Measuring food neophobia during pregnancy and its association with the infant's feeding method.

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This Master dissertation is based upon two papers, which will be referred to in text by their Roman numerals:

Paper I - Could the Food Neophobia Scale be adapted to pregnant women? A confirmatory factor analysis [Submitted to Appetite; under revision]

Paper II - Association between maternal Food Neophobia and the infant's milk feeding method in the first months of life [to be submitted]

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Abreviations

BIC: Bayesian information criterion

CFA: Confirmatory factor analysis

CFI: Comparative fit index

EFA: Exploratory factor analysis

ENA: Escala de neofobia alimentar

FNS: Food Neophobia Scale

OR: Odds ratio

95%CI: 95% confidence interval

P-FNS: Portuguese Food Neophobia Scale

R: Reversed scores

RMSEA: Route mean square error of approximation

SD: standard deviation

SRMR: Standardized root mean square residual

TLI: Tucker-Lewis Index

Resumo

Introdução

A neofobia alimentar é definida como um traço de personalidade que influencia a vontade em experimentar alimentos novos, e de acordo com estudos anteriores, pode afetar a qualidade da alimentação materna e subsequentemente as preferências alimentares da criança. A gravidez é um importante período sensível para a aprendizagem precoce de sabores e preferências alimentares, no entanto, é também um estado de alterações psicossociais que pode favorecer a neofobia alimentar. A escala de neofobia alimentar é amplamente utilizada em diversos países para medir a neofobia alimentar, no entanto, são necessárias análises psicométricas apropriadas que permitam a comparação dos resultados entre diferentes culturas e em grupos populacionais específicos. Segundo o nosso conhecimento, a maioria dos estudos foram realizados em crianças e populações adultas, sem qualquer referência a mulheres grávidas.

Objetivos

Esta tese ambiciona responder a dois objectivos específicos: i) Traduzir e testar as propriedades psicométricas da versão em português da escala de neofobia alimentar e identificar *clusters* de neofobia alimentar durante a gravidez (artigo I); ii) Avaliar a associação entre a neofobia alimentar materna e o método de aleitamento escolhido para a alimentação dos bebés no início da vida (artigo II).

Métodos

Os participantes no estudo são mulheres grávidas que se encontravam no último trimestre da gravidez e mães de crianças com menos de 6 meses de idade (n = 219) que foram convidadas para participar no estudo de intervenção *Taste* (projeto HabEat: http://www.habeat.eu/). A escala de neofobia alimentar foi autoadministrada (a neofobia alimentar foi reportada, tendo como período de referência o último trimestre de gestação) e é composta por 10 itens numa escala de 7 pontos. Para avaliar a dimensionalidade da escala realizou-se uma análise exploratória factorial. A análise factorial confirmatória foi realizada através diferentes índices para testar a adequação do modelo anteriormente obtido. A consistência interna da escala foi avaliada através do coeficiente alfa de *Cronbach*. Identificaram-se padrões de neofobia alimentar através do método *Model-based clustering*; e o número de classes latentes foi definido pelo critério de informação *Bayesiano*. As associações entre as pontuações de neofobia alimentar materna e o método de aleitamento planeado e utilizado foram avaliadas por regressão logística não condicional (*odds ratio* e respectivos intervalos de confiança a 95% - OR, IC95%), após ajuste para características maternas como a

idade, a escolaridade, o estado civil, o consumo de produtos hortofrutícolas e náuseas durante a gravidez.

Resultados

No primeiro artigo, após a exclusão do item 8 (com carga factorial <0.4) foi obtido um modelo com dois fatores, explicando 51 % da variância total. O alfa de Cronbach foi de 0,75 para o fator 1 (5 itens) e 0,71 para o fator 2 (4 itens). O modelo apresentou um bom ajuste global, confirmado pelos índices de ajuste: X2: 70,369, p <0,001; TLI = 0,876, CFI = 0,911, RMSEA = 0,088 e SRMR = 0,051. Os itens 1, 4, 6, 9 e 10 apresentaram correlações mais fortes com o primeiro factor (maior vontade em experimentar alimentos novos; traço de personalidade menos neofóbico) e os itens 2, 3, 5, 7 correlacionam-se mais com o segundo fator (menor vontade em experimentar alimentos novos; traço de personalidade mais neofóbico). Quanto maior a escolaridade, idade e o consumo de produtos hortofrutícolas, menor foi a tendência neofóbica, medida pela escala de neofobia alimentar. Foram identificados três grupos (*clusters*) de neofobia alimentar, caracterizando traços neofobicos em mulheres grávidas: neofílico moderado, neofóbico moderado e extremamente neofóbico; os pontos de corte foram fornecidos para serem usados em futuros estudos.

Na segundo artigo, mães com pontuações mais elevadas de neofobia alimentar tiveram cerca de 50% mais probabilidade de planear alimentar os seus bebés exclusivamente com leite materno durante a primeira semana de vida (exclusivamente com leite materno versus não exclusivamente: OR = 1,46, IC95%: 1,01-2,13). Em análise multivariada, a associação não se manteve significativa, mas permaneceu forte (OR = 1,36, IC95%: 0,89-2,06). Não foram encontradas associações significativas entre as pontuações de neofobia materna e os métodos de aleitamento planeados para períodos mais longos (primeiro mês e primeiros 3 meses), nem para o método realmente usado para alimentar os bebés nos primeiros 3 meses de vida (OR = 0,99 IC 95%: 0,75-1,30).

Conclusões

A versão em português da escala de neofobia alimentar apresenta os requisitos básicos de uma medida válida e confiável de neofobia alimentar durante a gravidez e permite a identificação correta de grupos de neofobia durante a gravidez, que pode ser reproduzido por outros investigadores que queiram utilizar a versão portuguesa da escala de neofobia alimentar em grávidas.

Este estudo sugere também que neofobia alimentar durante a gravidez não parece estar associada com o método de aleitamento escolhido para a alimentação do

lactente, no curto espaço de tempo. No entanto, a neofobia alimentar materna pode ter um efeito importante em outras práticas de alimentação e outros eventos relacionados com a gravidez, associações que futuramente deverão ser exploradas em estudos longitudinais.

Abstract

Introduction

Food neophobia is defined as a personality trait that influences the willingness to consume novel foods, and according to previous evidence can impact mother's diet quality and subsequent infant's food preferences. Pregnancy is an important sensitivity period for early flavor and food preference learning, but is also a state of psychosocial changes that can favor food neophobia. The Food Neophobia Scale (FNS) is widely used in different countries to measure food neophobia, however appropriate psychometric analyses are required to allow cross-cultural comparisons and to use the scale in specific target groups. To our knowledge, most studies have been conducted among children and adult populations, with no reference to pregnant women.

Objectives

This thesis aims to answer two specific objectives: i) To translate and test the psychometric properties of a Portuguese version of the FNS (P-FNS), and to identify clusters of food neophobia during pregnancy (paper I); ii) To evaluate the association between maternal food neophobia scores and the milk feeding method chosen for feeding infants early in life (paper II).

Methods

Participants are pregnant women who were in their final trimester of pregnancy and mothers of infants less than 6 months old (n=219) who were asked to take part of the Taste intervention study (HabEat project: http://www.habeat.eu/). After a careful translation and back-translation, the FNS was self-administered (food neophobia was reported having as reference period their last trimester of pregnancy) and consists of 10 items with a 7-point rating scale. An exploratory analysis was performed to evaluate the scale's dimensionality. Confirmatory factor analysis was performed by using different indexes to test the fit of the model previously obtained. The internal reliability of the scale was tested using the Cronbach's alpha coefficient. A Model-based clustering was used to identify patterns of food neophobia; the number of latent classes was defined according to the Bayesian information criterion. The associations between maternal food neophobia scores and the planned and actual milk feeding method were assessed by unconditional logistic regression (odds ratio and respective 95% confidence intervals – OR, 95%CI), after adjusting for maternal age, education, marital status, fruit and vegetables intake and nausea sickness during pregnancy.

Results

In paper I, after excluding item 8 (with factor loading <0.4), a two-factor model solution was obtained, explaining 51% of the total variance. Cronbach's alpha was 0.75 for factor 1 (5 items) and 0.71 for factor 2 (4 items). A good global of fitness of the model was confirmed by fit indexes: X2: 70.369, p<0.001; TLI=0.876, CFI= 0.911, RMSEA=0.088 and SRMR=0.051. Items 1, 4, 6, 9 and 10 loaded into the first factor (i.e. more willingness to try new foods; less neophobic traits) and items 2, 3, 5 and 7 loaded into a second factor (i.e. more neophobic traits). As higher the education, age and fruit and vegetable intake lower the neophobia tendency, measured by the P-FNS. Three patterns (i.e. clusters) of food neophobia, characterizing neophobia traits of pregnant women were identified: moderate neophilic, moderate neophobic, and extreme neophilic, and cut-off points were provided to be used in future research.

In paper II, mothers with higher food neophobic scores had approximately 50% more likelihood of planning to feed infants with breast milk only during the first week of infant's life (exclusively with breast milk vs. not exclusively: OR=1.46, 95%CI: 1.01 – 2.13). In multivariate analyses, the association was no longer significant, but remained strong (OR=1.36, 95%CI: 0.89 - 2.06). No significant associations were found neither between the food neophobia scores and the planned milk feeding methods for longer periods (first month and first 3 months) nor the actual method used to feed the infants in the first 3 months of life (OR=0.99 95%CI: 0.75 - 1.30).

Conclusions

The P-FNS has basic requirements of a valid and reliable measure of food neophobia during pregnancy and allows identifying properly food neophobic clusters during pregnancy, which can be reproduced by other investigators who want to use the P-FNS among pregnant women.

This study also suggests that food neophobia during pregnancy does not seem to be associated with the milk feeding method chosen for early infant's feeding. However, maternal food neophobia could have an important effect in other feeding practices and pregnancy-related outcomes that should be explored in future longitudinal research.

1. Early life establishment of Food Preferences

From a biological point of view, early life food intake is strongly influenced by internal cues of hunger and satiety of newborns, however later food intake is largely determined by food preferences (Russell and Worsley 2008).

Food preferences are developed from genetically determined predispositions to like sweet and salty flavours and to dislike sour or bitter tastes (Benton 2004). Food preferences influenced by innate factors can be perceived already during pregnancy. *In utero,* the fetus can inhale and swallows the amniotic fluid that contains many substances and flavors derived from the mother's diet and environmental exposures. A study found that the injection of a sweet-tasting stimulus into the amniotic fluid stimulated fetal swallowing (Snoo 1937). On the other hand, another study found that injection of a bitter-tasting stimulus inhibited fetal swallowing (Ventura and Worobey 2013). These reactions have been interpreted as positive and negative hedonic responses to sweet and bitter tasting stimuli, respectively (Ventura and Worobey 2013).

In newborns, taste experiences produce similar responses to those experienced *in utero*. It seems that the preference for sweet, as measured by the observation of facial expressions, is universally present in neonates. Sweet and umami solutions motivated behaviors in newborns that were interpreted as positive responses, with almost all newborns reacting more positively to sugar solutions than to water, whereas sweeter solutions were preferred over less sweeter solutions (Barr, Pantel et al. 1999). It was also observed that newborns increased the rate of sucking and the ingestion of larger volumes in response to sweet and umami solutions compared to bitter, sour, salty and neutral stimuli (Ventura and Worobey 2013). A neutral facial response to salty solutions is exhibit at birth. It seems that the preference for a salty taste emerges later at around four months of age (Beauchamp, Cowart et al. 1986). Interestingly, it was observed that children of mothers who experienced increased vomiting episodes during pregnancy had infants with greater preference for salty solutions (Birch 1999). In general, compared with adults, children like higher concentrations of sugar and salt and are more sensitive to bitter tastes (Benton 2004). In fact, newborns expressed negative facial expressions in the presence of bitter tastes or sour substances, exhibiting behaviors that were interpreted to be negative hedonic responses; with a disruption in sucking behavior (Birch 1999; Ventura and Worobey 2013).

The variation in bitterness perception seems to be related with the ability to taste compounds such as the phenylthiocarbamide (PTC) and the 6-n-propylthiouracil (PROP), that are present, for example, in several vegetables, contributing to explain

why children, in general, do not like vegetables. Adults and young children with greater sensitivity to the bitter taste of PTC and PROP reported lower preferences, acceptance and consumption of bitter foods such as bitter vegetables like grapefruit juice, green tea and soy products (Tsuji, Nakamura et al. 2012) or raw spinach and broccoli (Wardle and Cooke 2008). Also, individuals who perceived PROP as extremely bitter are more sensitive to sweet tastes (Drewnowski, Henderson et al. 1997).

This pattern of food preferences may be interpreted from an evolutionary perspective, where human survival depends on correctly discriminating foods. In nature, sweetness is an indicator of sugar content, and therefore calories dense, while bitter tastes are associated with foods that are potentially poisonous and toxic (Mennella, Jagnow et al. 2001; Wardle and Cooke 2010). Thus, these innate food preferences seem to be related to the adaptive capacity to survive to varied and uncertain food environments in the past (Ventura and Worobey 2013). However, in the modern "obesogenic" environment, this once beneficial mechanism has become maladaptive, given the abundance and easy access to high sugar, energy-dense and salty foods (Brug, Tak et al. 2008).

Although large part of food preferences development occurs during early childhood, being partially genetic determined (Drayna 2005), food preferences can change over later childhood, adolescence and adulthood.

