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PhD THESIS

Early life diet and child psychomotor and behavioral development: the "Rhea" mother-child cohort in Crete, Greece

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BA Chemistry

MSc Medicinal Chemistry

MSc Public Health Nutrition

Τομέας Κοινωνικής Ιατρικής

Τμήμα Ιατρικής

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ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

Προοπτική μελέτη του ρόλου της διατροφής κατά τη βρεφική και παιδική ηλικία στην ψυχολογική, κινητική και συμπεριφορική ανάπτυξη των παιδιών-Μελέτη Μητέρας Παιδιού Κρήτης, Μελέτη ΡΕΑ

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Στον πατέρα μου...τον μεγαλύτερο υποστηρικτή μου!

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Abstract in Greek

Περίληψη

Εισαγωγή: Γενετικοί και περιβαλλοντικοί παράγοντες έχει βρεθεί πως διαδραματίζουν σημαντικό ρόλο στη διαμόρφωση και ανάπτυξη του εγκεφάλου τόσο κατά την εμβρυική όσο και στη μεταγεννητική ζωή. Η διατροφή αποτελεί έναν από αυτούς τους παράγοντες που μπορεί να επιδρά στην έκφραση των γονιδίων του εγκεφάλου μέσω επιγενετικών μηχανισμών. Στα πρώτα χρόνια της ζωής μέχρι την προσχολική ηλικία, συμβαίνουν σημαντικές αλλαγές στον εγκέφαλο που θέτουν τις βάσεις για τη μετέπειτα συμπεριφορική και γνωσιακή ανάπτυξη του ατόμου. Ερευνητική υπόθεση της παρούσας διατριβής αποτελεί η μελέτη του πιθανού ρόλου της διατροφής μέχρι την προσχολική ηλικία στην παιδική νευροανάπτυξη.

Ειδικοί Στόχοι:

- Να μελετηθεί η διατροφή στη διάρκεια της εγκυμοσύνης διερευνώντας παράγοντες, όπως είναι το χαμηλό βάρος γέννησης, ο πρόωρος τοκετός, και η διάρκεια κύησης, που καθορίζουν την μελλοντική ψυχοκινητική ανάπτυξη του παιδιού.
- Να διερευνηθεί η συσχέτιση της διάρκειας του θηλασμού με την ψυχοκινητική ανάπτυξη των παιδιών.
- Να διερευνηθούν οι διατροφικές συνήθειες στην πρώιμη παιδική ηλικία (2-4 ετών), με τη χρήση σταθμισμένου ημι-ποσοτικού ερωτηματολογίου διατροφής καθώς και το πώς αυτές επηρεάζονται απο διάφορους κοινωνικο-δημογραφικούς παράγοντες.
- Να μελετηθεί η σχέση της διατροφής του παιδιού με την ψυχοκινητική του ανάπτυξη στην ηλικία των 4 ετών.
- Να διερευνηθεί η επίδραση που έχουν οι διατροφικές συμπεριφορές των παιδιών στους διάφορους τομείς της ελλειματικής προσοχής στην ηλικία των 4 ετών.

Μεθοδολογία: Για τη μελέτη της διατροφής στη διάρκεια της εγκυμοσύνης πραγματοποιήθηκε μια μελέτη (case study) σε συνεργασία με 19 Ευρωπαϊκές μελέτες μητέρας-παιδιού με συνολικό πληθησμό 151.880 έγκυες γυναίκες. Στα δεδομένα που συλλέχθηκαν από τις διάφορες χώρες πραγματοποιήθηκε εναρμονισμός και μετανάλυση. Τα δεδομένα για τη μελέτη του ρόλου της διατροφής στην νευροανάπτυξη

των παιδιών στα πρώτα χρόνια της ζωής προήλθαν από τη μελέτη Μητέρας Παιδιού Κρήτης, Μελέτη ΡΕΑ. Χρησιμοποιήθηκαν δεδομένα για περίπου 1.000 έγκυες γυναίκες και των παιδιών τους. Πληροφορίες για τις πρακτικές θηλασμού συλλέχθηκαν με ερωτηματολόγια στους 9 και 18 μήνες ζωής. Η συλλογή των διατροφικών δεδομένων των παιδιών στην ηλικία των 4 ετών πραγματοποιήθηκε με τη χρήση ημι-ποσοτικών ερωτηματολογίων διατροφής. Για την εκτίμηση της διατροφικής συμπεριφοράς των παιδιών χρησιμοποιήθηκε ένα ερωτηματολόγιο (Children’s Eating Behaviour Questionnaire (CEBQ)) που συμπληρώθηκε από τους γονείς, οι οποίοι απάντησαν σε ερωτήσεις που αφορούν σε συμπεριφορές του παιδιού τους σε σχέση με το φαγητό. Ειδικά εκπαιδευμένοι ψυχολόγοι αξιολόγησαν την ψυχοκινητική ανάπτυξη των παιδιών χρησιμοποιώντας τις Κλίμακες βρεφικής και νηπιακής ανάπτυξης (Bayley III) στους 18 μήνες και τις Κλίμακες Εκτίμησης Παιδικών Δεξιοτήτων (McCarthy Scales of Children's Abilities) στην ηλικία των 4 ετών. Επίσης στην ηλικία των 4 ετών έγινε αξιολόγηση ελλειμματικής προσοχής-υπερκινητικότητας των παιδιών (Gilliam, 1995) με κλίμακα που συμπληρώθηκε από τους γονείς αυτών.

Αποτελέσματα: 1) Στη διάρκεια της εγκυμοσύνης προέκυψε από τη συνεργασία 19 Ευρωπαϊκών μελετών μητέρας-παιδιού ότι οι γυναίκες που καταναλώνουν ψάρι περισσότερο από μια φορά την εβδομάδα έχουν μικρότερο κίνδυνο να γεννήσουν πρόωρα. Επιπρόσθετα, οι έγκυες γυναίκες που καταναλώνουν ψάρι πάνω από τρείς φορές την εβδομάδα βρέθηκε ότι είναι πιο πιθανό να γεννήσουν μωρά με αυξημένο βάρος κατά 15.2 γραμμάρια σε σχέση με εκείνες που καταναλώνουν ψάρι λιγότερο από μια φορά την εβδομάδα. 2) Η διάρκεια του θηλασμού (σε μήνες) βρέθηκε να συσχετίζεται θετικά με όλες τις κλίμακες της νευροανάπτυξης, εκτός της αδρής κινητικότητας, όταν τα παιδιά ήταν 18 μηνών. Για κάθε επιπλέον μήνα θηλασμού τα παιδιά παρουσίασαν καλύτερη επίδοση στην γνωστική κλίμακα, την κλίμακα κατανόησης του λόγου, την κλίμακα έκφρασης του λόγου και την κλίμακα λεπτής κινητικότητας. Επιπλέον, βρέθηκε ότι τα παιδιά που θήλασαν πάνω από 6 μήνες παρουσίασαν καλύτερη επίδοση στην κλίμακα λεπτής κινητικότητας σε σύγκριση με τα παιδιά που δεν θήλασαν ποτέ. 3) Στην προσχολική ηλικία προσδιορίστηκαν 3 διατροφικά πρότυπα: το ‘Μεσογειακό’, το ‘Πρόχειρο’ και το ‘Δυτικό’ πρότυπο διατροφής. Τα αποτελέσματα αυτής της ανάλυσης έδειξαν ότι τα παιδιά που πήγαιναν παιδικό σταθμό και περνούσαν περισσότερο χρόνο με τη μητέρα τους (≥ 2 ώρες/ημέρα) ήταν πιο πιθανό να ακολουθούν το ‘Μεσογειακό’ πρότυπο διατροφής. Το ‘Πρόχειρο’ πρότυπο σχετίστηκε θετικά με το χαμηλό εκπαιδευτικό επίπεδο των γονιών και την πρώιμη εισαγωγή σε στερεά τροφή για βρέφη (<6 μήνες). Τέλος, το ‘Δυτικό’ διατροφικό πρότυπο είχε θετική συσχέτιση με το παθητικό κάπνισμα και την παρακολούθηση τηλεόρασης. 4) Στην ανάλυση όπου ελέγχθηκε η συσχέτιση των διατροφικών προτύπων με την νευροανάπτυξη των παιδιών στην ηλικία των 4 ετών, βρέθηκε ότι τα παιδιά που ακολουθούσαν το ‘Πρόχειρο’ πρότυπο διατροφής είχαν χαμηλότερο σκορ στην κλίμακα της λεκτικής ικανότητας καθώς και στη γενική γνωστική ικανότητα. Αρνητική ήταν και η επίδραση του ‘Δυτικού’ προτύπου διατροφής σε όλες σχεδόν τις κλίμακες της νευροανάπτυξης, χωρίς όμως να είναι η συσχέτιση αυτή στατιστικά σημαντική κατόπιν ελέγχου πολλών κοινωνικο-δημογραφικών χαρακτηριστικών των γονιών και των παιδιών τους. 5) Στην προσχολική ηλικία βρέθηκε ότι οι διατροφικές συμπεριφορές των παιδιών σχετίζονται θετικά με συμπτώματα του συνδρόμου ελλειματικής προσοχής. Πιο συγκεκριμένα, οι δεκτικές προς το φαγητό συμπεριφορές (π.χ. συναισθηματική υπερφαγία) είχαν θετική συσχέτιση με την παρορμητικότητα, την ανικανότητα συγκέντρωσης και την υπερκινητικότητα που παρουσίαζαν αυτά τα παιδιά.

Συμπεράσματα: Συνοψίζοντας όλα τα παραπάνω, η διατροφή στα πρώτα χρόνια της ζωής φαίνεται να επηρεάζει την ψυχοκινητική ανάπτυξη των παιδιών. Η διερεύνηση των διατροφικών συνηθειών και η έγκαιρη διαπίστωση ψυχοκινητικών δυσκολιών ή συμπτωμάτων ελλειματικής προσοχής αποτελούν αναγκαίο εργαλείο στα χέρια των επιδημιολόγων και διατροφολόγων προκειμένου να επιτευχθεί η προάσπιση της δημόσιας υγείας τόσο σε επίπεδο πρόληψης όσο και παρέμβασης. Περαιτέρω παρακολούθηση των παιδιών σε μεγαλύτερες ηλικίες είναι απαραίτητες προκειμένου να επιβεβαιωθούν και ισχυροποιηθούν τα παραπάνω συμπεράσματα.

Abstract in English

Introduction: During fetal and early postnatal life genetic and environmental factors play an equally critical role in the shaping of brain growth and development. Environmental determinants such as nutrition can have direct effect on gene expression in brain through epigenetic mechanisms. The preschool years is a time of rapid and dramatic changes in the brain, and it is time for acquisition of fundamental cognitive and behavioral changes. The research hypothesis of the present thesis is that early life nutrition starting in pregnancy up to preschool age may be associated with children's neurodevelopment.

Specific Objectives:

- To evaluate the association of diet during pregnancy with fetal growth, length of gestation, birth weight as strong predictors of children's future neurodevelopment.
- To investigate the role of breastfeeding initiation and duration in infant mental and psychomotor development, in Crete, Greece.
- To identify the dietary patterns followed by Greek preschool children with the use of validated Food Frequency Questionnaire (FFQ) and to examine the influence of multiple socio-demographic characteristics and lifestyle factors on children's diet.
- To examine the impact of children's dietary patterns on cognitive and psychomotor development at preschool age.
- To investigate in depth the association of eating behaviours with Attention Deficit Hyperactivity Disorder symptoms in a population-based sample of Greek preschool children.

Methods: In order to investigate diet during pregnancy, a case study was performed in collaboration with 19 European birth cohort studies including 151.880 pregnant women. A 2 stage approach was used to assess the association of fish intake during pregnancy with birth outcomes. First associations were analyzed at a cohort level and second, cohort specific effect estimates were combined by using meta-analysis.

Data originated from the Rhea mother-child cohort were used to examine the role of nutrition on children's neurodevelopment up to preschool age, including 1000 women and their children. Information on breastfeeding practices was collected at the 9th month postpartum, and this information was updated at the age of 18 months with the use of

questionnaires. Dietary assessment at 4 years of age was performed with the use of a validated semi-quantitative FFQs. For the assessment of children's eating behaviour in preschool age we used the Children's Eating Behaviour Questionnaire (CEBQ). This instrument was designed to be completed by parents referring to children's eating behavior. Children's neurodevelopment was assessed by trained psychologists, with the use of Baykey III at 18 months and with the McCarthy Scales of Children's Abilities at age 4 years. The ADHD symptoms in preschool children were assessed with the 36-item ADHD interview test (ADHDT) as developed by Gilliam in 1995. Parents completed the interview, which is based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for ADHD.

