.5)	255 (47.1)	543 (47.2)	319 (46.7)	361 (48.4)	394 (44.2)	
Overweight	1391 (31.8)	115 (31.1)	148 (27.4)	344 (29.9)	226 (33.1)	255 (34.2)	303 (34.0)	
Obese	936 (21.4)	116 (31.4)	135 (25.0)	246 (21.4)	130 (19.0)	125 (16.8)	184 (20.7)	
Mean (SD)	26.5 (5.1)	27.9 (6.0)	26.8 (5.4)	26.4 (5.2)	26.2 (5.0)	26.0 (4.7)	26.4 (4.9)	<0.001
Waist circumference (cm) Mean (SD)	84.4 (12.4)	88.1 (13.9)	85.4 (12.6)	84.2 (12.8)	83.6 (11.7)	83.0 (11.6)	84.6 (11.7)	<0.001
Waist-to-height ratio Mean (SD)	0.53 (0.08)	0.56 (0.09)	0.54 (0.08)	0.53 (0.08)	0.53 (0.08)	0.52 (0.07)	0.53 (0.08)	<0.001

SD, standard deviation; P25, percentile 25; P75, percentile 75;

[a]except if otherwise specified;

[b]according to WHO cut off points for BMI (28).

Note: in each variable, differences in total number are due to missing data.

Table3: Participants' characteristics according to type of breastfeeding.

		Breastfeeding type			
	Total (n=1335)	**Did not breastfeed (n=370)**	**Exclusive (n=456)**	**Mixed (n=509)**	p
	n (%)[a]	n (%)[a]	n (%)[a]	n (%)[a]	
Age at follow-up (years)					
<25	79 (5.9)	21 (5.7)	34 (7.5)	24 (4.7)	0.101
25-29	223 (16.7)	63 (17.0)	87 (19.1)	73 (14.4)	
30-34	437 (32.8)	111 (30.0)	142 (31.2)	184 (36.3)	
≥35	593 (44.5)	175 (47.3)	192 (42.2)	226 (44.6)	
Mean (SD)	33.6 (5.7)	34.1 (6.1)	33.1 (5.8)	33.8 (5.2)	0.045
Educational level at follow-up (years)					
≤6	327 (24.5)	114 (30.8)	126 (27.6)	87 (17.1)	<0.001
7-9	376 (28.2)	102 (27.6)	137 (30.0)	137 (26.9)	
10-12	354 (26.5)	82 (22.2)	119 (26.1)	153 (30.1)	
>12	278 (20.8)	72 (19.5)	74 (16.2)	132 (25.9)	
Median (P25-P75)	11 (8-13)	9 (6-12)	9 (6-12)	12 (9-13)	<0.001
Marital status at follow-up					
Married/cohabiting	1147 (85.9)	327 (88.4)	373 (81.8)	447 (87.8)	0.008
Single/divorced/widow	507 (11.9)	43 (11.6)	83 (18.2)	62 (12.2)	
Household income at follow-up (€/month)					
≤1000	439 (32.9)	134 (36.2)	172 (37.7)	133 (26.1)	0.001
1001-1500	391 (29.3)	102 (27.6)	138 (30.3)	151 (29.7)	
>1500	481 (36.0)	128 (34.6)	136 (29.8)	217 (42.6)	
Does not know/ Prefers not to answer	24 (1.8)	6 (1.6)	10 (2.2)	8 (1.6)	
Pre-pregnancy BMI (kg/m^2)[b]					
Underweight	50 (4.1)	16 (4.8)	19 (4.5)	15 (3.2)	0.679
Normal weight	740 (60.8)	199 (59.6)	249 (59.6)	292 (62.8)	
Overweight	293 (24.1)	76 (22.8)	105 (25.1)	112 (24.1)	
Obese	134 (11.0)	43 (12.9)	45 (10.8)	46 (9.9)	
Mean (SD)	24.4 (4.6)	24.7 (5.1)	24.2 (4.3)	24.2 (4.5)	0.217
Mode of delivery					
Vaginal	821 (62.2)	222 (60.8)	309 (68.5)	290 (57.7)	0.002
Caesarian	1572 (37.2)	143 (39.2)	142 (31.5)	213 (42.3)	
Smoking during pregnancy					
Smoker	243 (18.2)	71 (19.2)	91 (20.0)	81 (15.9)	0.295
Stopped smoking	201 (15.1)	50 (13.5)	78 (17.1)	73 (14.3)	
Ex-smoker	157 (11.8)	40 (10.8)	56 (12.3)	61 (12.0)	
Never smoked	734 (55.0)	209 (56.5)	231 (50.7)	294 (57.8)	
BMI (kg/m^2)[b]					
Underweight	13 (1.0)	4 (1.1)	5 (1.1)	4 (0.8)	0.018
Normal weight	593 (44.4)	135 (36.5)	214 (46.9)	244 (47.9)	
Overweight	386 (28.9)	115 (31.1)	133 (29.2)	138 (27.1)	
Obese	343 (25.7)	116 (31.4)	104 (22.8)	123 (24.2)	
Mean (SD)	26.9 (5.5)	28.0 (6.0)	26.6 (5.2)	26.6 (5.3)	<0.001
Waist circumference (cm) Mean (SD)	85.7 (13.1)	88.1 (13.9)	84.9 (12.8)	84.7 (12.6)	<0.001
Waist-to-height ratio Mean (SD)	0.54 (0.08)	0.56 (0.09)	0.53 (0.08)	0.53 (0.08)	<0.001

SD, standard deviation; P25, percentile 25; P75, percentile 75.

[a]except if otherwise specified.