In adults, food preferences are influence by a more complex combination of factors that comprise sensory experiences and beliefs such as taste, satiety, food price, food convenience and others (Drewnowski 1997). Biological needs are also influenced by age, sex, heredity and physical and social factors like culture, social and economic factors (Krondl, Krasnegor et al. 1990), which all together influence adults' of food preferences and consequently food choices and food consumption. Thus, the development of food preferences involves a complex interplay between biological, cultural, environmental and social factors (Scaglioni, Salvioni et al. 2008).

2. The Food Neophobia concept

Food neophobia is a personal trait, associated with food preferences (Russell and Worsley 2008), that has been defined as a reluctance to eat novel foods (Pliner and Hobden 1992). Parents also report the rejection of known and previously accepted foods, but the rejection of a substantial amount of familiar food (as well novel) has been defined as Picky Eating, and tends to result in a diet with low variety (Dovey, Staples et al. 2008). Thus, food neophobia and Picky Eating are two related traits, but theoretical

and behavioral distinct concepts, with different factors predicting their severity and expression (Pliner and Salvy 2006). Picky eating is differentiated from food neophobia through the novelty value of the food presented.

Food neophobia is thought to have an adaptive value; the predisposition to respond with a neophobic behavior to new foods is highly established when the transition from an exclusive milk diet to the omnivore's diet occurs. Food neophobia seems to ensure the ingestion of foods that are familiar, reducing the possibility of poisoning from unfamiliar, toxic and allergenic foods, thus having a protective function. However, in the modern environment, the food safety is general guaranteed with food neophobia impacting negatively in human's diet variety (Knaapila, Tuorila et al. 2007). Rozin and colleagues have shown that distaste (dislike of the sensory characteristics of a food) appears to be the strongest driver of neophobia in young children, followed by a potential fear of negative consequences of eating (Fallona and Rozina 1983).

2.1 How to measure Food Neophobia?

Pliner and Hobden developed, in 1992, the Food Neophobia Scale (FNS), a psychometric instrument specifically designed to assess food neophobia in humans (Pliner and Hobden 1992). In this scale, food neophobia is conceptualized as a continuum along which individuals can be located in terms of their propensity to approach or avoid novel foods or novel eating situations (Koivisto and Sjoden 1996). The scale is a self-ten-item questionnaire, where individuals indicate their level of agreement/disagreement with ten statements about foods and eating situations; a lower score represents a more willingness trait to try or choose new foods (food neophilia), while a higher score represents those less willing to try new foods (food neophobia).

The FNS has been widely used in a number of studies from different countries. Since this scale was originally developed in Canada, using a convenience sample of Canadian college students (Pliner and Hobden 1992), it is important to test the psychometric properties of the scale in others languages in order to allow comparisons between different countries, cultures, age-groups, clinical populations, and its use in different sociocultural contexts. Several authors have tested and validated the FNS in adults (Tuorila, Lähteenmäki et al. 2001; Ritchey, Frank et al. 2003; Knaapila, Tuorila et al. 2007) and children (Pliner 1994), producing different adapted instruments, which vary in the total number of items.

The FNS is the most common measure used for assessing food neophobia. However, there are others measurements of food neophobia that include the Food Attitude Scale (FAS) and the revised version FAS-R, which measure familiarity to food, where participants rate their willingness to taste foods on a pre-defined list (Raudenbush and Frank 1999). Loewen and Pliner also developed the Food Situational Questionnaire (FSQ) to access food neophobia in children, in this questionnaire items described hypothetical situations in which novel foods might be encountered and children are asked to report how they would feel about tasting or eating them (Pliner 1994).

2.2 Determinants of Food Neophobia

Recent works studied food neophobia as a hereditable personal trait. Cooke et al. in a twin study estimated heritability of neophobia as 78% (Cooke, Haworth et al. 2007). Additionally, Knaapila *et. al.* estimated the heritability of food neophobia using a sample of Finnish families and a twin sample of British female, and the results from both populations suggest that approximately two thirds of variation in food neophobia is genetically determined (Knaapila, Tuorila et al. 2007).

Although food neophobia is a general characteristic of humans, neophobia changes during development in response to environmental experiences, suggesting that food neophobia could be changed and it is age-specific. In general, food neophobia tends to decline with age, with younger children being more neophobic than older, and children being more neophobic than their parents (Hursti Uk and Sjoden 1997). There is an exception to this general trend, where food neophobia seems to be minimal during the infancy, rising rapidly around the age of 2, peaking around the age of 4, and gradually decreasing thereafter (Dovey, Staples et al. 2008). During early infancy, this minimal neophobia could have an adaptive value because in this period the access to food is largely controlled by adults (the supply of solid food is essentially ensured by an adult caregiver, consequently the food security is generally guarantee). However, as children become increasingly independent (2-5 years-old) and more able to find food for themselves, increasing neophobia could serve as a protective function, reducing the risk of ingestion of poisoning foods (Birch 1999). Thus, as children age, their experiences with food are more varied and frequent, and few foods are novel, and therefore, they become less neophobic. Also, some studies observed an increase in food neophobia levels in the elderly period. In this stage, higher levels could be a response to a weakness health state, therefore the expression of neophobia may be

related to the perception of their health status, and not exactly as a reaction to novel foods (Dovey, Staples et al. 2008).

Studies have found gender differences in respect to FN. Some studies described that women are generally less neophobic than men (Tuorila, Lähteenmäki et al. 2001), while others consider that this difference does not exist when it comes to young people. Accordingly, Koivisto *et al.* reported that fathers and boys scored higher in several items of the scale than mothers and girls, although only a slight significant gender-difference was detected (Koivisto and Sjoden 1996). In other study, Koivisto *et al.* found that fathers showed significant higher total neophobia scores than did mothers (Hursti Uk and Sjoden 1997). Also, Tourila *et al.* found that Finnish men were more neophobic than woman (Tuorila, Lähteenmäki et al. 2001). Nordin *et al.*, in turn, suggested no gender-related differences in food neophobia (Nordin, Broman et al. 2004).

In respect to socio-demographic variables, food neophobia scores seem to decrease with increasing education and with the degree of urbanization. Individuals living in rural areas are more neophobic than their more urban counterparts (Flight, Leppard et al. 2003). In Tuorila *et al.* study, education was negatively related with food neophobia scores (Tuorila, Lähteenmäki et al. 2001). A higher education level and living in urban areas probably enhances the access, experience and exposure to novel food, and perhaps it helps to decrease the neophobic response.

Food neophobia seems also to negatively influence dietary intake and variety. Cooke *et al.* suggested that food neophobia impacts differentially on the consumption of different food types. In this study, children with the highest scores on the Child Food Neophobia Scale had lower consumption of fruit and vegetables, suggesting that neophobic children had less healthy diets compared to their less neophobic peers (Cooke, Carnell et al. 2006). Galloway *et al.* accessed the consequences of food neophobia in young girls, and have found that girls with both neophobia and pickness consumed fewer servings of vegetables than girls with neither food neophobia nor pickness (Galloway, Lee et al. 2003). Koivisto Hursti *et al.* found that higher food neophobia in mothers and children was associated with fewer uncommon foods being served (Koivisto and Sjoden 1996). In turn, Falgicia *et al.* found that neophobic children consumed higher amounts of saturated fat and consumed fewer unique types of food. In this study it was found that FN, has a negative impact in overall diet quality, with the overall Health Eating Index (HEI) score being significantly lower for the neophobic group compared with the average and neophilic groups (Falciglia, Couch et al. 2000). It was also found that energy and essential nutrient intake was comparable between neophobic and neophilic children, except for vitamin E. Fewer neophobic children met

two thirds of the recommended intake for vitamin E than neophilic children (Falciglia, Couch et al. 2000).

Parental attitudes and feeding styles also influence children's FN. Parents chose the feeding methods, control the foods that are available and accessible, create the contexts where food are offered, and have specific food likes and dislikes (Savage, Fisher et al. 2007). This cultural and selective food environment affords to children opportunities to learn about food selection and other related behaviors, influencing their food preferences. Generally, it has been observed that neophilic models can reduce food neophobia in children, whereas neophobic models can increase neophobic responses. Thus, a family resemble appears to exist between food likes and dislikes of the young children and those of their parents. For example, it was identified a range of modifiable features within the home food environment that were associated with fruit and vegetables consumption among preschool children (Wyse, Campbell et al. 2011). The parental intake, the availability and accessibility of fruit and vegetables in the home and the setting of mealtimes accounted for almost half of the variation in children's fruit and vegetable consumption (Rozin and Millman 1987; Eertmans, Baeyens et al. 2001).

In addition, some parent's feeding strategies to promote healthy and varied diet have been found to be counter-productive, with the overt control (a type of control perceived by the child, such as prohibiting the consumption of specific foods) and the offering of rewards having negative effects on food acceptance patterns in children (Scaglioni, Arrizza et al. 2011). Restricting access to foods (for example snacks high in sugar and fat) can have a negative effect at the long- term, because the restricted foods become more attractive and the preference for that kind of food increases (Brown and Ogden 2004). Also, when a food reward is used, the preference for the rewarded food increases, and the preference tends to decrease for the food, in which the food reward was used (Eertmans, Baeyens et al. 2001). Mothers who reported greater food restriction of daughters' snacks had daughters who selected and consumed more of the restricted foods when present in an environment where the foods were freely available to the child and the mothers were not present to restrict the intake (Fisher and Birch 1999).

Children's food acceptance also depends with of the context and consequences related with the ingestion of foods. When foods are offered in positive social environments they are preferred. On the other hand, when foods are present in negative contexts, with more negative emoting, the preference decreases (Savage, Fisher et al. 2007). Moreover, postingestive consequences of food ingestion affects food acceptance. When the consumption of new foods is followed by positive pleasant signals, learned food preferences are produced. In contrast, negative gastrointestinal

consequences, like nausea and vomiting, leads to learned food aversions. Once a food aversion is formed hardly disappears and food consumption will be rejected (Birch 1999).

2.3 Changing Food Neophobia

As children age, their genes and environment interact and can determine the development of a particular phenotype (Birch 1999). Foods preferences are malleable, and even negative food preferences can be modified through time and experience (Cooke, Haworth et al. 2007; Wardle and Cooke 2010). Children have the predisposition to learn preferences and to accept novel foods when they are offered repeatedly (Pliner, Pelchat et al. 1993; Ventura and Worobey 2013). After repeated opportunities to consume new foods, approximately five to ten exposures (the number of exposures required varied with child's age), the liking for new foods generally increases. Research demonstrated that an early introduction to fruit and vegetables during weaning was associated with a higher frequency of consumption of these foods in 2- to 6-years old children (Cooke, Wardle et al. 2004). With exposure and experience to novel foods, the initial neophobic response can be transformed into acceptance via 'learned safety' (Wardle, Herrera et al. 2003). However, these exposures require tasting foods. In some studies, the "mere exposure" or learning about the novel food, on repeated occasions, did not promote children preferences for that food (Birch, McPhee et al. 1987; Birch 1999).

Social context and behavior modeling are important factors that influence FN. Modeling has been shown to increase the acceptance of foods in a number of studies (Hobden and Pliner 1995; Hendy and Raudenbush 2000). An initially dislike for a food can be overcome by social influence from adults and peers, being the consumption of that food more rapidly accepted (Addessi, Galloway et al. 2005). Harper and Sanders long time ago conducted a study where young children tried an unfamiliar food more readily when an adult was eating it than when the food was merely offered. Both mothers and unfamiliar adults were successful models, although mothers' influences were more effective than unfamiliar adults (Harper and Sanders 1975). Birch, in 1980, investigated the influence of peer models' food selections and eating behaviors on preschoolers' food preferences, and it was found that when preschool children observed peer models selecting and eating vegetables (the target child's non-preferred food) the preference for and the intake of the non-preferred food increased (36).

Younger children (3-years-old) were more affected by peer modeling than older children (4-years-old) (Birch 1980). Also Addessi *et al.* found that young children are more likely to eat novel foods if others are eating the same type of food, than when others are merely eating a different food (Addessi, Galloway et al. 2005). In another study accessing model influence of adults on children's food preferences it was found that "silent" teacher was ineffective compared with "enthusiastic" teacher, which promoted an increased acceptance of novel foods. However the "enthusiastic" teacher compared with a competing peer model was no longer effective (Hendy and Raudenbush 2000).

3. Pregnancy and the perinatal period as opportunity windows for the establishment of food preferences and neophobia to foods

During pregnancy, proper maternal diet is essential to meet with increased nutritional needs and metabolic demands of mother and fetus (Picciano 2003). Nutritional adequacy in quantity and quality has been associated with reduced risk of adverse pregnancy outcomes (Abu-Saad and Fraser 2010). Malnutrition, including both under and over nutrition, can greatly impact long term health status and life expectancy of mother and infant (Roseboom, de Rooij et al. 2006; Le Clair, Abbi et al. 2009). Maternal under nutrition leads to poor fetal development and higher risk of pregnancy complications. Experimental studies showed that over nutrition during pregnancy is associated with increased risk of preeclampsia and gestational diabetes (George, Uthlaut et al. 2010).