Results: 1) Diet during pregnancy was investigated in a case study performed in collaboration of 19 European birth cohort studies and it was observed that women who ate fish more than once a week during pregnancy had a lower risk of preterm birth compared with women who rarely ate fish (once a week or less). In addition, women with a higher intake of fish during pregnancy gave birth to neonates with higher birth weight by 15.2g for more than 3 times a week compared to those that consumed fish less than once a week. 2) Longer duration of breastfeeding duration (in months) was associated with increased scores in the scales of cognitive, language, and fine motor development at 18 months of age. For every additional month of breastfeeding children had increased scores in the neurodevelopmental scales. Children who were breastfed longer than 6 months had 4.44 points increase in the scale of fine motor development compared with those never breastfed. 3) Three dietary patterns were identified in preschool age: the 'mediterranean', the 'snacky and the 'western' pattern. Preschool attendance and increased time spent with the mother ($\geq$ 2 hours/day) was positively associated with the 'mediterranean' pattern. Lower parental education, maternal age and earlier introduction to solid foods (< 6 months) were positively associated with the 'snacky' pattern. Higher scores on the 'western' type diet were associated with exposure to passive smoking and TV watching. 4) The following study includes the investigation of the association of dietary patterns with child cognitive and psychomotor development in preschool children. In this analysis, children who followed the 'snacky' pattern had lower scores in the scale of verbal ability, general cognitive and cognitive functions of posterior cortex. In the minimally adjusted model, the 'western' type diet was also associated with lower scores in almost all neurodevelopmental scales but these associations were attenuated with further

adjustment for maternal and child characteristics. 5) In preschool age a positive association was observed between children's eating behavior with ADHD symptoms. In particular, food approach behaviors such as food responsiveness and emotional overeating were positively associated with impulsivity, inattention and hyperactivity.

Conclusions: In summary, findings in the present thesis support that nutrition in early life may affect neurodevelopment in infancy and childhood. The investigation of dietary patterns and the early recognition of neurodevelopmental problems or ADHD symptoms can provide an avenue for prevention and intervention policies and thus lead to more effective management of these problems in early childhood. The long term follow up is needed to better understanding the relation between nutrition and neurodevelopment.

PhD thesis publications

The current thesis consists of a compilation of six scientific publications: one validation study and five research papers.

Validation study:

1. **V. Leventakou**, V. Georgiou, L. Chatzi, K. Sarri, Relative validity of an FFQ for pre-school children in the mother-child 'Rhea' birth cohort in Crete, Greece, Public Health Nutr, February 18:421-7, 2015.

Research papers:

1. **V. Leventakou**, T. Roumeliotaki, D. Martinez, H. Barros, A. L. Brantsaeter, M. Casas, M. A. Charles, S. Cordier, M. Eggesbø, M. van Eijsden, F. Forastiere, U. Gehring, E. Govarts, T. I. Halldórsson, W. Hanke, M. Haugen, D. H.M. Heppe, B. Heude, H. M. Inskip, V. W.V. Jaddoe, M. Jansen, C. Kelleher, H. M. Meltzer, F. Merletti, C. Moltó-Puigmartí, M. Mommers, M. Murcia, A. Oliveira, S. F. Olsen, F. Pele, K. Polanska, D. Porta, L. Richiardi, S. M. Robinson, H. Stigum, M. Strøm, J. Sunyer, C. Thijs, K. Viljoen, T. G.M. Vrijkotte, A. H. Wijga, M. Kogevinas, M. Vrijheid, L. Chatzi, Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies, *Am J Clin Nutr*, March; 99(3):506-16, 2014.

2. **V. Leventakou**, T. Roumeliotaki, K. Koutra, M. Vassilaki, E. Mantzouranis, P. Bitsios, M. Kogevinas , L. Chatzi, Breastfeeding duration and cognitive, language, and motor development at 18 months of age: Rhea mother-child cohort in Crete, Greece. *J Epidemiol Community Health*, March; 69(3):232-9, 2015.

3. **V. Leventakou,** K. Sarri, V. Georgiou, V. Chatzea, E. Frouzi, A. Kastelianou, A. Gatzou, M. Kogevinas, L. Chatzi, Early life determinants of dietary patterns in preschool children: Rhea mother-child cohort, Crete, Greece. *Eur J Clin Nutr*, Jun 17, 2015, doi: 10.1038/ejcn.2015.93.

4. **V. Leventakou**, T. Roumeliotaki, K. Sarri, K. Koutra, M. Kampouri, A. Kyriklaki, M. Vassilaki, M. Kogevinas, L. Chatzi, Dietary patterns and their association with neurocognitive development in preschool children: the Rhea mother-child cohort study, *British J Nutr*, under revision.

5. **V. Leventakou**, N. Micali, V. Georgiou, K. Sarri, K. Koutra, S. Koinaki, M. Vassilaki, M. Kogevinas, L. Chatzi, Is there an association between eating behaviour and ADHD symptoms in preschool children? *J Child Psychol Psychiat*, under revision.

1. General Introduction

Early life nutrition has a significant impact on the maintenance of lifelong health (Godfrey and Barker 2000). The fetal origins of adult disease model, known as 'early life programming' was originally proposed by Barker in an effort to explain the observed associations between undernutrition of the fetus, low birth weight (<2.500 grams) and a higher risk for chronic diseases in later life such as cardiovascular disease, diabetes and metabolic syndrome (Barker 1995). Although low birth weight was initially considered as the primary indicator of altered fetal development within this model, other measures of fetal growth later emerged as equally relevant. Over recent decades, much progress has been made towards the understanding of the way in which metabolic tissues and physiological systems develop, and the impact of early life nutrition on these processes (Martinez, Cordero et al. 2012). A substantial body of epidemiological evidence suggests that an adverse intrauterine environment, elicited by maternal dietary or placental insufficiency, may "program" susceptibility of the fetus to later development of cardiovascular or metabolic diseases such as obesity, hypertension, insulin resistance and type 2 diabetes (Gluckman, Hanson et al. 2008; Symonds, Sebert et al. 2009), as well as neurocognitive disorders (Bale, Baram et al. 2010). This concept is known as 'early nutrition' programming, and has gained broad recognition among researchers (Demmelmair, von Rosen et al. 2006), as increasing evidence shows that diet, breastfeeding, and complementary feeding may have an irreversible impact on child development and long-term health (Lucas 2005; Demmelmair, von Rosen et al. 2006).

Psychiatric research has been inspired by the model of the developmental origins of health and disease (DOHaD) which links fetal development to an unfavorable environment with non-communicable diseases in later life (Gluckman, Hanson et al. 2010). Such research aims to provide insight in the etiology of mental disorders as a consequence of nutritional insults during pregnancy and early life. Recent epidemiological studies have shown that prenatally under nourished individuals (e.g. those exposed to Dutch Hunger Winter of 1944–45 and 1959-61 Chinese famines) are at higher risk to develop schizophrenia in adult life (Brown and Susser 2008; Xu, Sun et al. 2009). Various scenarios implicate the potential role of epigenetic programming in risk for schizophrenia related to nutritional deficiency. Individuals with prenatal exposure to famine, demonstrated reduced DNA methylation of the imprinted IGF2 (insulin-like growth factor II) gene compared to those non-exposed, supporting that early life programming causes epigenetic changes that persist over time (Heijmans, Tobi et al. 2008).

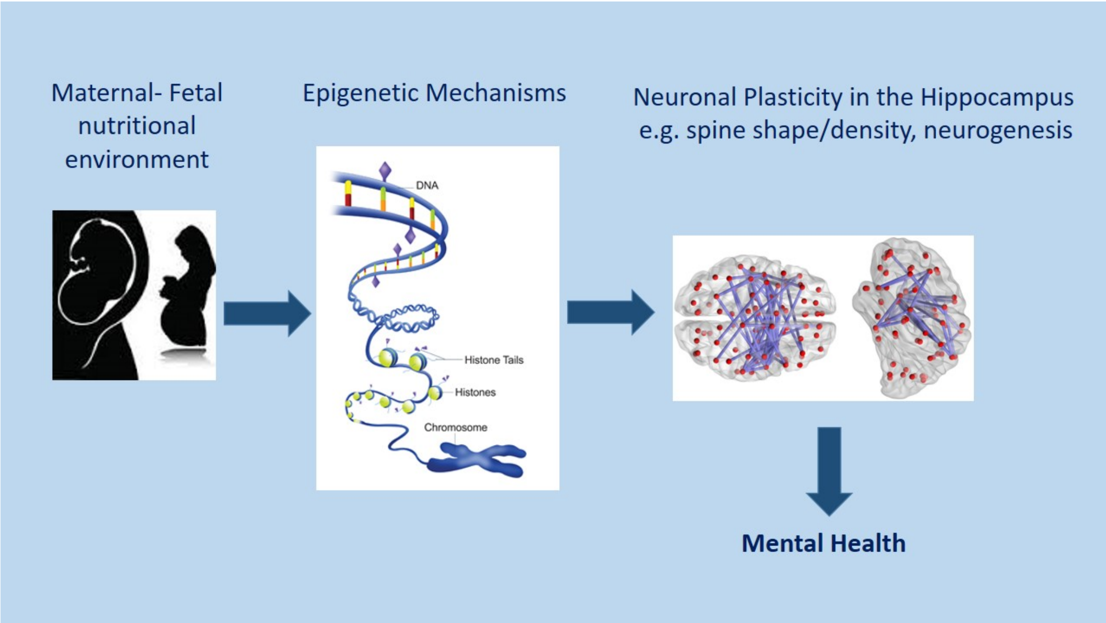

Figure 1. How early life nutrition may affect health, through epigenetic mechanisms.

There is increasing major public health interest about the concept of early nutrition programming, while statements reflecting this concept are appearing in policy documents, leaflets, research publications and other documents. The study design of population-based prospective cohort studies starting early in pregnancy or at birth, provide the opportunity to understand mechanisms involved in disease predisposition. Birth cohorts have important strengths in that they collect data prospectively on many covariates and follow children for several years after birth, thereby providing insights into developmental problems at birth as well as in the first years of life. Although associations between early nutritional status and later neurodevelopmental disorders have been established, little is known about their underlying causes, including the role played by maternal nutrition during pregnancy and infant feeding practices.

1.1 The impact of early life nutrition on child neurodevelopment

1.1.1 Diet during pregnancy

Current research has provided interesting findings supporting the association of poor nutrition during pregnancy with adverse developmental outcomes in children, including impaired cognitive functions, deficits in attention and behavioral problems (Liu and Raine 2006). Deficiencies of key micronutrients such as fatty acids, iron, iodine, zinc, choline and B vitamins during the brain growth spurt, which occurs in the last trimester of pregnancy and the first 2 years of life, may have lasting effects on neurodevelopment (Prado and Dewey 2014).

The n-3 fatty acid docosahexaenoic acid (DHA) and n-6 fatty acid arachidonic acid (AA) are the major long chain polyunsaturated fatty acids (LC-PUFAs). Their metabolism can influence many aspects of brain development, including neuronal migration, axonal and dendritic growth, and the creation, remodelling and pruning of synaptic connections. Animal studies have shown that both neural integrity and function can be permanently disrupted by deficits of n-3 fatty acids during fetal and neonatal development (Innis 2007). Reduced DHA has been associated with cognitive and behavioural dysfunctions in newborn infants (Uauy, Hoffman et al. 2003). Although both n-6 and n-3 fatty acids are essential, the n-3 fatty acids such as DHA appear to play a special role in highly active sites such as synapses and photoreceptors, and deficiencies have particularly been linked to visual and cognitive deficits. An enhanced prenatal AA and DHA intake is possible to lead to improved neurodevelopmental outcomes in infants (Hadders-Algra, Bouwstra et al. 2007) and children (Campoy, Escolano-Margarit et al. 2011; Escolano-Margarit, Ramos et al. 2011). However, recent reviews have concluded to no clear long lasting benefit of LC-PUFAs supplementation during pregnancy (Dziechciarz, Horvath et al. 2010; Gould, Smithers et al. 2013).

DHA is predominantly concentrated in fish, along with other nutrients considered to be beneficial for fetal growth and offspring neurodevelopment, including protein, selenium, iodine and vitamin D (Starling, Charlton et al. 2015). Fetal development and birth outcomes like SGA or preterm birth have been well documented to be the most significant predictors of poor long term neurodevelopment (Arcangeli, Thilaganathan et al. 2012; Xiong, Gonzalez et al. 2012). Recent randomized clinical trials (Hauner, Much et al. 2012; Carlson, Colombo et al. 2013), and reviews have suggested that maternal n-3 supplementation during pregnancy is associated with small but significant increases in the length of gestation and infant birth size(Szajewska, Horvath et al. 2006; Salvig and Lamont 2011), whilst other birth cohort studies have consistently reported positive associations between maternal fish intake in pregnancy and neurodevelopment in infancy or childhood (Daniels, Longnecker et al. 2004; Oken, Wright et al. 2005; Hibbeln, Davis et al. 2007; Oken, Osterdal et al. 2008; Oken, Radesky et al. 2008; Mendez, Torrent et al. 2009). At the same time, fish is a well-known route of exposure to pollutants such as dioxins, polychlorinated biphenyls (PCBs), methyl mercury, and other heavy metals which may adversely affect fetal growth and birth outcomes (Myers, Davidson et al. 2003; Oken, Radesky et al. 2008). Therefore, pregnant women are usually facing conflicting reports on the risks and benefits of fish intake, resulting in controversy and confusion over the place of fish consumption in a healthy diet in pregnancy.