[b]according to WHO cut off points for BMI (28).

Note: in each variable, differences in total number are due to missing data.

Table 4: Associations between duration of breastfeeding and body mass index, waist circumference and waist-to-height ratio, in women 4-5 years after delivery.

	BMI (kg/m^2)	**WC (cm)**	**WHtR**
	β (95%CI)	β (95%CI)	β (95%CI)
Breastfeeding duration			
Crude model			
Did not breastfeed	0[b]	0[b]	0[b]
≤ 1 month	-1.11 (-1.79 to -0.44)	-2.65 (-4.27 to -1.03)	-0.02 (-0.03 to -0.01)
1.1-4 months	-1.58 (-2.17 to -0.98)	-3.86 (-5.29 to -2.43)	-0.03 (-0.04 to -0.02)
4.1-6 months	-1.78 (-2.43 to -1.14)	-4.44 (-5.99 to -2.89)	-0.03 (-0.04 to -0.02)
6.1-12 months	-1.98 (-2.62 to -1.35)	-5.05 (-6.57 to -3.52)	-0.04 (-0.05 to -0.03)
> 12 months	-1.49 (-2.11 to -0.87)	-3.47 (-4.95 to -1.99)	-0.02 (-0.03 to -0.02)
	p<0.001[c]	*p<0.001*[c]	*p<0.001*[c]
Adjusted model [a]			
Did not breastfeed	0[b]	0[b]	0[b]
≤ 1 month	-0.63 (-1.01 to -0.24)	-1.27 (-2.42 to -0.13)	-0.01 (-0.02 to -0.00)
1.1-4 months	-0.92 (-1.26 to -0.58)	-2.15 (-3.16 to -1.13)	-0.01 (-0.02 to -0.01)
4.1-6 months	-0.96 (-1.32 to -0.59)	-2.43 (-3.53 to -1.34)	-0.02 (-0.02 to -0.01)
6.1-12 months	-0.77 (-1.14 to -0.41)	-2.26 (-3.35 to -1.17)	-0.02 (-0.02 to -0.01)
> 12 months	-0.76 (-1.11 to -0.40)	-1.80 (-2.85 to -0.74)	-0.01 (-0.02 to -0.01)
	p=0.009[c]	*p=0.007*[c]	*p<0.001*[c]

β, regression coefficient; 95%CI, 95% confidence interval; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio.

[a] model adjusted for parity (continuous variable), mode of delivery (categorical variable), pre-pregnancy BMI (continuous variable), smoking status during pregnancy (categorical variable), age (continuous variable) and educational level in years of schooling (continuous variable).

[b] reference class.

[c] p for trend

Table 5: Associations between type of breastfeeding and body mass index, waist circumference and waist-to-height ratio, in women 4-5 years after delivery.

	BMI (kg/m^2)	**WC (cm)**	**WHt ratio**
	β (95%CI)	β (95%CI)	β (95%CI)
Type of breastfeeding			
Crude model			
Did not breastfeed	0[b]	0[b]	0 [b]
Exclusive	-1.41 (-2.16 to -0.67)	-3.20 (-4.99 to -1.42)	-0.02 (-0.03 to -0.01)
Mixed	-1.42 (-2.14 to -0.69)	-3.42 (-5.17 to -1.68)	-0.02 (-0.03 to -0.01)
Adjusted model [a]			
Did not breastfeed	0[b]	0[b]	0 [b]
Exclusive	-0.86 (-1.30 to -0.41)	-1.60 (-2.86 to -0.35)	-0.01 (-0.02 to -0.00)
Mixed	-0.78 (-1.21 to -0.34)	-1.65 (-2.89 to -0.42)	-0.01 (-0.02 to -0.00)

β, regression coefficient; 95%CI, 95% confidence interval; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio.

[a] model adjusted for parity (continuous variable), mode of delivery (categorical variable), pre-pregnancy BMI (continuous variable), smoking status during pregnancy (categorical variable), age (continuous variable) and educational level in years of schooling (continuous variable).

[b]reference class.

GENERAL CONCLUSIONS

In this thesis, the effect of breastfeeding both on child and maternal adiposity, 4 to 5 years after birth was studied.

Although most of these Portuguese women breastfeed their children immediately after birth, as only about 8% of our children were not breastfed, the overall duration of breastfeeding (median duration of 22 weeks) was below the WHO and AAP recommendations.

A high prevalence of overweight/obesity was observed in this sample. The prevalence of overweight/obesity in these pre-school aged children was 20.7%, and 31.8% of the mothers were overweighed and 21.4% were obese.

The expected protective effect of breastfeeding on child adiposity was not observed in this sample, and similar results have been described in previous studies that assessed this association in children of the same age.

We believe that further studies, with longer follow up periods are needed to clarify this relationship, as the protective effect of breastfeeding on childhood adiposity may only manifest later in the child life-course.

On the other hand, we found a significant inverse association between duration of breastfeeding and maternal adiposity, 4 to 5 years after delivery. This inverse effect was independent of social and obstetrical maternal characteristics. Similar results have been reported previously, supporting the protective effect of breastfeeding in postpartum maternal adiposity both in the short and medium/long term. Also, in this sample, the protective effect of breastfeeding was observed regardless if it was exclusive or mixed breastfeeding.

This study contributes to the understanding of the role of breastfeeding on child and maternal adiposity in the first years after birth/delivery. The recommendation of encouraging mothers to breastfeed their infants, at least during 6 months, irrespective if it is exclusive or mixed with formula or other type of food, must be reinforced in order to achieve the optimal growth and health of the child, but also because it seems to play an important role for a better long-term health of the mother.

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