Likewise, a maternal adequate nutrition status during lactation is also important, influencing normal growth and infant's development (Haileslassie, Mulugeta et al. 2013). Breast milk is commonly perceived as the ideal source of nutrition for infant's feeding, particularly at the first six months of life (Kramer and Kakuma 2002). Breast fed children have lower morbidly and mortality rates (Perera, Ranathunga et al. 2012). Breast milk enables passive immunity, with significant protective effects in gastrointestinal infections and others (Aguiar and Silva 2011), and also seems to show long-term benefits in adulthood, such as a decreased risk of obesity and cardiovascular diseases (Owen, Martin et al. 2005; Guardamagna, Abello et al. 2012). Mother's benefits are related with more rapid recovery from pregnancy, lower risk factor of postpartum hemorrhage, osteoporosis, heart disease, premenopausal female cancers and decreased risk of type 2 diabetes mellitus (Breastfeeding 2012). Despite advantages and recommendations, the rates of breastfeeding are generally low and

high rates of abandonment have been described including in Portugal (Aguiar and Silva 2011).

Parents play a pivotal role in the development of children's food neophobia, and consequently on their food preferences. Indeed these influences may even begin during pregnancy. Flavors from the mother´s diet enter the amniotic fluid and are ingested by the fetus, thus the neonate experience with cultural taste patterns begins long before his first exposure to solid foods. This prenatal exposure probably leads to greater acceptance and enjoyment of these foods during the weaning process (Beauchamp and Mennella 2009). In one study, infants of mothers who had drank carrot juice regularly throughout their pregnancy or lactation, exhibited fewer negative facial expressions when being fed a carrot-flavored cereal compared with a plain cereal (Beauchamp and Mennella 2009). Moreover, those infants who were exposed to carrots prenatally were perceived by their mothers as enjoying the carrot-flavored cereal more compared with the plain cereal. Infants whose mothers drank water during pregnancy and lactation exhibited no such difference. Thereafter, during breastfeeding a wide range of flavors of mother's diet appears dissolved in breast milk. Breast-fed infants seem more willing to accept a novel vegetable, on first presentation compared with formula-fed infants, generally less receptive to accept new flavors (Mennella, Jagnow et al. 2001). One possible explanation is that breast-fed infants are exposed to a largest variety of flavors dissolved in mother's milk than formula fed babies, familiar to experience the monotony of flavors presents in infant formula (Gerrish and Mennella 2001). Also, during weaning, infants who were exposed to a variety of different vegetables were more likely to accept a new vegetable than infants only exposed to one type of vegetable during the same period (Galloway, Lee et al. 2003). However it is possible that not only the good practices are transmitted, Bayol *et al.*, in an experimental study using rats, showed that the offspring of mothers fed with 'junk food' during pregnancy and lactation showed a preference for a 'junk food' diet, with an exaggerated preference for fatty, sugary and salty foods (Bayol, Simbi et al. 2008). While flavors are transmitted via breast milk and this may contribute to increase fruit and vegetables intake, if mothers who breast feed have a poor diet, these habits may be transmitted to the child.

Pregnancy is a critical period of human development, where significant anatomical, physiological, biochemical and endocrine changes take place (Abduljalil, Furness et al. 2012). In this period different stages of development with specific features arise. The first trimester is characterized by embryological and early fetal development; with a single embryological cell multiples into a fetus. During the second trimester, occurs a rapidly fetus growth, with organ development including their support

organ systems. Finally, the third trimester involves a rapid fetal growth, in which fetal finishes developing and becomes well prepared for the life outside the womb. Maternal physiological modifications in circulating blood volume, peripheral vascular compliance and resistance, myocardial function and contractibility, heart rate and rhythm, neurohormonal and respiratory system are some important adaptations that enable maternal organism to meet the increased metabolic demands of pregnancy (Heidemann and McClure 2003).

These different changes require adaption of pregnant woman. Time food choices are influenced by environmental factors such as cultural food practices and beliefs, internal factors such as food cravings and food aversions, and some digestive disorders such as reflux, nausea and vomiting that together may influence the intake of certain foods (Forestell and Mennella ; Kramer, Bowen et al. 2013). During this period dietary intake is particularly perceived as important, because pregnant woman needs to ensure an adequate food intake to satisfy the additional energy requirements for this period and, at the same time, needs to be caution, avoiding potentially toxic and hazardous food.

Some studies have reported small but significant relations between the food neophobia scores and anxiety emotionality traits in children and adults (Pliner and Hobden 1992; Galloway, Lee et al. 2003). In fact, pregnancy is considered as a stage of great vulnerability, and is associated with frequent mood changes, fatigue, exhaustion, sleepiness, and depressive reactions (Bjelica and Kapor-Stanulovic 2004). These ambivalent and contradictory feelings can predispose pregnant woman to a higher stage of anxiety, and hypothetically more neophobic behaviors.

In nowadays food environment, food neophobia paired with the innate predisposition to prefer sweet and salty flavors, and the innate tendency to reject bitter and sour tastes can compromise dietary quality of both adults and children (Koivisto and Sjoden 1996; Falciglia, Couch et al. 2000; Cooke, Carnell et al. 2006; Knaapila, Tuorila et al. 2007).

Early experiences with food are important factors to shape food preferences and, for these reasons, it seems crucial to find new windows of action that can mitigate the consequences of wrong food choices throughout life, such as obesity and cardiovascular diseases (Guardamagna, Abello et al. 2012). For this, pregnancy should be seemed as an important sensitivity period for early flavor and food preference learning. The ability to access food neophobia in pregnant could provide opportunities to healthcare professionals to develop significant nutritional interventions, which promote the development of healthy eating behaviors in the unborn child.

The Food Neophobia Scale (FNS) is widely used in different countries to measure neophobia to foods, however appropriate psychometric analyses are required to allow cross-cultural comparisons. To our knowledge, most studies have been conducted among children and adult populations, with no reference to pregnant women.

Thus, we set as objectives of this thesis:

i) To translate and test the psychometric properties of a Portuguese version of the FNS, and to identify clusters of food neophobia during pregnancy (Paper I).

ii) To evaluate the association between the scoring of pregnant women in the (P-FNS) and the milk feeding method of infants in the first months of life (Paper II)

Paper I

Could the Food Neophobia Scale be adapted to pregnant women?

A confirmatory factor analysis.

[submitted to Appetite]

Could the Food Neophobia Scale be adapted to pregnant women? A confirmatory factor analysis.

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Abstract

Background: The Food Neophobia Scale (FNS) is widely used in different countries, however appropriate psychometric analyses are required to allow cross-cultural comparisons. To our knowledge, most studies have been conducted among children and adult populations, with no reference to pregnant women. The objective of this study was to translate and test the psychometric properties of a Portuguese version of the FNS, and to identify clusters of food neophobia during pregnancy.

Methods: The FNS was translated into Portuguese by three health researchers, and back-translated into English by an independent native English speaker and professional translator. The scale was self-administered in a sample of 219 women from the baseline evaluation of the Taste intervention study (HabEat project: http://www.habeat.eu/), who attended medical visits in two hospitals from Porto, Portugal, reporting food neophobia during the last trimester of pregnancy. The FNS consists of 10 items with a 7-point rating scale. An exploratory analysis was performed to evaluate the scale's dimensionality, followed by a confirmatory factor analysis to test the fit of the previous model by using different indexes. Cronbach's alpha coefficient was calculated to evaluate the internal reliability of the scale. The construct validity was assessed by comparing the FNS scores by categories of education, age and fruit and vegetables intake by ANOVA. A Model-based clustering was used to identify patterns of food neophobia; the number of latent classes was defined according to the Bayesian information criterion.

Results: A two-factor model solution was obtained (after excluding item 8 with a factor loading <0.4), explaining 51% of the total variance. Cronbach's alpha was 0.75 for factor 1 (5 items) and 0.71 for factor 2 (4 items). Items 1, 4, 6, 9 and 10 loaded into the first factor (i.e. more willingness to try new foods; less neophobic traits) and items 2, 3, 5 and 7 loaded into a second factor (i.e. more neophobic traits). A good global of fitness of the model was confirmed by fit indexes: TLI=0.876, CFI= 0.911, RMSEA=0.088 and SRMR=0.051. The higher the education, age, and fruit and vegetables intake the lower the neophobic tendency, measured by the Portuguese FNS. Three patterns (i.e. clusters) of food neophobia, characterizing neophobia traits of pregnant women were identified: Moderate Neophilic, Moderate Neophobic, and Extreme Neophilic (cut-off points were provided).

Conclusion: The Portuguese version of the FNS has the basic requirements of a valid and reliable measure of food neophobia and permits the identification of clusters of neophobic traits during pregnancy.

Key-words: Food Neophobia Scale; pregnant women; psychometrics; factor analysis; Portugal

Abbreviations

BIC: Bayesian information criterion
CFA: Confirmatory factor analysis
CFI: Comparative fit index
EFA: Exploratory factor analysis
FNS: Food Neophobia Scale
P-FNS: Portuguese version of the Food Neophobia Scale
R: reversed scores
RMSEA: Route mean square error of approximation
SRMR: Standardized root mean square residual
SD: standard deviation
TLI: Tucker-Lewis Index

Introduction

Food neophobia, defined as reluctance to eat unfamiliar foods, is a personality trait that influences food choices and consequently food acceptance and consumption (Tuorila et al., 2001; Flight et al., 2003). Food neophobia has been associated with the "Omnivore's Dilemma", in which humans must decide whether or not to consume novel foods; that is, they must weigh up the possible benefit of consumption (receiving valuable nutrition) against the possibility of harm (ingesting poisons or toxins) (Pliner & Hobden, 1992; Dovey et al., 2008). In the broadly safe food environment of the 21st century, the protective function of neophobia is less salient than in the distant past. Rejection of new foods nowadays may have an adverse effect on food choices, compromising quality and variety of diet, since in the modern environment food safety is mostly guaranteed (Pliner & Melo, 1997; Cooke et al., 2006; Cooke et al., 2007).

To assess food neophobia, Pliner and Hobden (1992) developed the Food Neophobia Scale (FNS), a validated psychometric instrument specifically designed to assess this reluctance to consume new foods (Pliner & Hobden, 1992). This scale is a self-administrated ten-item questionnaire, where a lower score represents more willingness to try or choose new foods (food neophilia) and a higher score represents those less willing to try new foods; more neophobic. The FNS is the most common measure used for assessing food neophobia and it has been widely used, but since the scale was originally developed using a sample of Canadian students (Pliner & Hobden, 1992), care must be taken in interpreting results from different populations. In order to allow for cross-cultural comparison, its psychometric properties need to be tested in different countries.

Several validation studies have been conducted to explore the properties of the FNS (Tuorila et al., 2001; Ritchey et al., 2003; Schickenberg et al., 2008; Fernández-Ruiz, 2013) and the results from these different studies suggest that the FNS is a valid tool for assessing food neophobia in different populations. The scale has been used to identify individuals with more neophobic traits, who might require proper intervention and medical advice, supporting its usefulness and importance.

To our knowledge, this scale has not been used in the Portuguese population, and most studies have been conducted among children (with a different FNS version) (Koivisto & Sjödén, 1996; Koivisto & Sjödén, 1997; Falciglia et al., 2000; Cooke et al., 2003; Flight et al., 2003; Galloway et al., 2003; Cooke et al., 2006; Cooke et al., 2007; Dovey et al., 2008; Monneuse et al., 2008; Mustonen et al., 2012) and adult populations (Pliner et al., 1995; Pliner & Melo, 1997; Arvola et al., 1999; Nordin et al., 2004; Knaapila et al., 2007; Edwards et al., 2010; Knaapila et al., 2011) with no

reference to pregnant women. Since neophobia might affect both the quality and variety of diet (Falciglia et al., 2000), it seems relevant to explore food neophobia in pregnant woman, given the importance of nutrition for maternal health, foetal development and birth outcomes.

This study aims to translate, culturally adapt and test the psychometric properties of the FNS in a sample of Portuguese women who reported food neophobia in the last trimester of pregnancy. We also aim to identify clusters of food neophobia among pregnant women.

Methods

Participants

Participants were pregnant women who were in their final trimester of pregnancy and mothers of infants less than 6 months old (n=219). Pregnant women were consecutively approached between April-July 2011, before their attendance to medical visits in two hospitals from Porto (main public hospital and private antenatal clinic), and they were invited to take part in the baseline evaluation of the Taste intervention included in the HabEat project that aims to determine factors and critical periods in food habit formation and breaking in early childhood in several European countries (more detailed information could be find at http://www.habeat.eu/). All participants signed an informed consent form to participate in the study. The research protocol was approved by the local ethical committee (Ethical committee of São João Hospital/ University of Porto Medical School) and the study procedures complied with the Helsinki Declaration.

Data collection

The original FNS consists of 10 items with a 7-point rating scale ranging from (1) ‘strongly disagree' to (7) ‘strongly agree', with (4) corresponding to the neutral position 'neither agree nor disagree'.

The FNS, originally written in English, was translated into Portuguese by three health researchers, and the result was the Portuguese Food Neophobia Scale (P-FNS). This Portuguese version was back-translated into English by an independent native English speaker and professional translator (who was blinded to the original version) and it was compared with the original version of the FNS to ensure equivalence between the two versions. Discrepancies were decided by unanimous

agreement. Therefore, the instrument was piloted in a convenience sample (n=10) to evaluate its cultural adaptation.

The P-FNS was self-administered and had as reference period the third trimester of gestation. The 10 items of the P-FNS appear in the same order as in the original version (see appendix). Before analysis, the scores of 5 items marked with (R) were reversed to obtain ratings in the same direction (Pliner & Hobden, 1992). The total score could range from 10 to 70, as the original one.