As previously mentioned important nutrients during pregnancy associated with brain development also include iron, iodine and folic acid. Animal studies have reported that iron deficiency in utero or in early postnatal life alters neurogenesis and differentiation of brain cells and regions and also alters dopamine and norepinephrine metabolism, therefore affects neurodevelopmental functions(Lozoff, Beard et al. 2006). A recent systematic review that examined the effect of iron supplementation in pregnant women reported modest effects on their children's psychomotor development but no effects on their mental and behavioral development(Szajewska, Ruszczynski et al. 2010), whilst other studies have found no evidence on the beneficial role of maternal iron status on children's cognitive development (Zhou, Gibson et al. 2006). Iodine deficiency has also been extensively researched with many studies supporting that severe iodine deficits during pregnancy are associated with poor cognitive development, mental retardation and hearing impairment (Zimmermann 2009). However, findings are discrepant in cases of mild-moderate maternal iodine deficiency with no as clear effects as in severe deficiency(Zimmermann 2007). Finally, many researchers have investigated the role of folic acid during pregnancy. Animal studies have shown that folic acid plays an important role in early brain development as it influences neuronal and glial growth and proliferation, and can affect the synthesis and release of neurotransmitters such as dopamine, adrenaline and serotonin. Results from studies in humans suggest that folic acid use early in pregnancy may be associated with reduced risk for autistic disorder (Suren, Roth et al. 2013) and severe language delay in children (Roth, Magnus et al.

2011). High doses of folate early in pregnancy may also be associated with enhanced vocabulary development, communicational skills and verbal comprehension in infancy (Chatzi, Papadopoulou et al. 2012). However, further research is needed for more conclusive results and parameters such as and should take into account timing of nutrient deficiency, supplementation and severity of deficiency should be taken into account.

Maternal dietary habits have undergone many changes during the last years shifting to a more ‘western’ type diet that is widespread in modern societies. The dramatic increase in the consumption of processed foods high in saturated fats and added sugar have led to a situation in which developing foetus is exposed to an unfavourable environment. Animal studies have demonstrated that when rats were fed a high fat diet, it altered the development of the foetus hippocampus(Niculescu and Lupu 2009) and affected the activation of the sympathetic nervous system, leading to neurobehavioral disturbances(Fernandes, Grayton et al. 2012). A large population-based birth cohort study recently reported that children whose mothers followed an ‘unhealthy’ pattern (high in fat and sugar foods) during pregnancy, were at higher risk for externalizing problems(Jacka, Ystrom et al. 2013). This finding was also observed by another birth cohort reporting that the less ‘healthy’ maternal pattern (high in processed foods) was positively associated with higher levels of externalizing in offspring(Steenweg-de Graaff, Tiemeier et al. 2014).

These studies not only point out that maternal diet is a potential predictor of offspring’s health but also highlight the potential of dietary interventions during pregnancy as a key strategy for improving health in children. It seems plausible that mental illnesses later in life can be potentially preventable by promoting specific lifestyle factors and dietary behaviours in early life instead of using established psycho-therapeutic approaches.

1.1.2 Breastfeeding

Several studies have consistently pointed out that breastfeeding includes a range of nutritional advantages and provides many important health benefits to both babies and mothers (Hamosh 2001; Picciano 2001). The World Health Organization (WHO) considers exclusive breastfeeding for the first 6 months of life to be the optimal method for infant feeding, a recommendation that is also supported by the American Academy of Pediatrics (Gartner, Morton et al. 2005). Breastfeeding is also recommended for at least two years and for as long thereafter as mother and child desire it. However, the frequency and duration of breastfeeding in Crete, Greece is relatively low compared to WHO recommendations (Ladomenou, Kafatos et al. 2007; Vassilaki, Chatzi et al. 2014).

Breast milk contains the ideal amounts of fatty acids, lactose, water and amino acids for human digestion, brain development, and growth, as well as, many bioactive ingredients, such as cytokines, nucleotides, hormones and growth factors (Picciano 2001). One of the differences between human milk and commercially available formulas, is its high content of DHA, a major form of n-3 LC-PUFA, well known for its beneficial effects on neurotransmission, neurodevelopment, and particularly visual acuity(McCann and Ames 2005; Koletzko, Lien et al. 2008; Jensen and Lapillonne 2009). On the other hand, recent meta-analysis of randomized trials with infant formulas supplemented with long-chain polyunsaturated fatty acids (LC-PUFAs) have generally not found any significant effects on either mental and psychomotor (Simmer, Patole et al. 2011), or visual and cognitive development (Qawasmi, Landeros-Weisenberger et al. 2012; Qawasmi, Landeros-Weisenberger et al. 2013).

The role of breast milk on cognitive development has attracted research interest since the first observation made by Hoefer and Hardy in 1929 (Hoefer and Hardy 1929). Several systematic reviews have tried to summarize the effect of breastfeeding on child mental and psychomotor development but their findings are inconsistent (Anderson, Johnstone et al. 1999; Drane and Logemann 2000; Der, Batty et al. 2006). Some researchers support that breastfed children have higher scores on cognitive abilities, as compared to never breastfed (Anderson, Johnstone et al. 1999), while at the same time others support that the observed associations are heavily influenced by maternal cognitive ability, socioeconomic and psychosocial factors with little or no effect of breastfeeding itself on cognitive development (Drane and Logemann 2000; Walfisch, Sermer et al. 2013). In a large trial where prolonged and exclusive breastfeeding was promoted in the experimental group, the children from that group had better cognitive development compared to the control group at the age of 6.5 years (Kramer, Aboud et al. 2008). Most studies are in the same direction providing evidence for the positive effect of breastfeeding on children's neurodevelopment with only a few focusing at early ages (<2 years of age)(Sacker, Quigley et al. 2006; Dee, Li et al. 2007; Chiu, Liao et al. 2011; Guxens, Mendez et al. 2011). Further research is needed in order to support the beneficial role of breastfeeding initiation and duration on children's cognition and, thus, enhance national public health policies.

1.1.3 Diet in childhood

Early childhood is a significant period for the establishment of dietary habits, since dietary preferences are first established, laying the formation of adult eating habits (Birch, Savage et al. 2007; Ventura and Worobey 2013). Dietary choices in the first years of life have been associated with multiple health outcomes (Smithers, Golley et al. 2011). Unhealthy dietary patterns in early life, with high fat and low fibre foods, have been positively associated with greater risk of childhood obesity (Ambrosini 2014) and negatively associated with cognitive development (Northstone, Joinson et al. 2012). At the same time, many different factors are possible to influence food habits in a complex way. Home environment, parents, socioeconomical aspects and prevailing lifestyles are some of the factors that affect children's food preferences. As dietary patterns are likely to be age-specific, understanding early life dietary choices is of great interest in terms of developing strategies that will ensure healthy nutrition in early childhood.

Most studies on school-aged children have so far indicated the role of specific foods or nutrients on neurodevelopment (Bryan, Osendarp et al. 2004; McAfee, Mulhern et al. 2012). In particular, diets with inadequate intake of cereal, milk, meat and fish products contribute to low levels of micronutrients, such as omega-3 fatty acids, vitamin B12, folic acid, zinc, iron and iodine, that may be responsible for children's cognitive impairment (Anjos, Altmae et al. 2013). A diet low in protein intake is possible to cause impairment of neurotransmission and brain development. Undernourished children (<3 years of age) usually have impaired behaviour and lower school achievements, whereas protein supplementation studies have indicated the beneficial effects on their development(Grantham-McGregor and Baker-Henningham 2005). Other studies have also supported the important role of regular meals in order to continuously supply brain with the required glucose(Bellisle 2004). Glucose metabolism increases from birth up to 4 years of age and remains high until 9-10 years old. Thus, children are more vulnerable to the overnight fasting, and breakfast is crucial to provide the adequate fuel for brain in children. Holey and Dye recently supported with their review that having breakfast may enhance cognitive development, especially in malnourished children (Hoyland, Dye et al. 2009).

However, the study of isolated foods or nutrients fails to reveal their synergistic role on individual's diet. The use of dietary patterns allows the combination of foods usually eaten together and the exploration of the whole diet(Smith, Emmett et al. 2013). Currently the most common method to obtain dietary patterns is the Principal component analysis (PCA), a statistical method that groups individual foods and nutrients and describes specific patterns. A number of studies have examined dietary patterns in infancy and toddlerhood (Robinson, Marriott et al. 2007; Ystrom, Niegel et al. 2009; Smithers, Brazionis et al. 2012; Bell, Golley et al. 2013; Kiefte-de Jong, de Vries et al. 2013; Okubo, Miyake et al. 2014), with only a few being focused at preschool age (North and Emmett 2000; Aranceta, Perez-Rodrigo et al. 2003; Theodore, Thompson et al. 2006; Wall, Thompson et al. 2013). Although the type and number of identified patterns in preschoolers' vary among studies, three main patterns have been observed in literature. These patterns have been described as 'healthy', 'less healthy' and 'traditional'. Several parental socio-demographic and lifestyle characteristics have been associated with the adoption of specific dietary patterns in early childhood. Lower levels of maternal education (North and Emmett 2000; Aranceta, Perez-Rodrigo et al. 2003; Northstone and Emmett 2013; Wall, Thompson et al. 2013) and the presence of older siblings(North and Emmett 2000) have been associated with 'less healthy' diets. On the other hand, breastfeeding has been positively associated with the adoption of a 'healthy' dietary pattern in childhood (Grieger, Scott et al. 2011).

In addition, limited number of studies have investigated the impact of children's diet as a whole on cognitive development (Gale, Martyn et al. 2009; Northstone, Joinson et al. 2012; Smithers, Golley et al. 2012). Birth cohort studies in infants aged 6, 12, 15 and 24 months reported that those who followed the 'breastfeeding' and the 'home made' pattern had increased Intelligence Quotient (IQ) scores at 4 and 8 years of age(Gale, Martyn et al. 2009; Smithers, Golley et al. 2012). A cross sectional study performed by Theodore and colleagues observed that children with high consumption of breads and cereals and fish at 3.5 and 7 years of age had higher IQ scores(Theodore, Thompson et al. 2009). Half of the children included in this study were born small for gestational age and generalizability of the results to other populations is rather difficult. On the other hand, a ‘processed’ (high fat and sugar content) pattern of diet at 3 years of age was negatively associated with IQ assessed at 8.5 years of age in the ALSPAC cohort in UK(Northstone, Joinson et al. 2012). Since PCA method is data-driven, results from literature cannot be extrapolated to other populations. Thus, it is essential to investigate the association of diet as a whole, with children’s neurodevelopment in the Greek population.

1.1.4 Children’s eating behaviour

Attention Deficit Hyperactivity Disorder (ADHD) has a prevalence rate in children of 7.2% worldwide (Thomas, Sanders et al. 2015), with boys being more affected than girls(Silva, Colvin et al. 2014). Inattention, impulsivity and hyperactivity are well known symptoms of ADHD, predominantly detected in early childhood, that are likely to persist over time, into adolescence and adulthood. While the etiology of ADHD is multidimensional and still remains unclear, higher rates of eating pathology have implicated such as overeating (eating a large amount of food), or binge eating (overeating with loss of control).

There is considerable evidence suggesting that genetic(Banaschewski, Becker et al. 2010), environmental factors (Froehlich, Anixt et al. 2011; Silva, Colvin et al. 2014) and dietary factors (Millichap and Yee 2012; Heilskov Rytter, Andersen et al. 2015) are likely to be relevant with the occurrence of ADHD. For the last decade, most of the interest in childhood eating patterns has been driven by identifying risk patterns for later obesity (Cortese and Castellanos 2014). Several studies have pointed out the potential contributing role of disturbed eating behaviours such as overeating, binge eating, and bulimic behaviours to the association between obesity and ADHD (Cortese and Vincenzi 2012; Docet, Larranaga et al. 2012; Nazar, Suwwan et al. 2014). Existing findings have also highlighted the association between ADHD symptoms, overeating behaviours and adverse outcomes like excess weight gain (Davis, Levitan et al. 2006).