The questionnaire administered during the recruitment process also provided socio-demographic information and maternal fruit and vegetable intake, obtained by a food frequency questionnaire. Educational level was categorized into mandatory education (1-9 schooling years), high school education (10-12 schooling years) and university education (>12 schooling years). Three age categories were formed (≤25, 26-34, ≥35 years). Fruit and vegetables intake were dichotomized according to the World Health Organization recommendations (<5 vs. ≥5 servings/day).

Statistical analysis

An exploratory factor analysis (EFA) was performed to understand the underlying structure of the P-FNS version. The exploratory factor analysis was performed using the maximum likelihood estimation method together with the Geomin rotation (considering that we expected a correlation between factors). Factors were selected if their eigenvalue was higher than one. The items with absolute factor loading of 0.4 or higher were interpreted as having meaningful part on the whole domain.

This analysis was followed by confirmatory factor analysis (CFA) to test the fit of the model obtained from the EFA. The fit of the scale was assessed using different indexes: i) the Tucker-Lewis index (TLI) (Tucker & Lewis, 1973), ii) the Comparative fit index (CFI) (Bentler, 1990), iii) the Route mean square error of approximation (RMSEA) (Steiger, 1990), and iv) the Standardized root mean square residual (SRMR) (Hu & Bentler, 1999). The CFI and TLI indexes range from 0 to 1, with higher values indicating a better model fit. The RMSEA and SRMR indexes range from 0 to 1, with lower values indicating a better model fit. A good model fit is indicated by a CFI and TLI values of 0.90 or higher (Hu & Bentler, 1999) and values of RMSEA and SRMR close to 0 (Browne & Cudeck, 1993).

The internal reliability of the scale was tested using the Cronbach's alpha coefficient.

To test the construct validity of the P-FNS, the mean values of each subscale were compared according to age, education and fruit and vegetables intake categories (previous theoretical hypotheses) by using ANOVA.

A model-based clustering (Fraley & Raftery, 2002) was used to identify clusters of food neophobia. According to this method, data was assumed to be generated with multivariate normal distribution items. The multivariate normal distributions were parameterized by their means and covariances that determine their geometric features. Characteristics (orientation, volume and shape) of distributions were estimated from data, and can be allowed to vary between clusters, or constrained to be the same for all clusters. In this study, the number of latent classes (patterns of food neophobia) was defined according to the Bayesian information criterion (BIC). Starting from one single class and increasing one class at each step, the best solution was identified when the increase in the number of classes did not lead to a decrease in BIC. The interpretation of the clusters was obtained by a classification tree that identified the cut-offs to predict the clusters membership using the factors extracted from previous CFA.

To perform EFA and CFA, Mplus, version 5.2 was used. Data analysis for model-based clustering was conducted with the software R 2.14.1, using the package mclust (Fraley et al., 2012). To obtain the classification tree, rpart was used (Therneau, T., Atkinson, B., Ripley, B., & Ripley, M. B. (2012). Package 'rpart'. *Retrieve from http://cran. rproject. org/web/packages/rpart/rpart. pdf*).

The significance level was set at 5%. The missing values were treated as missing at random.

Results

Exploratory factor analysis

An initial EFA was performed to explore if the Portuguese version of the FNS in this population supports a single dimension, as did the original version of the FNS (Pliner & Hobden, 1992). In this analysis, it was identified one-factor solution model that explained 36.1% of the total variance (Table 1). Since item 8 "I am very particular about the foods I will eat" had a low factor loading (less than 0.4), it was decided to eliminate this item, and a second EFA was performed. This factor analysis revealed a two-factor model solution, explaining approximately 51% of the total variance, with factor 1 and factor 2 explaining 26.3% and 24.5% of the total variance, respectively. The respective scree plot supported this solution, indicating that a two-factor solution was the most appropriate model. The internal reliability coefficients from the two

subscales were calculated. Cronbach's alpha was 0.75 for subscale factor 1 (5 items) and 0.71 for subscale factor 2 (4 items), indicating that the P-FNS has good reliability.

Confirmatory factor analysis

In accordance with results from the EFA (Table 1), in the CFA it was assumed that items 1, 4, 6, 9 and 10 belong to factor 1 and items 2, 3, 5 and 7 belong to factor 2, and they were correlated with each other. Figure 1 shows the factor loadings supporting these relations. The global of fitness of the model was tested and it was confirmed by the following fit indexes: TLI=0.876, CFI= 0.911, RMSEA=0.088 and SRMR=0.051. These values suggest a good global of fitness of the P-FNS. The two factors were moderately correlated (r=-0.64), and items 1, 4, 6, 9 and 10 loaded into the first factor and items 2, 3, 5 and 7 loaded into a second factor. The first factor corresponds to the five reversed ordering items (i.e. more willingness to try new foods; less neophobic traits) and the second factor corresponds to the four positively ordering items (i.e. less willingness to try new foods: more neophobic traits).

Construct validity

Construct validity was assessed considering three theoretical hypotheses, based on previously described literature: as the higher the education (Tuorila et al., 2001), age (Dovey et al., 2008) and fruit and vegetables intake (Cooke et al., 2004), the lower the neophobia. To test these hypotheses, the mean values of each subscale according to these variables were compared (Table 2).

Educational level was positively related to factor 1 and negatively related to factor 2: more educated pregnant women scored significantly higher on factor 1 (representing more neophilic traits) and significantly lower on factor 2 (representing more neophobic traits). Older women scored lower on factor 2 (i.e. more neophobic traits) than younger women (≤25, 26-34, ≥35 years: 3.84, 3.63, 3.38 p=0.225). Pregnant women consuming at least 5 portions/day scored significantly higher on factor 1 (4.89 vs. 4.38, p=0.012) and lower on factor 2 compared with those consuming less servings, although the results were not significant (3.31 vs. 3.71, p=0.064). These associations are consistent with the theoretical hypothesis, supporting the construct validity of the P-FNS.

Identification of clusters of food neophobia

Clusters of food neophobia were identified based on the mean score on each factor (factor 1 representing more neophilic traits and factor 2 representing more neophobic traits). The number of clusters of food neophobia was defined according to the BIC, and the best solution was set at three clusters for characterizing neophobia traits of pregnant women (representing three mutually-exclusive groups of women sharing the same pattern). Figure 2 shows the mean scores of each pattern in the two factors, previously defined. Cluster 1 was characterized by moderate scores in all items (answers could range from 1 to 7), with a slightly higher score in factor 1 (mean 5.02 ± 0.84) than in factor 2 (mean 3.06 ± 0.78). Cluster 2 had also a moderate score in all items, but with a slightly higher score in factor 2 (mean 4.76 ± 1.05) than in factor 1 (mean 3.50 ± 1.17). In turn, cluster 3 had the highest score in factor 1 (mean 6.22 ± 0.44) and the lowest score in factor 2 (mean 1.59 ± 0.43) (Figure 3).

The interpretation of the clusters could be easily represented by a classification tree (Figure 3) that predicts the clusters membership using the factors extracted from the previous CFA. The classification tree shows the cut-off points in the two subscales (factor 1 and factor 2) that discriminate each cluster identified.

Discussion

In the present study, we aimed to describe the adaptation and validation process of the P-FNS to address the lack of available instruments to assess and explore food neophobia in Portuguese pregnant women.

Factor analysis revealed a two-factor model solution, explaining 51% of the total variance; factor 1 (with moderate-to-strong correlations with items 1, 4, 6, 9 and 10, representing more willingness to try new foods; less neophobic traits) and factor 2 (with moderate-to-strong correlations with items 2, 3, 5 and 7, representing less willingness to try new foods; more neophobic traits). Although the original scale was one-dimensional, as previously reported by Pliner and Hobden (Pliner & Hobden, 1992), previous studies in adults have already reported that this scale did not perform well with a single dimension (Tuorila et al., 2001; Ritchey et al., 2003; Fernández-Ruiz, 2013). Tourila et al, using exploratory factor analysis, found that items loaded into two factors; one factor related with the interest in trying new and ethnic foods and a second factor that may reflect a general concern with trying unknown foods (Tuorila et al., 2001), as suggested by our study. Additionally, Ritchey et al, by confirmatory factor analysis, validated the FNS and showed that the scale was not one-dimensional, but

two factors were found with data from North American, Swedish and Finnish adult populations (the latter, using the same population of Tourila work, previously described), and a shortened six-item version of the scale was proposed (Ritchey et al., 2003). Also in a Spanish validation study of the FNS in adults (Fernández-Ruiz, 2013), the items were split into two factors, one composed by the positive worded items (more willing to try new foods) and the second composed by the negative worded items.

In our sample, this two-factor model was supported based upon values of the fit indexes from the confirmatory factor analyses, indicating that the P-FNS has a good global fitness. Based on Cronbach's alpha coefficients, with values higher than 0.7 for each subscale, our results also suggest a good internal reliability of the P-FNS.

By the contrary, other studies have also tested the psychometric properties of an adapted Dutch version of the FNS, and showed adequate internal consistency and test–retest reliability of the FNS version used, but with a one-factor structure of the scale (Schickenberg et al., 2008). A French version of the FNS was successfully translated and its validity was confirmed; in this study a factor analysis also revealed a one-dimensional structure of the adapted questionnaire (Rubio et al., 2008).

The original FNS is a ten-item questionnaire, but during the validation process, we noticed that item 8 "I'm very particular about the foods I'll eat" had a low factor loading (<0.4) and it was excluded from analysis. Although the translation process was carefully conducted (by three health researchers and a native English speaker) we found some inconsistencies in the response frequency to item 8 that were not in line with the frequencies of the remaining items. When exploratory factor analysis was performed our suspicion was confirmed (the factor loading was below 0.4, meaning that does not have a meaningful part on the whole domain). Other studies also detected some problems related to item 8. Tuorila el at, reported that this item may be related to a concern caused by dietary restrictions rather than to neophobia or neophilia (Tuorila et al., 2001). Also, Koivisto and Sjodén reported that item 8 may not clearly reflect the trait of neophobia (Koivisto & Sjödén, 1996), suggesting that it has been changed during the translation process. In our study, we cannot rule out a translation problem (one potential solution is to replace the word "particular", in Portuguese "exigente" by selective, in Portuguese "seletivo"), but it may also have been due to an interpretation problem. Since we are dealing with maternal food neophobia during pregnancy, and given the general importance and attention that diet receives in this life stage, item 8 might be viewed in a very particular way by this population. For all these reasons, it was decide to eliminate item 8 from the P-FNS.

In this study, the theoretical hypotheses were confirmed: as the higher the education, age and fruit and vegetables intake the lower the neophobia, measured by the P-FNS.

Education seems to improve the access and exposure to various stimuli, events and cultural knowledge, which may influence and reduce neophobia levels (Flight et al., 2003). Although the evidence about food neophobia levels among different age groups is not as consistent as with education, several studies support that food neophobia is inversely correlated with age (Dovey et al., 2008). Cooke et al reported that food neophobia appears to be minimal in infancy, raising rapidly at age two and gradually tailing off thereafter (Cooke et al., 2003). In our study, the age range was not very large, omitting the effect of extreme classes (the very young and the elderly). Cooke et al. also suggested that neophobia impacts differentially the consumption of different food types (Cooke et al., 2006) and that could be observed in our investigation; pregnant women consuming at least 5 portions/day of fruit and vegetables scored significantly more in factor 1 (more neophilic traits) and lower in factor 2 (more neophobic traits) compared with those consuming less servings.

In the present study, the mean score of the FNS was 4.53 (SD=1.36) for factor 1 and 3.56 (SD=1.42) for factor 2. To assure comparability with other results, we decided to divide the mean score of the FNS by the number of items used in each scale. An overall rate of 3.52 was observed, representing a global average of 31.7 divided by 9 items, SD=10.8, which seems to be slightly higher than previous studies conducted in other populations. Pliner and Hobden (Pliner & Hobden, 1992), in their validation study, reported an average food neophobia score of 3.45 (34.5/ 10 items; SD=11.86), among Canadian students. For Korean respondents, the mean score was 3.35 (33.5/10 items; SD=9.0) (Rubio et al., 2008), while among Spanish adults (Fernández-Ruiz, 2013) the food neophobia mean score was 3.17 (31.7/10 items; SD=10.98). For the Dutch adult population, the mean score was 3.02 (30.2/10 items; SD=9.7) (Schickenberg et al., 2008). Lower scores were found for American students with a mean score of 2.98 (29.8/10 items; SD=11.7) (Olabia et al., 2009). These different scores could be related with the different cultural and socio-demographic backgrounds, which could influence food neophobia level. However, it is interesting to note that our study participants had the highest mean score of food neophobia. In this sense, it would be interesting to extend the study to other population groups, to observe whether this is a general characteristic of the Portuguese population, or if these scores are due to the specific target group under study.

Most studies analyze food neophobia scores based on the factor loadings of the two factors identified (or one factor, when appropriate). According to this, each

individual is represented in both factors, but with higher scores in one of them. To simplify future data analysis, and using a clustering-based approach, we identified three clusters of food neophobia, representing three mutually-exclusive groups of women sharing the same pattern: moderate neophilic, moderate neophobic, and extreme neophilic. These designations were arbitrary and were decided based on scoring of each factor. Cluster 1 was characterized by moderate scores in all items, with a slightly higher score in factor 1 than in factor 2 (moderate neophilic). Cluster 2 had also a moderate score in all items, but with a slightly higher score in factor 2 (moderate neophobic), and cluster 3 had the highest score in factor 1 and the lowest score in factor 2 (extreme neophilic). The classification tree shows the cut-off points in the two subscales (factor 1 and factor 2) that discriminate each cluster identified, and could thus be reproduced by other investigators who want to use the P-FNS among pregnant women.

Some limitations of the present study deserve discussion. It was not possible to measure test-retest reliability of the P-FNS. Women were reporting during the last trimester of their pregnancy, meaning that we were unable to administer another test at a later stage of pregnancy. Although the P-FNS data were self-reported (usually leading to lower social desirability bias), responses could have been influenced by social desirability. Women's reports may be affected by their own beliefs in what pregnant women should ideally eat to provide better nutrition to their baby. Also, food neophobia could be trimester-specific, and so it would be interesting to administer the P-FNS in each trimester. Finally, our results are focused on food neophobia during pregnancy, so the generalization of results for other populations should be made with caution.

Conclusions

The findings of this study support the use of the P-FNS as a valid and reliable measure is able to identify clusters of food neophobia during pregnancy. This validation study provides sufficient evidence that the P-FNS could be a very useful instrument in health research and clinical interventions in pregnant women.

Future studies could use the P-FNS to assess the impact of maternal food neophobia on child's behaviors and growth.

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Table 1: Items of the Portuguese version of the Food Neophobia Scale, having a reference period the last trimester of pregnancy: mean values, factor loadings and explained variance obtained from the exploratory factor analysis.

	Mean	SD	Loadings of one-factor solution	Loadings of two-factors solution	
			F1	F1	F2
1. I am constantly sampling new and different foods. (R)	3.890	1.762	0.400	**0.781**	0.281
2. I don't trust new foods.	2.760	1.530	-0.526	--	**0.719**
3. If I don't know what's in a food, I won't try it.	4.380	2.113	-0.532	0.131	**0.756**
4. I like foods from different countries. (R)	4.540	2.039	0.685	**0.641**	-0.214
5. Foreign food looks too weird to eat.	3.43	1.903	0.734	-0.231	**0.672**
6. At dinner parties, I will try a new food. (R)	4.74	1.810	0.653	**0.740**	--
7. I am afraid to eat things I have never had before.	3.78	2.10	-0.711	-0.199	**0.660**
8. I am very particular about the foods I will eat.*	5.25	1.681	-0.334	-	-
9. I will eat almost anything. (R)	5.05	1.916	0.556	**0.532**	-0.143
10. I like to try new ethnic restaurants. (R)	4.38	2.130	0.730	**0.632**	-0.287
% Explained variance			36.1	26.3	24.5
				Total: 50.8	

*Item 8 was excluded from the two-factor solution model.

Higher loadings are in **bold type**.

(R): reversed items.

SD: standard deviation.

Table 2: Mean scores of the two factors according to education, age and servings of fruit and vegetables intake of pregnant women.

	n (%)	Factor 1[a]	Factor 2[b]
	219	Mean (SD)	Mean (SD)
Education (schooling years)			
1-9	45 (20.5)	4.00 (1.28)	4.09 (1.07)
10-12	58 (26.5)	4.65 (1.31)	3.72 (1.59)
>12	112 (51.1)	4.68 (1.38)	3.36 (1.39)
Missing	4 (1.8)		
p- value		**0.013**	**0.002**
Age (years)			
<25	48 (21.9)	4.42 (1.40)	3.84 (1.27)
26-34	106 (48.4)	4.46 (1.30)	3.63 (1.56)
≥35	65 (29.7)	4.69 (1.45)	3.38 (1.28)
Missing	0		
p-value		0.483	0.225
Servings of fruit and vegetables in the last 3 months			
< 5 portions/day	128 (58.4)	4.37 (1.30)	3.66 (1.43)
≥ 5 portions/ day	85 (38.8)	4.78 (1.44)	3.48 (1.42)
Missing	6 (2.7)		
p-value		**0.031**	0.362

[a] *Factor 1 represents more neophilic traits and*

[b] *Factor 2 represents more neophobic traits*

SD: standard deviation

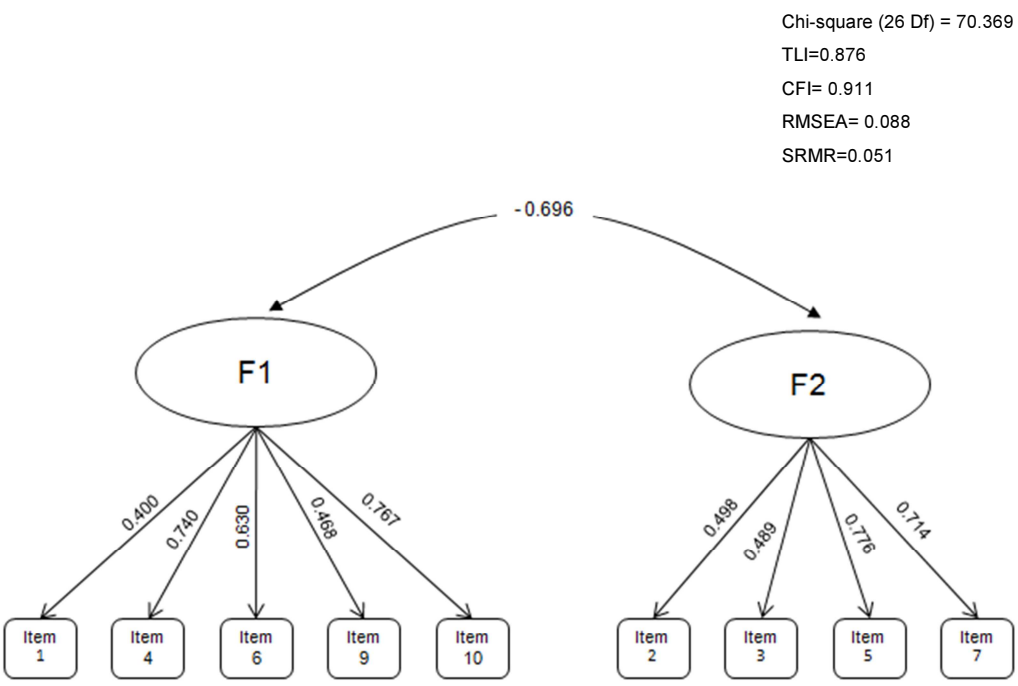

Figure 1: Confirmatory factor analysis for the two-factor model of the Portuguese version of the Food neophobia Scale (P-FNS).

Factor 1 (F1) represents the more neophilic trait and factor 2 (F2) represents the more neophobic trait.

The factor loadings are the values of the correlation coefficient between the items and factors.

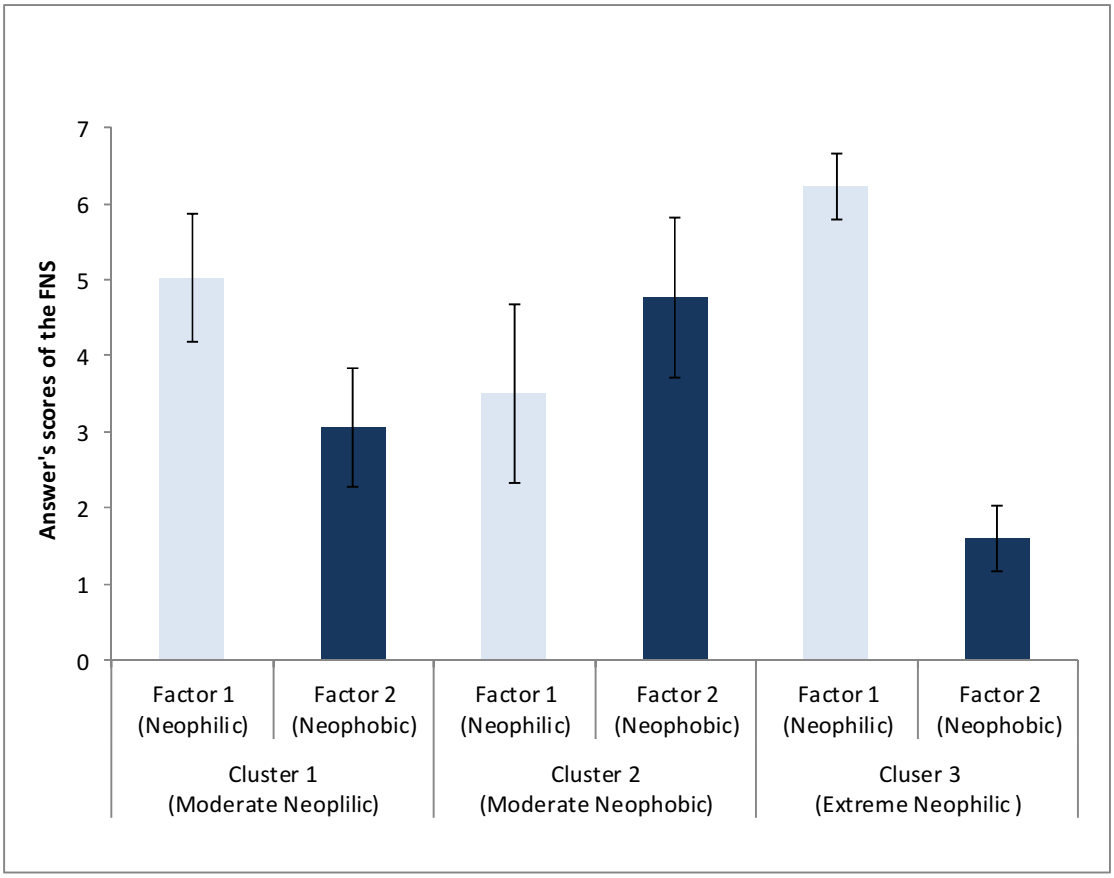

Figure 2: Mean scores in the Portuguese version of the Food Neophobia Scale (FNS) in each cluster by factor 1 and factor 2.

Bars represent means and lines the respective standard deviation.

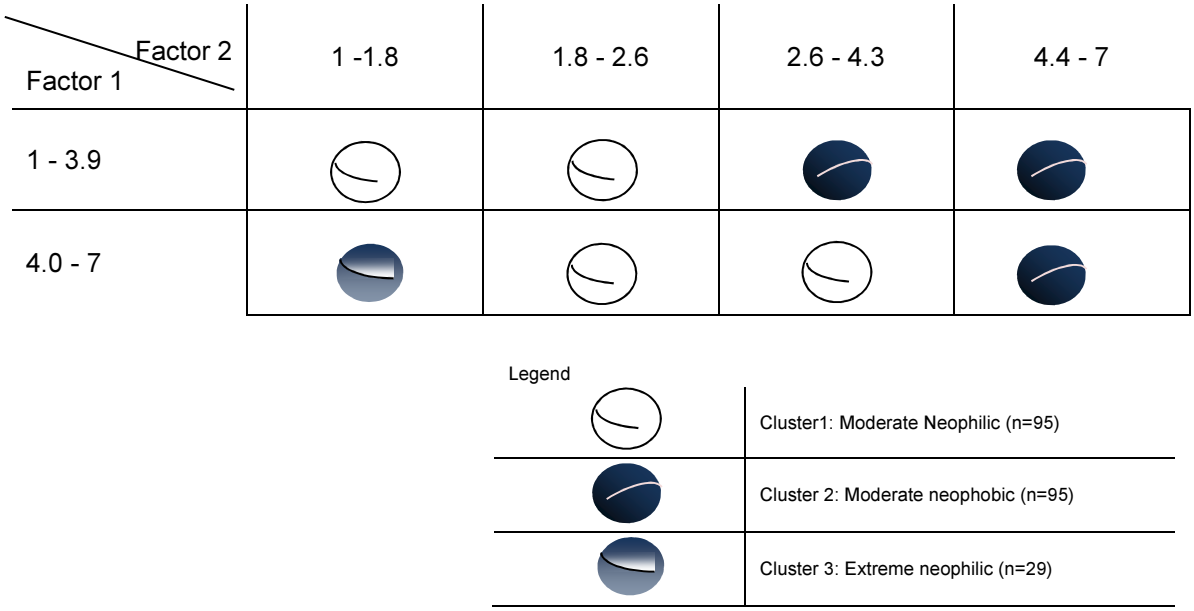

Figure 3: Classification tree showing the cut-off points in the two subscales (factor 1 and factor 2) that discriminate each cluster identified.

Appendix

Description of correspondence between the original items of the Food Neophobia Scale (Pliner and Hobden, 1992) and the Portuguese version of the Food Neophobia Scale (P-FNS).