The investigation of children’s behavioural characteristics is also very challenging, since early life behavioural styles are strong predictors of individual’s psychopathology in later life (Caspi, Harrington et al. 2003). Longitudinal studies focusing on early life behavioural characteristics are essential in order to determine whether specific individual characteristics are associated with the development of disturbed eating patterns in childhood and their maintenance into adolescence and adulthood (Hartmann, Czaja et al. 2010). An in depth understanding of the association between ADHD and its core features and disturbed eating patterns in children is lacking, despite evidence of this association. A very recent population study, including male participants aged 6-10 years old, reported that children with ADHD had irregular meals, ate more than 5 times per day and consumed a lot of sweetened drinks during the day(Ptacek, Kuzelova et al. 2014). A positive association between ADHD and binge eating has been shown cross-sectionally in children aged 10 years old (Cortese, Bernardina et al. 2007; Reinblatt, Leoutsakos et al. 2014) and longitudinally(Sonneville, Calzo et al. 2015). To our knowledge, there is one study that has evaluated the association between eating behaviours with childhood psychopathology which included emotional, behavioural and pervasive developmental disorders. This study showed that picky eating was positively associated with all domains of psychopathology among children aged 5 to 7 years, but unfortunately ADHD was not studied in depth (Micali, Simonoff et al. 2011).

Although it is difficult to fully decipher the direction of the association between ADHD, eating behaviours and obesity, an in depth understanding of this association can not only help preventative efforts for weight gain and obesity but can also help us understand the risk mechanisms.

2. Hypothesis and aims of the present thesis

Hypothesis

The main hypothesis of the present thesis is that maternal and infant nutrition have a critical role in children's brain function and neurodevelopment later in life. The identification of dietary factors along with a large number of confounding factors that can result in both immediate and long-term neurological impairment are of particular importance. The publications included in the present thesis are focused in different critical periods of life from pregnancy up to early childhood.

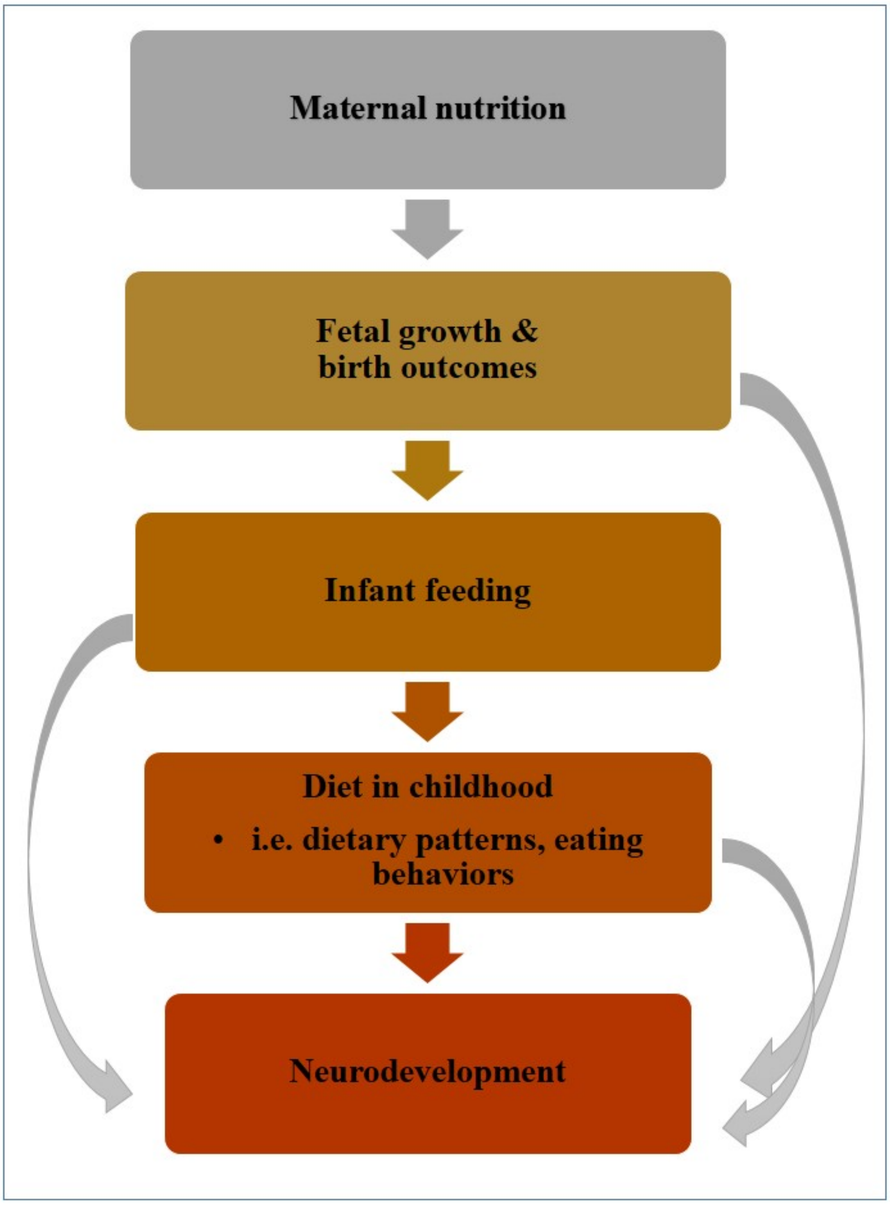

Figure 2. An overview of the research hypothesis studied in this thesis.

Aims

Although, the important role of nutrition in neurodevelopment is well documented, diet from pregnancy through childhood and its association with neurodevelopment in infancy and childhood is an area that remains under researched. Findings from current literature remain inconclusive, with numerous inconsistencies and limitations. Stronger evidence is required to support optimal maternal diet that will shield the offspring's development. Few birth cohorts have enough statistical power to examine the effects of sub-categories of fish intake during pregnancy and adverse birth outcomes, as early predictors of later neurodevelopment. The pooled analysis of existing studies conducted in different countries provide a practical approach when studying the health effects of during pregnancy leading to more robust evidence on the risks or benefits of nutrition in pregnant women. In addition, further research is needed on infant feeding practices since most of the studies, which have examined the effect of breastfeeding on cognitive development, have included participants from highly selected groups, such as preterm-born infants or with low birth weight, and it is not clear whether these results can be extrapolated to general population. Furthermore, nutrition in childhood has been investigated by limited number of studies. Current research has mainly focused to examine the role of isolated foods or nutrients instead of dietary patterns on children's neurodevelopment. To our knowledge, no studies have so far investigated in depth the association of eating behaviours with ADHD.

The concept that diet of pregnant women, infants and children could have long lasting effects on neurodevelopment has major implications for public health practice. Interventions to enhance better nutrition in early life is possible to lead to more cost-effective approaches in the prevention and management of mental health problems in later life. In addition the identification of specific population groups with dietary deficiencies may warrant targeted nutrition policies.

Given this gap in research the overall aim of the present thesis is to evaluate the effect of diet during pregnancy, infancy and early childhood on neurodevelopment within the only Greek mother-child birth cohort study to date, the RHEA study and other European population based birth cohort studies.

The specific objectives are:

- To evaluate the association of diet during pregnancy with fetal growth and length of gestation as strong predictors of children's neurodevelopment, in the framework of a European collaboration project (Paper 1)
- To evaluate the levels of breastfeeding initiation and duration in Crete, Greece and investigate the role of breastfeeding duration in infant mental and psychomotor development, in the Rhea mother-child cohort (Paper 2)
- To identify the dietary patterns followed by Greek preschool children with the use of a validated Food Frequency Questionnaire (Paper 3) and to examine the

influence of multiple socio-demographic characteristics and lifestyle factors on children's diet, in the Rhea mother-child cohort (Paper 4),

- To examine the impact of children's dietary patterns on cognitive and psychomotor development at preschool age, in the Rhea mother-child cohort (Paper 5)
- To investigate in detail for the first time the association of eating behaviours with ADHD symptoms in a population-based sample of Greek preschool children (Paper 6)

3. Methods

This section provides a summary of the methods used in the research papers included in this thesis. This thesis is based on data are derived from a large European collaboration project and from the Rhea mother-child cohort. Further details can be found in the papers presented in the results section.

3.1 Study Design

European collaboration project

A case study was conducted within the framework of CHICOS (Child Cohort Research Strategy for Europe, www.chicosproject.eu). The aim of CHICOS project is to improve child health across Europe by developing an integrated strategy for mother-child cohort research in Europe. A total of 29 European birth cohorts were eligible to participate in this study, identified from the European inventory of birth cohorts (www.birthcohorts.net), or from cohort's individual websites and published articles (assessed until June 2011). Seven cohorts did not reply to the invitation, and three cohorts declined to participate. The participating cohorts targeted the general population and altogether covered births from 1996 to 2011. All official procedures (personal identifiers were removed, data transfer agreements were signed) were followed for all datasets to be transferred to the University of Crete. A total of 151,880 live born singleton births were included with available data (non-missing values) on the exposure, outcome, and confounding variables. In total, 27 subjects were excluded from the present analysis due to extreme values on gestational age (<20 weeks or ≥45 weeks), and birth weight (>7000 grams); 79 subjects were excluded due to implausible combination of the above. Informed consent was obtained from all study participants as part of the original studies and ethical approval was obtained from the local authorized Institutional Review Boards.

Figure 3. Participating cohorts in the European collaboration project included in the present thesis

The Rhea mother-child birth cohort study

The "Rhea" birth cohort study is a prospective cohort that started in February 2007 in Crete, Greece. Briefly, pregnant women, residents in the study area, aged 16 years or above, with no communication handicap were included in the study. Pregnant women (Greek and immigrant) who became pregnant within a 12-month period, starting in February 2007, were contacted and asked to participate in the study. The first contact was made before 15 weeks' gestation, at the time of the first major ultrasound examination and participants were invited to provide blood and urine samples and to participate in a face-to-face interview. Women were contacted again at various times during pregnancy, at birth, at 8-10 weeks after delivery, and for children's follow-up at 9th, 18th months, and at 4 years of age. Face-to face completed questionnaires together with self-administered questionnaires and medical records were used to obtain information on dietary, environmental, and psychosocial exposures during pregnancy and early childhood. The study was approved by the Ethical Committee of the University Hospital of Heraklion (Crete, Greece), and all participants provided written informed consent after complete description of the study.

During the study recruitment period 1,765 eligible women were approached, 1,610 (91%) agreed to participate, and 1,388 (86%) were followed up until delivery. In each research paper the number of participants differentiates according to the available data for the particular analysis.

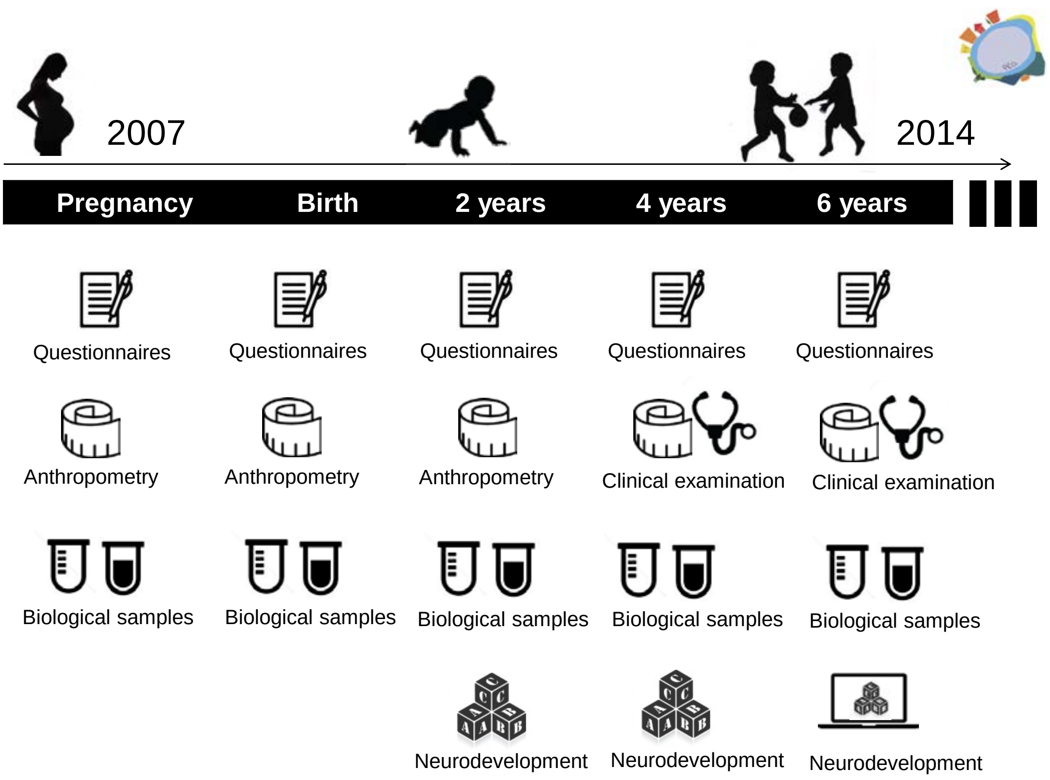

Figure 4. Overview of the data collected in the Rhea cohort study.