Items of the original FNS	Items of the P- FNS
1. I am constantly sampling new and different foods. (R)	1. Estou constantemente a experimentar alimentos novos e diferentes. (R)
2. I don't trust new foods.	2. Não confio em alimentos novos.
3. If I don't know what's in a food, I won't try it.	3. Se não souber o que está num alimento/ comida, eu não experimento.
4. I like foods from different countries. (R)	4. Gosto de alimentos/comidas de diferentes países. (R)
5. Foreign food looks too weird to eat.	5. Os alimentos/comidas de outros países parecem muitos estranhos para se comer.
6. At dinner parties, I will try a new food. (R)	6. Em jantares de festa, eu costumo experimentar novos alimentos/comidas. (R)
7. I am afraid to eat things I have never had before.	7. Receio experimentar coisas que nunca comi antes.
8. I am very particular about the foods I will eat.	8. Sou muito exigente com os alimentos/ comidas que vou comer.
9. I will eat almost anything. (R)	9. Eu como quase de tudo. (R)
10. I like to try new ethnic restaurants. (R)	10. Eu gosto de experimentar novos restaurantes étnicos (cozinha internacional. (R)

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R- Reversed item

Paper II

Association between maternal Food Neophobia and the infant's milk feeding method in early life

[To be submitted]

Association between maternal Food Neophobia and the infant's milk feeding method in early life

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Abstract

Background: Food neophobia can impact mother's diet quality and subsequent infant's food preferences. Pregnancy is a state of psychosocial changes that can favor food neophobia, thus we put the hypothesis that maternal food neophobia could influence feeding practices, such as the milk feeding method used with infants. The objective of this study was to evaluate the association between maternal food neophobia scores and the milk feeding method chosen (breast or bottle milk) for infant's feeding during the first 3 months of life.

Methods: Participants were pregnant women who were in their final trimester of pregnancy and mothers of infants less than 6 months old (n=219) who were asked to take part of the Taste intervention study (HabEat project: http://www.habeat.eu/). Data on socio-demographic, anthropometric and behavioural features, occurrences during pregnancy and the feeding method intention were obtained by a self-completed questionnaire. Mothers also self-completed the Portuguese version of the Food Neophobia Scale (P-FNS), previously validated. A sub-sample of mothers were re-evaluated approximately 6 months after birth (n=105) and self-completed a questionnaire that provided information on the actual feeding method chosen for infants during the first 3 months. The associations between maternal food neophobia scores and the planned and actual milk feeding method were assessed by unconditional logistic regression (odds ratio and respective 95% confidence intervals - OR, 95%CI), after adjusting for maternal age, education, marital status, fruit and vegetables intake and nausea sickness during pregnancy.

Results: Mothers with higher food neophobia scores had approximately 50% more likelihood of planning to feed their babies exclusively with breast milk during the first week of infant's life (OR=1.46, 95%CI: 1.01 – 2.13). In multivariate analyses, the association was no longer significant, but remained strong (OR=1.36, 95%CI: 0.89 - 2.06). No significant associations were found neither between the food neophobia scores and the planned milk feeding methods for longer periods (first month and first 3 months) nor the actual method used to feed the infants during the first 3 months of life (OR=0.99 95%CI: 0.75 – 1.30).

Conclusion: Although more neophobic mothers might plan feeding infants exclusively with breast milk in the short time, no robust associations were observed between food neophobia scores and the planned milk feeding method for longer periods, nor the actual method used to feed infants in early stages of life.

Abreviations

BMI: body mass index

OR: odds ratio

P-FNS: Portuguese Food Neophobia Scale

R: Reversed

SD: standard deviation

Introduction

During pregnancy a proper maternal diet is essential to meet with increased nutritional needs and metabolic demands of mother and fetus (Picciano 2003) and can greatly impact health status and life expectancy of both (Roseboom, de Rooij et al. 2006; Le Clair, Abbi et al. 2009; Abu-Saad and Fraser 2010). Likewise, a maternal adequate nutritional status during lactation is important, influencing normal growth and infant's development (Haileslassie, Mulugeta et al. 2013). In fact, prenatal flavor experiences, transmitted by the amniotic fluid or mother's milk, can modify subsequent liking and influence infant's food preferences (Beauchamp and Mennella 2011) and could increase food acceptance of the same flavors at the weaning process (Cooke and Fildes 2011). Breast milk is commonly perceived as the ideal source of nutrition for infant's feeding, particularly at the first 6 months of life (Kramer and Kakuma 2002). Breast milk is characterized by a great variability of flavor experiences, and compared with bottle fed, breast fed infants experience a wide range of flavors that depend directly on the dietary choices made by the mother (Beauchamp and Mennella 2011). Despite its advantages and recommendations, the rates of breastfeeding are generally low and high rates of abandonment have been described including in Portugal (Aguiar and Silva 2011).

During pregnancy, significant physiological, psychological and social changes occur (Abduljalil, Furness et al. 2012) that require adaption of pregnant woman. Food choices are influenced by environmental factors such as cultural food practices and beliefs, internal factors such as food cravings and food aversions, and some digestive disorders such as reflux, nausea and vomiting that together may influence the intake of certain foods (Forestell and Mennella 2008; Kramer, Bowen et al. 2013). During this period, dietary intake is particularly perceived as important, because pregnant woman needs to ensure an adequate food intake to satisfy the additional energy requirements and, at the same time, needs to be caution, avoiding potentially toxic and hazardous food.

The reluctance to eat unfamiliar foods is called as Food Neophobia, which can strongly influence diet's quality (Knaapila, Tuorila et al. 2007). Individuals that present food neophobia are more likely to have poor dietary variety and a lower consumption of vegetables (Falciglia, Couch et al. 2000; Cooke, Carnell et al. 2006; Dovey, Staples et al. 2008). Some studies also reported small but significant relations between the food neophobia scores and anxiety emotionality traits in children and adults (Pliner and Hobden 1992; Galloway, Lee et al. 2003). Pliner and Hobden examining temperament in children found that emotionality was significantly related to food neophobia (Pliner

and Hobden 1992). Galloway *et al* found a highly significant correlation between a trait measure of food neophobia and anxiety (Galloway, Lee et al. 2003).

In fact, pregnancy is considered as a stage of great vulnerability, and is associated with frequent mood changes, fatigue, exhaustion, sleepiness, and depressive reactions (Bjelica and Kapor-Stanulovic 2004). These ambivalent and contradictory feelings can predispose pregnant woman to a higher stage of anxiety. For these reasons and given that food neophobia is a highly hereditable personality trait (Cooke, Haworth et al. 2007; Knaapila, Tuorila et al. 2007) we hypothesized that physiological and psychosocial changes occurring during pregnancy can predispose the more neophobic women to express a higher neophobic response during this stage. Although pregnancy can be a sensitive period for more neophobic responses, the existing information is still very scarce, and to our knowledge no study has evaluated neophobia in pregnant women.

Since food neophobia can impact mother's diet quality and subsequent infant's food preferences, and since pregnancy is a state of psychosocial changes that can favor food neophobia, we put the hypothesis that maternal food neophobia could influence the planning of the milk feeding method and their actual feeding during the first months of life. Thus, our aim was to evaluate the association between maternal food neophiba scores and the milk feeding method chosen (breast or bottle milk) for early infant's feeding.

Key-words: Food Neophobia, pregnancy, breastfeeding

Methods

Participants

Participants were pregnant women who were in their final trimester of pregnancy and mothers of infants less than 6 months old (n=219). Pregnant women were consecutively approached between April-July 2011, before their attendance to medical visits in two hospitals from Porto (main public hospital and private antenatal clinic), and they were invited to take part in the baseline evaluation of the Taste intervention study, included in the HabEat project that aims to determine factors and critical periods in food habit formation and breaking in early childhood in several European countries (more detailed information could be find at http://www.habeat.eu/).

All participants signed a consent form to participate in the study. The research protocol was approved by the local ethical committee (Ethical committee for Health of the São João Hospital/ University of Porto Medical School) and the study procedures complied with the Helsinki Declaration and the current national legislation.

Data collection

Mothers completed questionnaires about themselves and their infants at baseline and at a follow-up (~6 months after birth).

At baseline the questionnaire provided information on: i) socio-demographic information, such as age (grouped into <25; 25-30; >30 years), education (<9; 9-12; ≥12 years) and marital status (married vs. not married); ii) behavioural features, such as smoking during pregnancy (smoker vs. non-smoker) and fruit and vegetables intake (<5 vs. ≥5 portions/day); iii) anthropometrics, such as self-reported weight and height before pregnancy to calculate the body mass index (BMI) (BMI grouped into <25.0; 25.0-29.9; ≥30.0 kg/m^2); iv) occurrences during pregnancy, such as nausea sickness, health problems, food restriction, food cravings, gone off foods; v) perinatal conditions: primipara (yes vs. no). Mothers were also asked about their intention to feed their babies during the first week, first month and first 3 months and answers were categorized into exclusively breastfeeding vs. not exclusively breastfeeding (bottle feeding or both). Mothers also gave their opinion about previous statements of mothers about infant's feeding in a 5-point Likert scale (1= totally disagree and 5=totally agree) (questions are described in detail in table 3).

The questionnaire administered during the follow-up evaluation provided information about mother's feeding routines. In this questionnaire mothers were asked about which feeding methods they have used in the first 3 months: a) breastfeeding only, b) mostly breastfeeding with some bottle-feeding, c) Equally breastfeeding and

bottle-feeding, d) mostly bottle-feeding and some breastfeeding, e) almost all bottle-feeding f) bottle-feeding only and g) other, these variables were recode into exclusive breastfeeding and not exclusive breastfeeding.

The Food Neophobia Scale

At baseline, mothers self-completed the Portuguese version of the Food Neophobia Scale (P-FNS), having as reference period the third trimester of gestation. The 10 items of the P-FNS appear in the same order as in the original version (scores ranging from 10 to 70) (see appendix). Before analysis, the scores of 5 items marked with (R) were reversed to obtain ratings in the same direction (1). The total score could range from 10 to 70, as the original one.

In a previous study from our research group, the FNS was translated into Portuguese and the psychometric properties of the Portuguese version were tested (submitted to Appetite, currently under revision). In this validation study, a two-factor model solution was obtained, explaining approximately 51% of the total variance, with factor 1 and factor 2 explaining 26.3% and 24.5% of the total variance, respectively. Factor 1 represents a less neophobic trait (more willingness to try new foods) and Factor 2 represents a more neophobic trait (less willingness to try new foods). This previous study supports that the scale has basic requirements of a valid and reliable measure of food neophobia during pregnancy.

Statistical analysis

The distribution of maternal food neophobia scores (by factor 1 and factor 2) according to socio-demographic characteristics (table 1), events or feelings that mothers felt during pregnancy (table 2), feelings that other mothers have said about feeding infants (statements a-g) and the planned milk feeding method for infants (statements h-j) (table 3) are presented as mean and standard deviation (SD). Mean comparisons between two independent samples were assessed using the Student's t test or analysis of variance (ANOVA) for more than two groups. Proportions were compared using the chi-square test or Fisher exact test, when appropriate.

Unconditional binary logistic regression models were run to assess the magnitude of the association between maternal food neophobia scores (factor 1 and factor 2) and the feelings about feeding infants (statements a-g), the planned milk feeding method (statements h-j) and the actual milk method chosen for infant's feeding (statement k) (table 4). Models were presented unadjusted (model 1) and after adjustment for variables that in a first-step were significantly associated with maternal

FN: maternal education level, age, fruit and vegetables intake and nausea sickness experience during pregnancy.

Significance level was set at 5%. The analyses were performed using the software Statistical Package for Social Sciences (SPSS), version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Table 1 describes the distribution of maternal food neophobia scores according to mother's characteristics. Maternal food neophobia scores significantly differ according to education; as higher the number of schooling years, the higher were the scores in factor 1 (neophilic factor) and the lower were the scores in factor 2 (neophobic factor). Married women had also significantly lower scores in the neophobic factor compared with those not married (3.52 (SD=1.43) vs. 4.15 (SD=1.18), p=0.026). Women who reported a consumption of fruit and vegetables of 5 or more portions a day in the previous 3 months had significantly higher scores in factor 1 (<5 vs. ≥ 5 portions/day: 4.37 (SD=1.30) vs. 4.78 (SD=1.44), p=0.031) and lower scores in factor 2, although they were not significantly different from the scores of low consumers of fruit and vegetables.

No significant differences in food neophobia scores were observed according to the remaining characteristics, but a clear tendency was observed with maternal age; as older, the higher the scores in the neophilic factor and the lower the scores in the neophobic factor. On the contrary, obese women had lower scores in the neophilic factor and higher scores in the neophobic factor; the latter was also observed for smokers during pregnancy. Mothers for the first time reported higher neophobic scores.

Table 2 shows the distribution of maternal food neophobia scores according to events and feelings that they felt during pregnancy. A significant difference in food neophobia scores was observed only according to nausea or sickness; those who reported this experience had significantly higher neophobic scores (yes vs. no: 3.76 (SD=1.36) vs. 3.34 (SD=1.48), p=0.031). No significant differences in maternal food neophobia scores were found according to the remaining events or feelings felt during pregnancy. However, it was observed that women who reported health problems had lower scores in factor 1 and higher scores in the neophobic factor (factor 2), and that women who reported food cravings and gone of foods also presented slightly higher neophobic scores.

Table 3 presents the distribution of maternal food neophobia scores according to feelings about infant's feeding. In general, women who agreed with statements that reflect a more positive attitude towards breastfeeding (table 3; statements b, d and f), had higher scores in the neophobic factor (factor 2), compared with those who disagree. In turn, who agreed with statements that reflect a less positive attitude toward breastfeeding (i.e. breastefeeding is difficult, hampers mother's freedom or bottle-feeding is better) (table 3; statements a, e and g) had, in general, lower scores in the neophobic factor and higher food neophobia scores in the neophilic factor. The only

significant difference found in the food neophobia scores was obtained for women who agreed with the statement “Bottle-feeding allows the father to share the care of the baby more”, who reported significantly higher neophobic scores (agree vs. not agree: 3.74 (SD=1.39) vs. 3.32 (SD=1.45), p=0.042).