3.2 Dietary assessment

3.2.1 In the European collaboration project

Fish intake during pregnancy

Exposure variables were measured as frequency (times a week) of total fish, fatty fish, lean fish, and seafood (other than fish) intake during pregnancy derived from cohort specific food frequency questionnaires (FFQ) or specially designed questionnaires for fish consumption during pregnancy. Salmon, herring, mackerel, trout, sardines, Greenland halibut, anchovy, gurnard, and tuna were classified as fatty fishes, while, cod, pollack, plaice, flounder, garfish, and similar species were classified as lean fishes. Assessments for standardised categories of fish intake [more than 1 but less than 3 times a week ($1<\text{times}<3$), and 3 or more times a week (≥ 3 times)] and birth outcomes compared with a reference category [once a week or less (≤ 1 times)] were based on the calculation of tertiles of total fish intake in the pooled database, in an attempt to create a universal categorisation among cohorts.

3.2.2 In the Rhea mother-child birth cohort study

Breastfeeding

Breastfeeding initiation, duration, use of infant formula, complementary food, and other kinds of bottle food were collected at the 9th month post-partum and updated at the 18th month follow-up questionnaire. Mothers were asked if they had ever breastfed their child (or placed the child on their breast to feed). In case of never breastfed, the reason was recorded. If women initiated breastfeeding further information on breastfeeding intensity and duration was asked, as well as information regarding the first time they breastfed their infant and the duration of breastfeeding. Breastfeeding was also categorized according to the WHO breastfeeding definitions as exclusive, predominant, and complementary breastfeeding.

Diet in preschool age

Dietary assessment at 4 years of age was performed with the use of an FFQ, a semi-quantitative food frequency questionnaire designed to assess habitual dietary intake in preschool children. This questionnaire was previously validated with the use of 3-day weighed food record (FRs) that the mothers kept for their children. Primary caregivers were phone-interviewed by a dietitian trained in a standard protocol in order to complete the questionnaire. The questionnaire contained questions on 118 food items which were aggregated into 17 food groups. Parents could choose from one or two portion sizes and report the child's intake in terms of times per day, week, month, and year or never. Seasonality of consumption was also reported in all food items. FFQ data were converted into daily intake of foods and nutrients using the UK food tables (McCance & Widdowson's The Composition of Foods, 6th summary edition) and standard greek recipes for complex mixed dishes. The dietary intake data were analyzed by using a software program developed at the Department of Applied Information Technology and Multimedia, TEI-Crete, Heraklion, Crete, Greece

Eating behaviour in preschool age

Children's eating behaviour in preschool age we used the Children's Eating Behaviour Questionnaire (CEBQ) as developed by Wardle et al (Wardle, Guthrie et al. 2001). This instrument was designed to be completed by parents referring to children's eating styles and it assesses behaviour in 8 different areas. Food approach behaviours consist of food responsiveness, enjoyment of food, desire to drink, emotional overeating and food avoidant behaviours consist of satiety responsiveness, emotional under eating, slowness in eating and food fussiness. The cross-cultural adaption of the 34-item version of the CEBQ was performed according to internationally recommended methodology, using the following guidelines: forward translation, backward translation, cognitive debriefing process, and pretesting. Primary caregivers/parents were interviewed on the phone by a dietician following a standard protocol in order to complete the CEBQ questionnaire.

3.3 Neurodevelopmental assessment

Infancy

The children's mental and psychomotor development was assessed at 18 months (±6 weeks) using the 3rd edition of Bayley Scales of Infant and Toddler Development (Bayley-III). The Bayley-III assesses infant and toddler development across three domains: (i) The Cognitive Scale (COG), (ii) The Language Scale which is composed of the Receptive Communication (RC) and the Expressive Communication (EC) subtest, and (iii) The Motor Scale which is divided into the Fine Motor (FM) and the Gross Motor (GM) subtest (Bayley 2006). All testing was done at the Medical School of the University of Crete and two public hospitals in Heraklion, in the presence of the mother. Total administration time was approximately 90 minutes.

Preschool age

Children's neurodevelopment was assessed by two trained psychologists, with the age appropriate instrument MSCA, developed for children aged 2½ - 8½. In brief, MSCA test aims to identify possible developmental delay in different skills with the use of six scales: the Verbal scale, the Perceptual-Performance scale, the Quantitative scale, the General Cognitive scale the Memory and the Motor scale (McCarthy 1972). Executive function and cognitive functions of posterior cortex are two additional scales derived from the MSCA test (Barkley 2001; Julvez, Ribas-Fito et al. 2007).

The ADHD symptoms in preschool children were assessed with the 36-item ADHD interview test (ADHDT) as developed by Gilliam et al (1995)(Gilliam 1995). Mothers completed the interview, which is based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for ADHD. The instrument is designed to identify and evaluate ADHD in ages 3-23 years, and contains three subscales; (i) Hyperactivity, (ii) Inattention, and (iii) Impulsivity. It, also, provides an index for total ADHD difficulties. The ADHDT was translated and adapted to the Greek population (Maniadaki and Kakouros 2002).

3.4 Statistical analysis

In the European collaboration project

In this study associations were firstly analyzed at a cohort level. Secondly, cohort-specific effect estimates were combined by random and fixed effects meta-analysis. Means and standard deviations were used to describe normally-distributed variables, and medians and interquartile ranges were used to describe non normally-distributed variables, for continuous variables. Linear and log-binomial regression models were used for continuous and binary outcome measures respectively. Fish intake variables were used as continuous variables [effect estimated per 1 unit (times a week) increments] or categorized in three categories [≤1 time (reference category); 1<times<3; ≥3 times].

Meta-analyses were performed combining the cohort-specific estimates of the association between fish intake variables and each birth outcome. Heterogeneity was assessed using the Q test and by I^2 statistic If the result of the *Q*-test was statistically significant ($p < 0.05$), or I^2>25%, we used random effects analyses Exposure-response slopes derived for each cohort were plotted together with the summary slope from the meta-analysis using forest plots of β coefficients or relative risks with 95% confidence intervals (CIs). Several sensitivity analyses were performed. First, we estimated the effect on birth weight and low birth weight neonates after restriction to term deliveries.

Meta-analyses was repeated to determine the influence of any particular cohort effect, leaving out one cohort at a time. Potential effect modification by maternal smoking, and pre-pregnancy weight status was explored in stratified analyses.

In the Rhea mother-child birth cohort study

In summary, bivariate associations between normally distributed continuous dependent variables (Bayley scores) and categorical independent variables were studied using either Student t-test or ANOVA. Bivariate associations between non-normally continuous exposure variable (breastfeeding duration) and independent variables (predictors) were studied using non-parametric statistical methods (Mann-Whitney, Kruskal-Wallis), whereas associations of categorical exposure variables and independent variables were tested using Pearson's Chi-square test. Pearson's r or Spearman's rho correlation coefficient was used to estimate the strength of the association between continuous dependent and independent variables.

Multivariable linear regression models were implemented to examine the associations between the exposure and the outcome after adjusting for several confounders. Potential confounders/determinants related with the exposure and outcomes of interest in the bivariate models with p value <0.10 as well as *a priori* selected confounders were included in the multivariable models. Effect modification was evaluated using the likelihood ratio test. Fractional polynomials were used to evaluate the linearity of the relationship between exposure variables and neurodevelopmental outcomes using graphical representation. Estimated associations were described with β-coefficients and 95% CI. All hypothesis testing was conducted assuming a 0.05 significance level and a 2-sided alternative hypothesis. All statistical analyses were performed using SPSS Statistics 19 software (SPSS Inc, Chicago, IL, USA).

A more detailed description of the statistical methods used can be found in the following section, in each paper.

4. Results

4.1 In the European collaboration project

4.1.1 Paper 1. Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies

Main Findings:

- Our findings support the beneficial role of moderate fish intake during pregnancy in the risk of preterm birth. Women who ate fish more than once a week during pregnancy had a lower risk of preterm birth compared with women who rarely ate fish (once a week or less).
- Women with a higher intake of fish during pregnancy gave birth to neonates with higher birth weight by 8.9g for 1<times<3 a week, and by 15.2g for ≥3 times a week, independent of gestational age. The association was greater in smokers, and in overweight or obese women.

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Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies[1–4]

Vasiliki Leventakou, Theano Roumeliotaki, David Martinez, Henrique Barros, Anne-Lise Brantsaeter, Maribel Casas, Marie-Aline Charles, Sylvaine Cordier, Merete Eggesbø, Manon van Eijsden, Francesco Forastiere, Ulrike Gehring, Eva Govarts, Thorhallur I Halldórsson, Wojciech Hanke, Margaretha Haugen, Denise HM Heppe, Barbara Heude, Hazel M Inskip, Vincent WV Jaddoe, Maria Jansen, Cecily Kelleher, Helle Margrete Meltzer, Franco Merletti, Carolina Moltó-Puigmartí, Monique Mommers, Mario Murcia, Andreia Oliveira, Sjúrður F Olsen, Fabienne Pele, Kinga Polanska, Daniela Porta, Lorenzo Richiardi, Siân M Robinson, Hein Stigum, Marin Strøm, Jordi Sunyer, Carel Thijs, Karien Viljoen, Tanja GM Vrijkotte, Alet H Wijga, Manolis Kogevinas, Martine Vrijheid, and Leda Chatzi

ABSTRACT

Background: Fish is a rich source of essential nutrients for fetal development, but in contrast, it is also a well-known route of exposure to environmental pollutants.

Objective: We assessed whether fish intake during pregnancy is associated with fetal growth and the length of gestation in a panel of European birth cohort studies.

Design: The study sample of 151,880 mother-child pairs was derived from 19 population-based European birth cohort studies. Individual data from cohorts were pooled and harmonized. Adjusted cohort-specific effect estimates were combined by using a random- and fixed-effects meta-analysis.

Results: Women who ate fish >1 time/wk during pregnancy had lower risk of preterm birth than did women who rarely ate fish (≤1 time/wk); the adjusted RR of fish intake >1 but <3 times/wk was 0.87 (95% CI: 0.82, 0.92), and for intake ≥3 times/wk, the adjusted RR was 0.89 (95% CI: 0.84, 0.96). Women with a higher intake of fish during pregnancy gave birth to neonates with a higher birth weight by 8.9 g (95% CI: 3.3, 14.6 g) for >1 but <3 times/wk and 15.2 g (95% CI: 8.9, 21.5 g) for ≥3 times/wk independent of gestational age. The association was greater in smokers and in overweight or obese women. Findings were consistent across cohorts.

Conclusion: This large, international study indicates that moderate fish intake during pregnancy is associated with lower risk of preterm birth and a small but significant increase in birth weight. *Am J Clin Nutr* 2014;99:506–16.

INTRODUCTION

The fetal and infant period is a particularly critical developmental period, and there is evidence that has suggested that nutritional perturbations during this period have long-term effects on offspring health (1, 2). Fish is a rich source of nutrients such as polyunsaturated n−3 fatty acids, protein, selenium, iodine, and vitamin D, which are considered to be beneficial for fetal growth and development (3) but, in contrast, is also a well-known route of exposure to pollutants such as dioxins, polychlorinated biphenyls, methylmercury, and other heavy metals, which may adversely affect fetal growth and gestational length (4, 5). Findings from prospective birth cohort studies on the relation between fish intake during pregnancy and fetal growth have been discrepant, with reports of either positive or null (6–15) or negative (5, 16–18) effects. These divergent results have been compatible with a hypothesis that there is a differential influence by different types or constituents of fish on fetal growth and birth size. Furthermore, individual studies have not often been

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able to detect small effect sizes. Several recent randomized clinical trials (19–21), and 3 systematic reviews have suggested that maternal n−3 supplementation during pregnancy is associated with small but significant increases in the length of gestation and infant birth size (22–24). In contrast, in 2004, the advice jointly issued by 2 US Federal Government agencies for pregnant women or women likely to become pregnant was to restrict their overall consumption of seafood to 340 g/wk (ie, 2 portions/wk) and avoid fetal exposure to trace amounts of several pollutants (25). In this context, pregnant women are faced with conflicting reports on risks and benefits of fish intake, which results in controversy and confusion over the place of fish consumption in a healthy diet in pregnancy. We pooled and harmonized individual data from 151,880 mother-child pairs in

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19 European birth cohort studies to study the association of fish intake during pregnancy with fetal growth and the length of gestation.

SUBJECTS AND METHODS

Subjects

European population-based birth cohorts were able to participate if they included children born from 1990 onward, had information on fish intake during pregnancy, and as a minimum, were at least gestational age and had weight at birth. We identified 29 European birth cohorts from the European inventory of birth cohorts (www.birthcohorts.net) or from cohort's individual websites and published articles (assessed until June 2011). Seven cohorts did not reply to the invitation, and 3 cohorts declined participation for reasons not related to the current hypothesis. Participating cohorts targeted the general population and, altogether, covered births from 1996 to 2011. A data-transfer agreement document was signed by each cohort, and data sets, with personal identifiers removed, were transferred to the University of Crete. Each data set was checked for inconsistencies and completeness, and a total of 151,880 liveborn singleton births were included with available data (nonmissing values) on exposure, outcome, and confounding variables. In total, 27 subjects were excluded from the current analysis because of extreme values on gestational age (<20 or ≥45 wk) and birth weight (>7000 g); 79 subjects were excluded because of an implausible combination of gestational age and birth weight (26). Informed consent was obtained from all study participants as part of the original studies, and ethical approval was obtained from the local authorized institutional review boards. Characteristics of cohorts included in the current analysis are shown in **Table 1**.

Exposure assessment: fish intake during pregnancy

Exposure variables were measured as the frequency (times/wk) of total fish, fatty fish, lean fish, and seafood (other than fish) intake during pregnancy derived from cohort-specific food-frequency questionnaires or specially designed questionnaires for fish consumption during pregnancy (Table 1). Salmon, herring, mackerel, trout, sardines, Greenland halibut, anchovy, gurnard, and tuna were classified as fatty fishes, whereas cod, pollack, plaice, flounder, garfish, and similar species were classified as lean fishes. All cohorts assessed fish intake during pregnancy, except in the Endocrine disruptors: longitudinal study on pregnancy abnormalities, infertility, and childhood (France) cohort, where the period of assessment covered the year before pregnancy.

Assessments for standardized categories of fish intake [>1 but <3 times/wk and ≥3 times/wk] and birth outcomes compared with a reference category (≤1 time/wk) were based on the

TABLE 1
Description of participating cohorts[1]

Cohort	Recruitment period	Provided data on birth outcomes[2]	Provided data on fish intake	Method of dietary assessment	Subjects included[3]
		n	*n*		*n*
ABCD, Amsterdam, NL	2003–2004	7850	7825	Questionnaire	7719
DNBC, nationwide, DK	1996–2002	87,477	63,948	FFQ	57,921
EDEN, Nancy, Poitiers, FR	2003–2005	1905	1838	FFQ	1765
FLEHS I, Flanders, BE	2002–2004	1164	1093	FFQ	1056
GASPII, Rome, IT	2003–2004	606	589	FFQ	536
Generation R, Rotterdam, NL	2001–2006	3366	3366	FFQ	2678
Generation XXI, Porto, PT	2005–2006	357	359	FFQ	276
HUMIS, regional, NO	2003–2009	1734	1696	FFQ	1552
INMA, Asturias, Gipuzkoa, Sabadell, Valencia, ES	2003–2008	2473	2606	FFQ	2295
KOALA, regional, NL	2000–2003	2740	2740	FFQ	2707
Lifeways Cross Generation, Dublin, IR	2001–2003	1068	−1089	FFQ	662
LucKi, regional, NL	2006–current	600	587	FFQ	543
MoBa, nationwide, NO	1999–2008	62,099	62,099	FFQ	58,926
NINFEA, nationwide, IT	2005–current	2553	2268	Questionnaire	2213
PELAGIE, Brittany, FR	2002–2006	3321	3308	Questionnaire	3228
PIAMA, nationwide, NL	1996–1997	3930	3922	Questionnaire	3335
REPRO-PL, nationwide, PL	2007–2011	917	917	FFQ	902
RHEA, Heraklion, GR	2007–2008	1390	1060	FFQ	970
SWS, Southampton, UK	1998–2007	2642	2642	FFQ	2596
Pooled data	—	—	—	—	151,880

[1] ABCD, Amsterdam Born Children and their Development study; BE, Belgium; DK, Denmark; DNBC, Danish National Birth Cohort; EDEN, study on the pre and early postnatal determinants of child health and development; ES, Spain; FFQ, food-frequency questionnaire; FLEHS I, Flemish Center of Expertise on Environment and Health Studies; FR, France; GASPII, Genetic and Environment: Prospective Study on Infancy in Italy; Generation R, The Generation R Study; GR, Greece; HUMIS, Norwegian Human Milk Study; INMA, Infancia y Medio Ambiente–Environment and Childhood Project; IR, Ireland; IT, Italy; KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg Birth Cohort Study; Lifeways, Lifeways Cross Generation Cohort Study; LucKi, Luchtwegklachten bij Kinderen Cohort Study; MoBa, Norwegian Mother and Child Cohort Study; NINFEA, Nascita e INFanzia: gli Effetti dell'Ambiente; NL, Netherlands; NO, Norway; PELAGIE, Endocrine disruptors: Longitudinal study on pregnancy abnormalities, infertility, and childhood; PIAMA, Prevention and Incidence of Asthma and Mite Allergy; PL, Poland; PT, Portugal; REPRO-PL, Polish Mother and Child Cohort Study; RHEA, Mother Child Cohort in Crete; SWS, Southampton Women's Survey; UK, United Kingdom.

[2] Subjects with available information on birth weight or gestational age as provided by cohorts.

[3] Subjects with full information on exposure variables, birth weight, gestational age, and selected confounding variables.

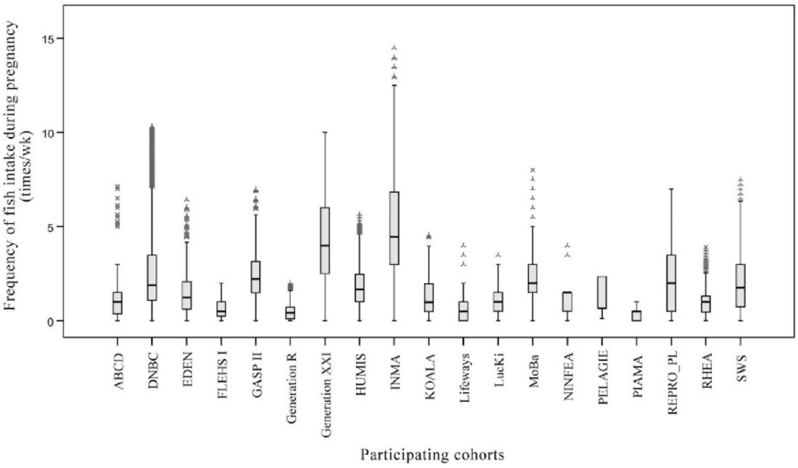

FIGURE 1. Distribution of the frequency of fish intake during pregnancy in participating cohorts. The symbol (▵) denotes outliers (>1.5 IQRs) of the distribution of fish intake during pregnancy for each cohort; the symbol (×) denotes extreme outliers (>3 IQRs) of the distribution of fish intake during pregnancy for each cohort. ABCD, Amsterdam Born Children and their Development study; DNBC, Danish National Birth Cohort; EDEN, study on the pre and early postnatal determinants of child health and development; FLEHS I, Flemish Center of Expertise on Environment and Health Studies; GASPII, Genetic and Environment: Prospective Study on Infancy in Italy; Generation R, The Generation R Study; HUMIS, Norwegian Human Milk Study; INMA, Infancia y Medio Ambiente–Environment and Childhood Project; KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg Birth Cohort Study; Lifeways, Lifeways Cross Generation Cohort Study; LucKi, Luchtwegklachten bij Kinderen Cohort Study; MoBa, Norwegian Mother and Child Cohort Study; NINFEA, Nascita e Infanzia: gli Effetti dell'Ambiente; PELAGIE, Endocrine disruptors: longitudinal study on pregnancy abnormalities, infertility, and childhood; PIAMA, Prevention and Incidence of Asthma and Mite Allergy; REPRO-PL, Polish Mother and Child Cohort Study; RHEA, Mother Child Cohort in Crete; SWS, Southampton Women's Survey.

calculation of tertiles of total fish intake in the pooled database in an attempt to create a universal categorization in cohorts. However, 6 cohorts [Flemish Center of Expertise on Environment and Health Studies, the Generation R Study, Generation XXI, Endocrine disruptors: longitudinal study on pregnancy abnormalities, infertility, and childhood; Prevention and Incidence of Asthma and Mite Allergy; and Mother-Child Cohort in Crete (RHEA)[5]] had at least one category that contained <5% of participants and, therefore, were excluded from this categorical dose-response analysis.

Birth outcomes

All cohorts provided information on birth weight, gestational age, and infant sex obtained from birth records, medical birth registries, or parental-completed questionnaires. Gestational age was estimated as the interval between the start of the last menstrual period (LMP) and delivery when available and, if this estimation was not inconsistent by ≥7 d, by using an ultrasound- based estimation (72% of births). The ultrasound-based estimation (20.8%) of gestational age was only used if the LMP was unavailable or if the LMP was inconsistent by ≥7 d with the ultrasound-based measurement taken in the first trimester of pregnancy. Finally, an obstetrician estimation (7.2%) was only used if the LMP and ultrasound-based measures were unavailable. Other continuous anthropometric measures provided by cohorts were birth length (available for 15 cohorts) and head circumference (available for 14 cohorts). Neonatal weights were defined as small for gestational age if they were below the 10th percentile of the cohort-specific growth curves stratified by gestational length and sex (available for 17 cohorts). The same method was used to define small-for-gestational-age neonates for length (available for 10 cohorts) and head circumference (available for 8 cohorts). Low birth weight was defined as any newborn with a birth weight <2500 g, whereas high birth weight was defined as a birth weight >4000 g. Preterm birth was defined as being born <37 wk of gestation.

Other variables

Potential confounding variables were defined as similarly as possible in cohorts given the information that was available. In all cohorts, information on maternal age at delivery (continuous in y), maternal prepregnancy BMI [continuous in kg/m^2 and categorized as normal weight (≥18.5 to <25), overweight (≥25 to <30), and obese (≥30)] and maternal height (continuous in cm) were collected by using questionnaires filled in during pregnancy or at birth, medical or national registries, or ad hoc measurements. Maternal educational level (low, medium, or high), maternal country of birth (country of the cohort or foreign country), maternal smoking during pregnancy (yes or no), and parity (multiparous or primiparous) were collected by using questionnaires filled in during pregnancy or at birth or medical birth registries.

[5]Abbreviations used: HUMIS, Norwegian Human Milk Study; INMA, Infancia y Medio Ambiente; LCPUFA, long-chain PUFA; LMP, last menstrual period; RHEA, Mother-Child Cohort in Crete.

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TABLE 2

Crude and adjusted combined associations of fish and seafood intake during pregnancy with birth weight and low-birth-weight neonates[1]

			Birth weight (g)			Low birth weight (<2500 g)		
	Cohorts	Subjects	Crude β (95% CI)	Adjusted β (95% CI)	P-heterogeneity	Crude RR (95% CI)	Adjusted RR (95% CI)	P-heterogeneity
Fish intake (times/wk)	19	151,880	1.98 (0.59, 3.26)[2]	1.46 (0.45, 2.46)[2]	0.31	1.00 (0.96, 1.04)[3]	1.00 (0.96, 1.04)[3]	0.06
Categories of fish intake[4]								
>1 but <3 times/wk	13	140,337	28.76 (21.59, 35.93)[2]	8.93 (3.31, 14.56)[2]	0.84	0.97 (0.94, 1.14)[3]	0.90 (0.77, 1.20)[3]	0.005
≥3 times/wk	13	140,337	36.79 (28.74, 44.85)[2]	15.20 (8.86, 21.54)[2]	0.67	0.97 (0.95, 1.18)[2]	0.91 (0.81, 1.02)[2]	0.44
Fatty fish (times/wk)	13	131,651	2.24 (−3.46, 7.94)[2]	2.38 (0.51, 4.25)[2]	0.97	0.99 (0.96, 1.01)[2]	0.98 (0.95, 1.02)[2]	0.31
Lean fish (times/wk)	12	129,886	1.02 (−5.09, 7.12)[3]	0.76 (−2.45, 3.98)[3]	0.11	1.03 (0.97, 1.1)[3]	1.05 (0.97, 1.13)[3]	0.004
Seafood (other than fish) (times/wk)	16	138,148	−7.23 (−18.95, 4.49)[3]	−3.92 (−14.31, 6.48)[3]	0.01	1.03 (0.95, 1.1)[2]	1.00 (0.92, 1.10)[2]	0.32

[1] β Coefficients (95% CIs) and RRs (95% CIs) were estimated by using a random- or fixed-effects meta-analysis by cohort. Linear and log-binomial regression models, respectively, were adjusted for maternal age, prepregnancy BMI, maternal height, education level, smoking during pregnancy, parity, infant sex, gestational age, and gestational age squared. P-heterogeneity values were estimated by using Cochran's Q test.