Table 4 summarizes the maternal food neophobia scores according to the planned milk feeding method and the actual method used for infant’s feeding during the first 3 months of life. Mothers who were planning breastfeeding her babies exclusively had, in general, significantly higher food neophobia scores, but the results were only significant when the planning was for the first week (exclusively breastfeeding vs. not exclusively breastfeeding: 3.64 (SD=1.42) vs. 2.93 (SD= 1.22), p=0.042). Regarding to the actual feeding method chosen to feed the infants during the first 3 months, mothers who exclusively breastfed had slightly higher scores in factor 1 (neophilic factor), but with no statistical significance.

Table 5 describes the univariate and multivariate associations between the food neophobia factors (neophilic and neophobic) and the feelings about feeding infants (statements a-g), the planned milk feeding method (statements h-j) and the actual milk feeding method chosen for infant’s feeding.

In univariate analysis, women with higher scores in factor 2 (neophobic factor) were more likely to agree with the statement “Bottle-feeding allows the father to share the care of the baby more” (OR=1.23, 95%CI: 1.01 – 1.51). After adjustment for potential confounders (model 2), the association lost the statistical significance. Additionally, those women with higher neophobic scores had also more probability of agreeing with statements that reflect a more positive attitude towards breastfeeding (statements d and f), but the associations were not significant.

Regarding to planned milk feeding method, mothers with higher neophobic scores had more probability of planning feeding their babies exclusively with breast milk, but the association was only significant when considering the planning for the first week. Thus, mothers with higher neophobic scores had about 50% more likelihood of planning to feed their babies with breast milk only (OR=1.46, 95%CI: 1.01 – 2.13). After adjustment for potential confounders (model 2), the association was no longer significant, but remained strong (OR=1.36, 95%CI: 0.89 - 2.06).

No significant associations between the food neophobia scores and the actual method used to infant’s feeding during the first 3 months of life were found (OR=0.99, 95%CI: 0.75-1.30).

Discussion

To our knowledge this was the first study conducted among pregnant women exploring the association of maternal food neophobia with parental feeding practices, namely the milk feeding method selected to feed infants in the first stages of life. Although more neophobic mothers might plan feeding infants exclusively with breast milk in the short time (i.e. first week), no robust associations were observed neither between food neophobia scores and the planned milk feeding method for longer periods nor the actual method used to feed infants during the first 3 months.

Pregnancy is a critical period to mothers, when physiological, psychological and social changes occur (Abduljalil, Furness et al. 2012) and it is a period of great vulnerability that can predispose women to a higher state of anxiety (Bjelica and Kapor-Stanulovic 2004). Based on that, we hypothesized that pregnancy could be a sensitive period for more adaptive neophobic responses. Accordingly, in the present study, more neophobic women (higher scores in factor 2) were 50% more likely to choose exclusive breastfeeding, compared to not exclusively breastfeeding, as the feeding method for the first week of infant's life. These data suggest inherent protective effect of maternal food neophobia by breastfeeding their infants. Exclusive breastfeeding is described as an ideal method for feeding infants during the first 6 months of life (Kramer and Kakuma 2002). However, when we explored the association between maternal food neophobia and the actual milk feeding method, we did not find any association. In fact, when we compared the planned feeding method with the actual feeding chosen for infants we found that from those who planned to exclusive breastfeed infants during the first 3 months, only 60% actual stick with that plan (50.7% vs. 43.4%, p=0.022). In this way, it is possible to preclude some disagreement between what was planned and what was actually made. These differences may be indicative of the complex interplay of factors that influence the decision to breastfeed. Breastfeeding attitudes, health professional advice and familial, work and societal environment are factors that could influence the mother's decision of breastfeeding, impacting the experience and duration of breastfeeding (Arora, McJunkin et al. 2000; Atchan, Foureur et al. 2011).

In accordance, when analysing the association of maternal food neophobia with statements regarding the feeding methods, those mothers with higher neophobic scores had more probability of agreeing that breastfeeding is better to infants, with higher probability of agreeing with the statement "breast milk is better for the baby" and "a mother who does not breast feed is inferior", which can suggest that more neophobic pregnant women are, in theory, more concerned with infant's feeding, but do not put in

practice their beliefs, and also have in general worse health behaviours (such as a low consumption of fruit and vegetables).

In this study, maternal food neophobia scores were also studied according to socio-demographic characteristics. It was found that more educated women had significantly higher scores in factor 1 (representing a more neophilic trait) and lower scores in factor 2 (representing a more neophobic trait). Similar results were found in the Tourila et al. investigation, showing that a high education predicted lower food neophobia among an adult population (Tuorila, Lähteenmäki et al. 2001). Other studies suggested this same inverse association through the effect of parental education on children's food neophobia scores. Children, whose parents were less educated, had higher neophobic mean scores than those children whose parents were more educated (Tuorila, Lähteenmäki et al. 2001; Schickenberg, van Assema et al. 2008; Mustonen, Oerlemans et al. 2012). Education is likely to enhance the access and exposure to various stimuli and events and it can contribute to low neophobia responses.

Although food neophobia is generally defined as personality trait that influences the willingness to try new foods (Pliner and Melo 1997) (and so a relatively enduring trait), it is not a static condition and may vary through life. Food neophobia can change with age being apparently minimal through infancy, picking at age of 4 years-old, tending to decline thereafter (Birch, McPhee et al. 1987). In this study, despite the limited age range of mothers, neglecting the extreme categories (the younger and the older women), a potential inverse relation was suggested between food neophobia and age; the older women had higher scores in factor 1 (representative of a more neophilic trait) and lower scores in factor 2 (representative of a more neophobic trait).

Furthermore, women who reported a consumption less than 5 portions per day of fruit and vegetables had significantly lower scores in factor 1 and higher scores in factor 2 (high neophobia). Earlier investigations demonstrated that food neophobia was a good predictor of lower food acceptance, lower diet variety, and lower consumption of fruit and vegetables (Skinner, Carruth et al. 2002; Cooke, Wardle et al. 2004; Cooke, Carnell et al. 2006; Russell and Worsley 2008). Skinner found that food neophobia was associated with reduced preferences for all food groups, especially for vegetables (Skinner, Carruth et al. 2002). Cooke et al. found that food nephobia in young children was strongly related to a lower consumption of fruit and vegetables, also in a study with 4-5 year old children, the same authors demonstrated that food neophobia was associated with lower consumption of fruit, vegetables and protein foods (Cooke, Wardle et al. 2004; Cooke, Carnell et al. 2006). In the same line with these investigations, Russell found that food neophobia appears to have a differential effect

on diet, with the strongest effect for vegetables, meat and fruit (Russell and Worsley 2008).

In this study, women who reported having experienced nausea or sickness during pregnancy had significantly higher scores in the neophobic factor (factor 2). An explanation for this could be because when individuals experience nausea or vomiting this may result in an increase reluctance of trying new foods (Cooke, Haworth et al. 2007). According to Nordin, nauseas may have an effect on the formation of learned food aversions, and thus contributing for general food rejection (Nordin, Broman et al. 2004).

Some limitations of the present study should be highlighted. Our results are focused on food neophobia during pregnancy, so the generalization of results for other populations should be made with caution. We hypothesized that pregnancy, for the significant physiological, psychological and social changes that brings can lead pregnant women to a more neophobic behavior, but since we were not able to evaluated maternal food neophobia before pregnancy, behavioral changes could not be established. Furthermore, the relatively small sample size used in this study may therefore have been statistically under-powered to detect differences, reducing the precision of our estimates.

This study suggests that food neophobia during pregnancy does not seem to be associated with the milk feeding method chosen for early infant's feeding. However, maternal food neophobia could have an important effect in other feeding practices and pregnancy-related outcomes that should be explored in future longitudinal research. Additionally, future research should clarify if pregnancy exacerbates FN, and if confirmed, strategies should be conducted to minimize potential adverse effects of FN, already shown in other population groups.

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Table 1. Distribution of maternal food neophobia scores (mean and standard deviation by factor1 and factor2) according to their sociodemographic, anthropometric and behavioural characteristics

	n (%)	Factor 1 (Neophilic)	Factor 2 (Neophobic)
	219	Mean (SD)	Mean (SD)
Maternal education			
1-9 schooling years	45 (20.5)	4.00 (1.28)	4.09 (1.07)
10-12 schooling years	58 (26.5)	4.65 (1.31)	3.72 (1.59)
>12 schooling year	112 (51.1)	4.68 (1.38)	3.36 (1.39)
Missing	4 (1.8)		
p-value		**0.013**	**0.011**
Maternal age			
≤25 years	48 (21.9)	4.42 (1.40)	3.84 (1.27)
26-34 years	106 (48.4)	4.46 (1.30)	3.63 (1.56)
≥35 years	65 (29.7)	4.69 (1.45)	3.38 (1.28)
Missing	0		
p-value		0.483	0.225
Marital Status			
Married	189 (80.4)	4.55 (1.41)	3.52 (1.43)
Not married	28 (11.9)	4.30 (1.05)	4.15 (1.18)
Missing	2 (0.9)		
p-value		0.365	**0.026**
Pre-pregnancy Body Mass Index			
<25.0 kg/m^2	161 (73.5)	4.55 (1.39)	3.59 (1.46)
25.0-29.9 kg/m^2	35 (16.0)	4.58 (1.31)	3.42 (1.25)
≥30 kg/m^2	21 (9.6)	4.26 (1.36)	4.00 (1.23)
Missing	2 (0.9)		
p-value		0.635	0.322
Primipara			
Yes	126 (57.5)	4.52 (1.45)	3.70 (1.45)
No	92 (42.0)	4.52 (1.26)	3.50 (1.39)
Missing	1 (0.5)		
p-value		0.989	0.307
Smoking status during pregnancy			
Smoker	19 (8.70)	4.69 (1.20)	4.01 (1.30)
No smoker	199 (84.7)	4.50 (1.39)	3.57 (1.43)
Missing	1 (0.5)		
p-value		0.570	0.197
Fruit and vegetables intake in the previous 3 months			
< 5 portions/ day	128 (58.4)	4.37 (1.30)	3.66 (1.43)
≥ 5 portions/ day	85 (38.8)	4.78 (1.44)	3.48 (1.42)
Missing	6 (2.7)		
p-value		**0.031**	0.362

SD: standard deviation

Table 2. Distribution of maternal food neophobia scores (mean and standard deviation by factor1 and factor2) according to events and feelings that mothers felt during pregnancy

	n (%)	Factor 1 (Neophilic) Mean (SD)	Factor 2 (Neophobic) Mean (SD)
Nausea or Sickness			
Yes	132 (60.3)	4.40 (1.33)	3.76 (1.36)
No	82 (37.4)	4.71 (1.41)	3.34 (1.48)
Missing	5 (2.3)		
p-value		0.115	**0.031**
Self-reported major health problems			
Yes	32 (14.6)	4.46 (1.60)	3.86 (1.40)
No	180 (82.2)	4.53 (1.33)	3.55 (1.40)
Missing	7 (3.2)		
p-value		0.782	0.250
Food Restriction			
Yes	36 (16.4)	4.84 (1.49)	3.45 (1.43)
No	176 (80.4)	4.47 (1.35)	3.61 (1.43)
Missing	7 (3.2)		
p-value		0.140	0.536
Food Cravings			
Yes	66 (30.1)	4.67 (1.46)	3.67 (1.49)
No	150 (68.5)	4.67 (1.33)	3.55 (1.39)
Missing	3 (1.4)		
p-value		0.314	0.553
Gone off foods			
Yes	69 (31.5)	4.61 (1.43)	3.69 (1.44)
No	145 (66.2)	4.49 (1.35)	3.53 (1.42)
Missing	5 (2.3)		
p-value		0.562	0.450

SD: standard deviation

Table 3. Distribution of maternal food neophobia scores (mean and standard deviation by factor1 and factor2) according to feelings about infant's feeding (statements a-g)

	n (%) 219	Factor 1 (Neophilic) Mean (SD)	Factor 2 (Neophobic) Mean (SD)
a. Breast-feeding stops a mother from having the freedom to do what she wants			
Agree	32 (14.6)	4.79 (1.21)	3.40 (1.41)
Disagree	183 (83.6)	4.49 (1.39)	3.62 (1.43)
Missing	4 (1.8)		
p-value		0.263	0.425
b. Breast-feeding gives the mother a special relationship with her baby			
Agree	209 (95.4)	4.54 (1.37)	3.60 (1.40)
Disagree	7 (3.2)	4.43 (1.79)	3.32 (1.95)
Missing	3 (1.4)		
p-value		0.830	0.609
c. Bottle-feeding allows the father to share the care of the baby more			
Agree	141 (64.4)	4.55 (1.36)	3.74 (1.39)
Disagree	75 (34.2)	4.51 (1.40)	3.32 (1.45)
Missing	3 (1.4)		
p-value		0.856	**0.042**
d. Breast milk is better for the baby			
Agree	212 (96.8)	4.53 (1.36)	3.59 (1.41)
Disagree	4 (1.8)	4.40 (2.30)	3.06 (1.61)
Missing	3 (1.4)		
p-value		0.850	0.462
e. Bottle-feeding is more convenient for the mother			
Agree	27 (12.3)	4.46 (1.50)	3.44 (1.43)
Disagree	190 (86.8)	4.54 (1.36)	3.61 (1.42)
Missing	2 (0.9)		
p-value		0.772	0.547
f. A mother who does not breast feed is inferior			
Agree	8 (3.7)	4.27 (1.80)	4.47 (1.04)
Disagree	208 (95.0)	4.55 (1.35)	3.56 (1.42)
Missing	3 (1.4)		
p-value		0.572	0.075
g. Breast-feeding is difficult			
Agree	35 (16.0)	4.89 (1.39)	3.36 (1.45)
Disagree	177 (80.8)	4.45 (1.36)	3.67 (1.40)
Missing	7 (3.2)		
p-value		0.079	0.226