[2] Fixed-effects meta-analysis: P-heterogeneity ≥ 0.05 and $I^2 \leq 25\%$.

[3] Random-effects meta-analysis: P-heterogeneity < 0.05 or $I^2 > 25\%$.

[4] Reference category: ≤1 time/wk.

Statistical analysis

We used a 2-stage approach to assess the association of fish intake during pregnancy with birth outcomes. First, associations were analyzed at the cohort level. Second, cohort-specific effect estimates were combined by using a random- and fixed-effects meta-analysis.

Distributions of categorical variables were presented as frequencies and percentages. For continuous data, means ± SDs were used to describe normally distributed variables, and medians and IQRs were used to describe nonnormally distributed variables. Linear and log-binomial regression models were used for continuous and binary outcome measures, respectively. Fish-intake variables were used as continuous variables [effect estimated per 1-unit (times/wk) increments] or categorized in 3 categories [≤1 (reference category), >1 but <3, and ≥3 times/wk]. Adjustment for confounding variables was based on a priori selection of potential risk factors for reduced birth weight or gestational age, including maternal age at delivery (continuous in y), maternal height (continuous in cm), prepregnancy BMI (continuous), maternal education (low, medium, or high), smoking during pregnancy (yes and no), parity (multiparous or primiparous), and infant sex (boy or girl). Gestational age and the square of gestational age were included in models that assessed the association of fish intake during pregnancy with birth weight, birth length, head circumference, and low- and high-birth weight neonates. In 3 cohorts, the adjusted models did not include the full list of confounders because of the unavailability of information [the Luchtwegklachten bij Kinderen (LucKi) cohort provided no information on maternal age and education, the Lifeways Cross Generation cohort provided no information on prepregnancy BMI, and the Norwegian Human Milk Study (HUMIS) cohort provided limited information on parity before index pregnancy in the data set used for this study].

Meta-analyses were performed that combined cohort-specific estimates of the association between fish-intake variables and each birth outcome. Heterogeneity was assessed by using the Q test and by I^2 statistic (27, 28), which indicated the proportion of variability in the combined estimate attributable to the heterogeneity across cohorts. If the result of the Q test was statistically significant ($P < 0.05$), or I^2 was <25%, we used random-effects analyses (27, 28). Exposure-response slopes derived for each cohort were plotted together with the summary slope from the meta-analysis by using forest plots of β coefficients or RRs with 95% CIs.

Several sensitivity analyses were performed. First, we estimated the effect on birth weight and low-birth-weight neonates after restriction to term deliveries. To determine the influence of any particular cohort effect, we repeated the meta-analyses by leaving out one cohort at a time. In addition, a potential effect modification by maternal smoking and prepregnancy weight status was explored in stratified analyses. Statistical analyses were conducted with SPSS software (version 19; IBM Corp) and R Core Team v2.15.1 software (R Foundation for Statistical Computing).

RESULTS

The mean birth weight across cohorts ranged from 3.201 kg (RHEA cohort; Greece) to 3.595 kg (Danish National Birth Cohort; Denmark), and the mean gestational age ranged from

38.4 wk (RHEA cohort) to 40.1 wk (Danish National Birth Cohort). The proportion of preterm births ranged from 2.8% [Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg (KOALA) cohort; Netherlands] to 10.5% (RHEA cohort), whereas the proportion of low-birth-weight neonates ranged from 1.7% (Flemish Center of Expertise on Environment and Health Studies cohort; Belgium) to 6.4% (HUMIS cohort; Norway) (*see* Supplemental Table 1 under "Supplemental data" in the online issue). The number of boys in the whole population was higher than the number of girls (overall sex ratio: 1.05). The mean maternal age was ≥29 y in all populations. In the Generation XXI (Portugal), HUMIS (Norway), and Southampton Women's Survey (United Kingdom) cohorts, one-third of pregnant women were overweight or obese (BMI ≥25) before pregnancy. The proportion of women who smoked during pregnancy ranged from 7.7% (KOALA cohort) to 31% Infancia y Medio Ambiente (INMA) cohort; Spain] (*see* Supplemental Table 2 under "Supplemental data" in the online issue). The proportion of nulliparous women exceeded 50% in 9 populations (*see* Supplemental Table 2 under "Supplemental data" in the online issue). The median fish intake in the 19 study populations ranged from 0.4 times/wk (the Generation R study; Netherlands) to 4.5 times/wk (INMA cohort) (**Figure 1**; *see* Supplemental Table 3 under "Supplemental data" in the online issue). The median fatty fish intake in the Genetic and Environment: Prospective Study on Infancy in Italy (Italy), Generation XXI, INMA, and Polish Mother and Child Cohort Study (Poland) cohorts was more than double the overall median intake of 0.5 times/wk. The highest median lean fish (3.5 times/wk) and seafood intake other than fish (0.9 times/wk) was reported in the INMA cohort. Portion sizes of different fish types varied from 100 to 150 g across cohorts with available information on portion-size specification.

Fish intake and fetal growth

Fish intake in pregnancy was positively associated with birth weight and corresponded to a 1.5-g (95% CI: 0.5, 2.5-g) increase per 1-time/wk increase in fish intake (**Table 2**). In a subset of studies with available information on types of fish intake (131,651 participants) the corresponding β coefficient was 2.4 g (95% CI: 0.5, 4.3 g) for fatty fish types and 0.8 g (95% CI: −2.5, 4.0 g) for lean fish types (Table 2). Compared with women who rarely ate fish (≤1 time/wk), women with a higher intake of fish during pregnancy gave birth to neonates with a higher birth weight by 8.9 g (95% CI: 3.3, 14.6 g) for >1 but <3 times/wk and by 15.2 g (95% CI: 8.9, 21.5 g) for ≥3 times/wk (Table 2, **Figure 2**). No significant associations were shown between fish intake and low-birth-weight (Table 2), high-birth-weight, or small-for-gestational age for weight, length, and head circumference neonates (*see* Supplemental Table 4 under "Supplemental data" in the online issue). Findings did not change after the exclusion of extreme values of fish consumption (data not shown).

Fish intake and preterm birth

Fish intake was associated with a higher gestational age of 0.4 d (95% CI: 0.3, 0.6 d) for fish intake >1 but <3 times/wk and of

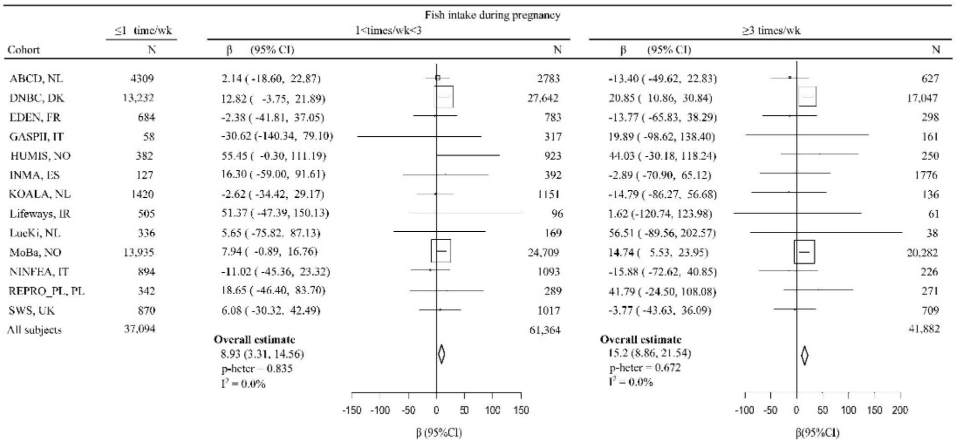


FIGURE 2. Adjusted associations of fish intake during pregnancy with birth weight. β Coefficients (95% CIs) by cohort were obtained by using linear regression models adjusted for maternal age, prepregnancy BMI, maternal height, education level, smoking during pregnancy, parity, infant sex, gestational age, and gestational age squared. Reference category was ≤1 time/wk. Overall estimates were obtained by using a random- or fixed-effects meta-analysis. p-heter values were estimated by using Cochran's Q test. ABCD, Amsterdam Born Children and their Development study; DK, Denmark; DNBC, Danish National Birth Cohort; EDEN, study on the pre and early postnatal determinants of child health and development; ES, Spain; FR, France; GASPII, Genetic and Environment: Prospective Study on Infancy in Italy; HUMIS, Norwegian Human Milk Study; INMA, Infancia y Medio Ambiente–Environment and Childhood Project; IR, Ireland; IT, Italy; KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg Birth Cohort Study; Lifeways, Lifeways Cross Generation Cohort Study; LucKi, Luchtwegklachten bij Kinderen Cohort Study; MoBa, Norwegian Mother and Child Cohort Study; NINFEA, Nascita e INFanzia: gli Effetti dell'Ambiente; NL, Netherlands; NO, Norway; p-heter, *P*-heterogeneity; PL, Poland; REPRO-PL, Polish Mother and Child Cohort Study; SWS, Southampton Women's Survey; UK, United Kingdom.

TABLE 3
Crude and adjusted combined associations of fish and seafood intake during pregnancy with gestational age and preterm birth[1]

			Gestational age (d)			Preterm birth (<37 wk gestational age)		
	Cohorts	Subjects	Crude β (95% CI)	Adjusted β (95% CI)	*P*-heterogeneity	Crude RR (95% CI)	Adjusted RR (95% CI)	*P*-heterogeneity
Fish intake (times/wk)	19	151,880	−0.02 (−0.09, 0.03)[2]	−0.02 (−0.09, 0.05)[2]	0.06	1.00 (0.97, 1.01)[2]	1.00 (0.97, 1.03)[2]	0.008
Categories of fish intake[3]								
>1 but <3 times/wk	13	140,337	0.47 (0.19, 0.74)[4]	0.41 (0.25, 0.57)[4]	0.24	0.89 (0.81, 0.93)[4]	0.87 (0.82, 0.92)[4]	0.36
≥3 times/wk	13	140,337	0.21 (0.03, 0.45)[4]	0.23 (0.05, 0.41)[4]	0.44	0.87 (0.82, 0.94)[4]	0.89 (0.84, 0.96)[4]	0.55
Fatty fish (times/wk)	13	131,651	0.15 (0.34, 0.03)[2]	0.14 (−0.31, 0.03)[2]	<0.001	1.04 (0.98, 1.10)[2]	1.04 (0.98, 1.09)[2]	0.02
Lean fish (times/wk)	12	129,886	−0.02 (−0.11, 0.07)[2]	−0.02 (−0.12, 0.08)[2]	0.06	0.99 (0.95, 1.04)[2]	1.00 (0.96, 1.05)[2]	0.03
Seafood (other than fish) (times/wk)	16	138,148	−0.02 (−0.17, 0.12)[4]	−0.03 (−0.18, 0.12)[4]	0.32	1.02 (0.96, 1.08)[4]	1.01 (0.96, 1.07)[4]	0.49

[1] β Coefficients (95% CIs) and RRs (95% CIs) were estimated by using a random- or fixed-effects meta-analysis by cohort. Linear and log-binomial regression models, respectively, were adjusted for maternal age, prepregnancy BMI, maternal height, education level, smoking during pregnancy, parity, and infant sex. *P*-heterogeneity values were estimated by using Cochran's *Q* test.
[2] Random-effects meta-analysis: *P*-heterogeneity < 0.05 or $I^2 > 25\%$.
[3] Reference category: ≤1 time/wk.
[4] Fixed-effects meta-analysis: *P*-heterogeneity ≥0.05 and $I^2 \leq 25\%$.

0.2 d (95% CI: 0.1, 0.4 d) for fish intake ≥3 times/wk (**Table 3**). Correspondingly, women who ate fish more than 1 time/wk during pregnancy had lower risk of preterm birth than did women who rarely ate fish (≤1 time/wk); the adjusted RR of fish intake >1 but <3 times/wk was 0.87 (95% CI: 0.82, 0.92), and for women who consumed fish ≥3 times/wk, the adjusted RR was 0.89 (95% CI: 0.84, 0.96) (Table 3, **Figure 3**).