SD: standard deviation

Table 4. Distribution of maternal food neophobia scores (mean and standard deviation by factor1 and factor2) according to the planned milk feeding method (statements h-j) and the actual milk method chosen for infant's feeding (statement k)

	n (%) 219	**Factor 1 (Neophilic)** Mean (SD)	**Factor 2 (Neophobic)** Mean (SD)
h. Planning to feed the baby for the first week			
Exclusively breastfeeding	196 (89.5)	4.57 (1.34)	3.64 (1.42)
Not exclusively breastfeeding	18 (8.2)	4.46 (1.55)	2.93 (1,22)
Missing	5 (2.3)		
p-value		0.738	**0.042**
i. Planning to feed the baby for the first month			
Exclusively breastfeeding	189 (86.3)	4.58 (1.34)	3.63 (1.43)
Not exclusively breastfeeding	24 (11.09	4.41 (1.46)	3.11 (1.23)
Missing value	6 (2.7)		
p-value		0.571	0.091
j. Planning to feed the baby for the first 3 months			
Exclusively breastfeeding	174 (79.5)	4.54 (1.39)	3.66 (1.33)
Not exclusively breastfeeding	40 (18.3)	4.50 (1.32)	3.22 (1.44)
Missing	5 (2.39		
p-value		0.863	0.074
K. Actual feeding method during the first 3st month	**n (%)** 105		
Exclusively breast milk	54 (24.7)	4.66 (1.50)	3.54 (1.33)
Not exclusively breast milk	51 (23.3)	4.35 (1.48)	3.56 (1.50)
Missing	0	0.282	0.942
p- value			

Table 5. Associations between maternal food neophobia scores (factor 1 and factor 2) and the feelings about feeding infants (statements a-g), the planned milk feeding method (statements h-j) and the actual milk method chosen for infant's feeding (statement k).

	Model 1 [a] OR (95% CI)	Model 2 [b] OR (95% CI)
a. Agrees that breast-feeding stops a mother from having the freedom to do what she wants		
Factor 1 (Neophilic)	1.18 (0.89 - 1.56)	1.20 (0.88 – 1.64)
Factor 2 (Neophobic)	0.90 (0.69 – 1.17)	0.83 (0.62 – 1.13)
b. Agrees that breast-feeding gives the mother a special relationship with her baby		
Factor 1 (Neophilic)	1.06 (0.62 - 1.83)	1.19 (0.67 – 2.12)
Factor 2 (Neophobic)	1.15 (0.67 – 1.99)	0.99 (0.55 – 1.79)
c. Agrees that bottle-feeding allows the father to share the care of the baby more		
Factor 1 (Neophilic)	1.02 (0.83 - 1,25)	1.08 (0.87 – 1.35)
Factor 2 (Neophobic)	**1.23 (1.01 – 1.51)***	1.15 (0.92 – 1.44)
d. Agrees that breast milk is better for the baby		
Factor 1 (Neophilic)	1.07 (0.52 – 2.19)	1.06 (0.51 – 2.19)
Factor 2 (Neophobic)	1.32 (0.63 – 2.78)	1.56 (0.64 – 3.80)
e. Agrees that bottle-feeding is more convenient for the mother		
Factor 1 (Neophilic)	0.96 (0.71 – 1.28)	0.93 (0.67 – 1.28)
Factor 2 (Neophobic)	0.92 (0.69 – 1.22)	0.95 (0.70 – 1.31)
f. Agrees that a mother who does not breast feed is inferior		
Factor 1 (Neophilic)	0.86 (0.52 – 1.44)	0.84 (0.50 – 1.40)
Factor 2 (Neophobic)	1.59 (0.94 – 2.67)	1.76 (1.02 – 3.05)
g. Agrees that breast-feeding is difficult		
Factor 1 (Neophilic)	1.29 (0.97 – 1.70)	1.22 (0.90 – 1.66)
Factor 2 (Neophobic)	0.85 (0.65 - 1.11)	0.88 (0.66 – 1.19)
h. Planning to feed the baby for the first week exclusively with breast milk (vs. not exclusively)		
Factor 1(Neophilic)	1.06 (0.74 – 1.52)	1.17 (0.80 – 1.70)
Factor 2(Neophobic)	**1.46 (1.01 – 2.13)***	1.36 (0.89 – 2.06)
i. Planning to feed the baby for the first month exclusively with breast milk (vs. not exclusively)		
Factor 1(Neophilic)	1.09 (0.80 – 1.49)	1.16 (0,83 – 1.62)
Factor 2 (Neophobic)	1.31 (0.96 – 1.80)	1.31 (0.92 – 1.87)
j. Planning to feed the baby for the first three months exclusively with breast milk (vs. not exclusively)		
Factor 1(Neophilic)	1.02 (0.80 – 1.31)	1.06 (0.81 – 1.39)
Factor 2 (Neophobic)	1.26 (0.98 – 1.62)	1.26 (0.95 – 1.67)
k. Actual feeding method with exclusively breast milk during the first 3 months (vs. not exclusively)*		
Factor 1(Neophilic)	1.16 (0.89 – 1.50)	1.17 (0.88 – 1.54)
Factor 2 (Neophobic)	0.99 (0.75 – 1.30)	0.95 (0.70 – 1.29)

Abbreviations: OR, odds ratio; 95%CI, 95% confidence intervals. Significant associations are taped in bold-type.

[a] Model 1, crude. [b] Model 2, adjusted for age, educational level, marital status, fruit and vegetables intake, and nausea sickness.

* Evaluated in a follow-up assessment ~6 months after birth.

General Discussion

Food neophobia is a personality trait that influences willingness to consume novel foods and is related with the development of food preferences (Tuorila, Lähteenmäki et al. 2001; Flight, Leppard et al. 2003). To assess reluctance to consume novel foods, Pliner and Hobden developed the food neophobia scale (FNS) using a convenience sample of Canadian college students (Pliner and Hobden 1992). Since the FNS is the most widely used instrument to assess food neophobia (Schickenberg, van Assema et al. 2008), and given cultural differences among countries, it is demanding to test the psychometrics properties of the FNS in other languages, to allow cross-national comparisons of the results.

This thesis had as first objective to translate and test the psychometric properties of a Portuguese version of the FNS (P-FNS) in pregnant women. In this validation study, a two factor model solution was obtained, with factor 1 representing more willingness to try new foods and factor 2 representing less willingness to try new foods (both explaining 51% of the total variance). Although the original scale was one-dimensional (Pliner and Hobden 1992), several studies conducted in adult populations have reported that the FNS did perform well with a single dimension (Tuorila, Lähteenmäki et al. 2001; Ritchey, Frank et al. 2003; Fernández-Ruiz 2013), thus a single global score should not be considered in analysis. This result was confirmed by estimating the global fitness of the model using fit indexes, and the values obtained indicate a good global of fitness of the P-FNS. Thus, the results of the present study suggest that the Portuguese version of FNS is a valid and reliable measure of food neophobia during pregnancy.

Nevertheless, it should be highlighted that possible due to the translation performed or due to the interpretation of that question, one item had to be excluded from analysis (i.e. item 8), since it does not have a meaningful part on the whole domain (factor loading <0.4), supporting that it is important to test the FNS in each population setting. To our knowledge, the validation studies have been conducted among children and adult populations, with no reference to pregnant women. Thus, this study allows rectifying a lack of available instruments to assess and explore food neophobia in Portuguese pregnant women. Another point that should be highlighted is that to compare the final score across studies we should be aware of the total number of items considered or only compare the mean score (already divided by the total number of items).

Moreover, most studies analyze food neophobia scores based on the factor loadings of the two factors identified (or one factor, when appropriate). According to this, each individual is represented in both factors, but with higher scores in one of them. To simplify future data analysis, and using a clustering-based approach, we

identified three clusters of food neophobia, representing three mutually-exclusive groups of women sharing the same pattern: moderate neophilic, moderate neophobic, and extreme neophilic. The classification tree allowed establishing the cut-off points in the two subscales (factor 1 and factor 2) that discriminate each cluster identified, and could thus be reproduced by other investigators who want to use the P-FNS among pregnant women.

Having a valid tool able to assess food neophobia during pregnancy (accomplished with study 1 of this thesis), we were able to assess the effect of maternal food neophobia in parental feeding practices, such as the milk feeding method chosen for infant's feeding in early stages of life (study 2). To assess this kind of association seems appropriate because pregnancy is a state where significant physiological, psychological and social take place (Abduljalil, Furness et al. 2012) and it is a period of great vulnerability that can predispose women to a higher state of anxiety (Bjelica and Kapor-Stanulovic 2004). This range of changes can favor the expression of a more neophobic behavior, so we hypothesize that that pregnancy could be a sensitive period for more adaptive neophobic responses. Food neophobia during pregnancy is very important, given the fact that food neophobia strongly influences diet's quality and the establishment of children's food preferences (Falciglia, Couch et al. 2000; Cooke, Carnell et al. 2006; Dovey, Staples et al. 2008; Beauchamp and Mennella 2011), and might also influence the planning and the actual milk feeding method chosen for their infants.

In the second study, we found that the more neophobic women were more likely to choose exclusive breastfeeding, compared to not exclusively breastfeeding, as the feeding method for the first week of infant's life. This is suggestive of an inherent protective effect of maternal food neophobia in relation to the decision of breastfeeding their infants. However, when we explored the association between maternal food neophobia and the actual milk feeding method, we did not find any association. In fact, when we compared the planned feeding method with the actual feeding chosen for infants we found that from those who planned to exclusive breastfeed infants during the first 3 months, only 60% actual stick with that plan. In this sense, disagreement between what was planned and what was actually made was observed. These differences may be indicative of the complex interplay of factors that influence the decision to breastfeed. Breastfeeding attitudes, health professional advice and familial, work and societal environment are factors that could influence the mother's decision of breastfeeding, impacting the experience and duration of breastfeeding (Arora, McJunkin et al. 2000; Atchan, Foureur et al. 2011).

In accordance, when analysing the association of maternal food neophobia with statements regarding the feeding methods, those mothers with higher neophobic scores had more probability of agreeing that breastfeeding is better to infants, with higher probability of agreeing with the statement " breast milk is better for the baby" and "a mother who does not breast feed is inferior", which can suggest that more neophobic pregnant women are, in theory, more concerned with infant's feeding, but do not put in practice their beliefs, and also have in general worse health behaviours (such as a low consumption of fruit and vegetables). Earlier investigations demonstrated that food neophobia was a good predictor of lower food acceptance, lower diet variety, and lower consumption of fruit and vegetables in children (Skinner, Carruth et al. 2002; Cooke, Wardle et al. 2004; Cooke, Carnell et al. 2006; Russell and Worsley 2008).

Some limitations of the present study deserve discussion. Women's reports may be affected by their own beliefs in what pregnant women should ideally eat to provide better nutrition to their baby, thus being affected by a social desirability bias. Also, food neophobia could be trimester-specific, and so it would be interesting to administer the P-FNS in each trimester and, additionally, to understand if women's food neophobia is affected by pregnancy, by measuring food neophobia before pregnancy. In fact, our results are focused on food neophobia during pregnancy, so the generalization of results for other populations should be made with caution. It should also be noted that the clusters of FN, identified in the first study, were not used in our second analysis due to the relatively low sample size of this study. By using the continuous factors (and not mutually exclusive groups of women, i.e. clusters) we gain some statistical power; even though it may have been statistically under-powered to detect differences, and have reduced the precision of our estimates.

The FNS was translated into Portuguese and its psychometric properties were tested, supporting that it is a valid and reliable measure of food neophobia during pregnancy. This study also allowed identifying three clusters of neophobic traits, which can be reproduced by other investigators who want to use the P-FNS among pregnant women. With the development of this valid tool it was possible to evaluate the association of food neophobia during pregnancy with the milk feeding method chosen for feed infants during the first 3 months of life. It was found that food neophobia during pregnancy does not seem to be associated with the milk feeding method chosen for early infant's feeding.

Nevertheless, maternal food neophobia could have an important effect in other parental feeding practices and pregnancy-related outcomes that should be explored in future longitudinal research. Additionally, future research should clarify if pregnancy exacerbates FN, and if confirmed, strategies should be conducted to minimize potential adverse effects of FN, already shown in other population groups. Meanwhile, the P-FNS can be a useful tool to be used by health professionals, to identify pregnant women more prone to neophobic behaviors, to be involved in intervention strategies that aimed at improving of food habits and health in general. Although food neophobia is an enduring trait, it is not a static condition and can change in response to environmental experiences such as exposure, modeling, nutritional knowledge, cultural and socio-economic contexts (Birch 1999).

During pregnancy, women may be particular receptive to nutritional counseling and food habits changes. For this, pregnancy should be perceived as an important sensitive period for early flavor and food preferences learning, where mothers should be informed about the consequences of their food choices.

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