Stratified and sensitivity analyses

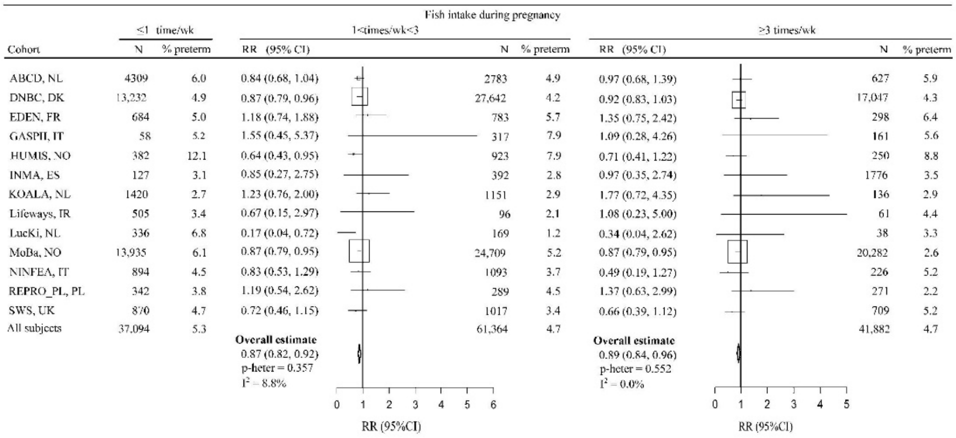
The association of fish intake during pregnancy with birth weight was more pronounced in pregnant women who smoked during pregnancy [β coefficient: 39.5 g (95% CI: 23.5, 55.5 g) for smokers who consumed fish >3 times/wk compared with <1 time/wk; *P*-interaction = 0.01); a significant increase was also observed in nonsmokers but was less pronounced (β coefficient: 10 g (95% CI: 3.2, 17 g) for fish intake ≥3 times/wk) (**Table 4**). A greater association of fish intake with birth weight was also observed in the stratum of women who were overweight or obese prepregnancy than in women with normal BMI prepregnancy (*P*-interaction = 0.03; Table 4).

A sensitivity analysis restricted to infants born at term (gestational age between 37 and 42 wk) showed no material changes in effect estimates for birth weight and low-birth-weight neonates (*see* Supplemental Table 5 under "Supplemental data" in the online issue). We observed similar effect estimates for birth weight and preterm birth, after excluding cohorts one by one, indicating that the overall effects were not produced by any particular population (*see* Supplemental Table 6 under "Supplemental data" in the online issue).

DISCUSSION

To our knowledge, this is the largest study conducted to assess the association of fish intake during pregnancy with birth weight and length of gestation, with the inclusion of >150,000 mother-child pairs. Our findings were consistent between cohorts and supported the evidence for a beneficial role of moderate fish intake during pregnancy in risk of preterm birth and a small but significant increase in birth weight. To what extent this slightly increased fetal growth in the group of women who frequently consumed fish during pregnancy is likely to be associated with future child development is unknown. The ongoing longitudinal follow-up of these population-based birth cohort studies will allow for the study the longer-term consequences of fish intake during pregnancy on child growth and development.

It is possible that the potential benefit of fish consumption could be attributed to its content of n−3 long-chain PUFAs (LCPUFAs). This possibility was supported by the fact that the most-pronounced effect on birth weight was observed for fatty fish types. n−3 LCPUFAs consist primarily of EPA (20:5n−3) and DHA (22:6n−3). Pregnancy is associated with a reduction in the maternal serum DHA percentage and its possible depletion in the maternal store (29). Because the synthesis of n−3 LCPUFAs in the fetus and placenta is low, both the maternal status and placental function are critical for their supply to the fetus (30). It was proposed that n−3 LCPUFAs might also reduce the activity of eicosanoid promoters of the parturition process, particularly prostaglandins F and E and increase the activity of eicosanoids with myometrial relaxant properties, such



Cohort	≤1 time/wk N	≤1 time/wk % preterm	1<times/wk<3 RR (95% CI)	1<times/wk<3 N	1<times/wk<3 % preterm	≥3 times/wk RR (95% CI)	≥3 times/wk N	≥3 times/wk % preterm
ABCD, NL	4309	6.0	0.84 (0.68, 1.04)	2783	4.9	0.97 (0.68, 1.39)	627	5.9
DNBC, DK	13,232	4.9	0.87 (0.79, 0.96)	27,642	4.2	0.92 (0.83, 1.03)	17,047	4.3
EDEN, FR	684	5.0	1.18 (0.74, 1.88)	783	5.7	1.35 (0.75, 2.42)	298	6.4
GASPII, IT	58	5.2	1.55 (0.45, 5.37)	317	7.9	1.09 (0.28, 4.26)	161	5.6
HUMIS, NO	382	12.1	0.64 (0.43, 0.95)	923	7.9	0.71 (0.41, 1.22)	250	8.8
INMA, ES	127	3.1	0.85 (0.27, 2.75)	392	2.8	0.97 (0.35, 2.74)	1776	3.5
KOALA, NL	1420	2.7	1.23 (0.76, 2.00)	1151	2.9	1.77 (0.72, 4.35)	136	2.9
Lifeways, IR	505	3.4	0.67 (0.15, 2.97)	96	2.1	1.08 (0.23, 5.00)	61	4.4
LucKi, NL	336	6.8	0.17 (0.04, 0.72)	169	1.2	0.34 (0.04, 2.62)	38	3.3
MoBa, NO	13,935	6.1	0.87 (0.79, 0.95)	24,709	5.2	0.87 (0.79, 0.95)	20,282	2.6
NINFEA, IT	894	4.5	0.83 (0.53, 1.29)	1093	3.7	0.49 (0.19, 1.27)	226	5.2
REPRO_PL, PL	342	3.8	1.19 (0.54, 2.62)	289	4.5	1.37 (0.63, 2.99)	271	2.2
SWS, UK	870	4.7	0.72 (0.46, 1.15)	1017	3.4	0.66 (0.39, 1.12)	709	5.2
All subjects	37,094	5.3	Overall estimate 0.87 (0.82, 0.92) p-heter = 0.357 I^2 = 8.8%	61,364	4.7	Overall estimate 0.89 (0.84, 0.96) p-heter = 0.552 I^2 = 0.0%	41,882	4.7

FIGURE 3. Adjusted associations of fish intake during pregnancy with preterm birth. RRs (95% CIs) by cohort were obtained by using log-binomial regression models adjusted for maternal age, prepregnancy BMI, maternal height, education level, smoking during pregnancy, and infant sex. Reference category was ≤1 time/wk. Overall estimates were obtained by using a random- or fixed-effects meta-analysis. p-heter values were estimated by using Cochran's *Q* test. ABCD, Amsterdam Born Children and their Development study; DK, Denmark; DNBC, Danish National Birth Cohort; EDEN, study on the pre and early postnatal determinants of child health and development; ES, Spain; FR, France; GASPII, Genetic and Environment: Prospective Study on Infancy in Italy; HUMIS, Norwegian Human Milk Study; INMA, INMA-Environment and Childhood Project; IR, Ireland; IT, Italy; KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg Birth Cohort Study; Lifeways, Lifeways Cross Generation Cohort Study; LucKi, Luchtwegklachten bij Kinderen Cohort Study; MoBa, Norwegian Mother and Child Cohort Study; NINFEA, Nascita e INFanzia: gli Effetti dell'Ambiente; NL, Netherlands; NO, Norway; p-heter, *P*-heterogeneity; PL, Poland; REPRO-PL, Polish Mother and Child Cohort Study; SWS, Southampton Women's Survey; UK, United Kingdom.

as prostacyclins, resulting in an increase in pregnancy duration (31, 32). A shift of the prostacyclin/thromboxane A balance to a more antiaggregatory and vasodilator state might also increase the placental flow and, as a consequence, fetal growth (33). Several randomized controlled trials showed that maternal intake of n−3 LCPUFA during pregnancy resulted in a slightly longer gestation period and somewhat higher birth size, and these results were also confirmed in 3 recent meta-analyses (22–24). Fish are also a good source of vitamin D and B complex and several essential aminoacids and trace elements (eg, selenium, calcium, magnesium, potassium, and iodine), which have been linked to potentially favorable birth outcomes (34–36).

Because the balance between the potential beneficial effect of n−3 LCPUFAs and deleterious effect of contaminants in fish intake such as toxins and metals is determined by the relative exposure, results may differ across populations consuming different types of seafood (4, 5, 37). The protective effect of fish intake on preterm birth was shown only in the categorical analysis and not in the continuous analysis. This result could mean that, for very high amounts of fish intake, the protective effect is attenuated and RRs get closer to 1 (a U-shaped association). We were not able to address potential effects at very high amounts of fish consumption through a stratified analysis, as there were only few women with high values of fish intake, and they were not present in all examined cohorts, which made international comparisons difficult. Moreover, we did not have the possibility to collect accurate information on amounts of polychlorinated biphenyls, mercury, and dioxins across all cohorts, which are contaminants that have been negatively associated with birth size and gestational length (38–40). In general, we would expect confounding from pollutants bioaccumulating in fish to bias the association between fish intake and birth weight toward the null. Therefore, any true effect size might be larger than the one reported in this article in the absence of correction for fish pollutants.

We observed a greater association of fish intake with birth weight in the stratum of pregnant women who smoked during pregnancy compared with nonsmokers. Cigarette smoking during pregnancy has been previously shown to cause a 100–300-g reduction in birth weight, whereas other research has indicated even an ∼500-g reduction in populations with certain metabolic gene polymorphisms (41). Numerous studies have shown that smokers generally have poorer-quality diets than do nonsmokers, with lower intakes of fish and antioxidant- rich foods, and the same applies for pregnant women (42). Therefore, the protective effect of regular fish intake during pregnancy in smokers might reflect a high fetal exposure to n−3 LCPUFAs and several antioxidant compounds and their property to counter the effect of oxidative stress damage by smoking on fetal tissues. In contrast, in the nonsmokers group, there was also observed a significant increase in birth weight, and the trend over categories was positive (although weaker compared with for smokers). This positive association provides stronger evidence that there is a true association between fish intake and birth weight because any residual confounding by smoking was excluded in this group.

The current study confirmed a more-pronounced association of fish intake during pregnancy with birth weight in overweight and

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TABLE 4
Adjusted combined associations of fish and seafood intake during pregnancy with birth weight (g) stratified by maternal smoking during pregnancy and maternal weight status prepregnancy[1]

	Smoking during pregnancy					Prepregnancy overweight/obesity status				
		Smokers		Nonsmokers			Normal		Overweight or obese	
	Cohorts	Subjects	β (95% CI)	Subjects	β (95% CI)	Cohorts	Subjects	β (95% CI)	Subjects	β (95% CI)
Fish intake (times/wk)	19	25,053	3.02 (0.78, 5.27)[2]	126,827	−0.31 (−2.69, 2.07)[3]	18	108,302	0.69 (−0.48, 1.85)[2]	42,916	1.72 (−1.95;5.39)[3]
Categories of fish intake[4]										
>1 but <3 times/wk	13	22,517	29.25 (14.95, 43.55)[2]	117,820	4.43 (−1.69, 10.55)[2]	12	99,290	2.45 (−4.12, 9.03)[2]	40,385	14.88 (3.84;25.92)[2]
≥3 times/wk	13	22,517	39.47 (23.46, 55.49)[2]	117,820	10.08 (3.18, 16.99)[2]	12	99,290	8.15 (0.74, 15.56)[2]	40,385	22.30 (9.88;34.73)[2]
Fatty fish (times/wk)	13	22,433	4.42 (0.20, 8.64)[2]	109,806	1.78 (−0.31, 3.86)[2]	12	56,238	0.74 (−1.42, 2.89)[2]	38,587	4.72 (0.94;8.51)[2]
Lean fish (times/wk)	12	21,966	3.41 (−0.05, 6.88)[2]	108,508	−1.45 (−5.94, 3.04)[3]	11	54,931	−1.04 (−5.27, 3.20)[3]	38,129	1.61 (−1.42;4.65)[2]
Seafood (other than fish) (times/wk)	16	23,096	−3.88 (−15.27, 7.52)[2]	115,052	−2.89 (−12.94, 7.17)[3]	15	97,626	−6.86 (−19.66, 5.95)[3]	39,860	1.77 (−15.72,19.26)[3]

[1] β Coefficients (95% CIs) were estimated by using a random- or fixed-effects meta-analysis by cohort. Linear regression models were adjusted for maternal age, prepregnancy BMI, maternal height, education level, parity, smoking during pregnancy, infant sex, gestational age, and gestational age squared.
[2] Fixed-effects meta-analysis: P-heterogeneity ≥ 0.05 and $I^2 \leq 25\%$.
[3] Random-effects meta-analysis: P-heterogeneity < 0.05 or $I^2 > 25\%$.
[4] Reference category: ≤1 time/wk.

obese women, which is a result that is similar to the findings by Drouillet et al (43) in the study on the pre and early postnatal determinants of child health and development birth cohort. The storage of n−3 LCPUFAs in maternal adipose tissue is of great importance because it represents a pool of fatty acids that can be used via placental transfer to supply the developing fetus (43, 44). Therefore, it might be possible that overweight and obese women with regular fish intake during pregnancy have an enhanced ability to release fatty acids from adipose tissue to sustain fetal growth.

Discrepant findings in earlier birth cohort studies on fish intake and birth outcomes have been puzzling (5–18). Reasons for the inconsistencies may be inadequate sample sizes, exposure misclassification, exposure profile heterogeneity [(ie, consumption frequencies compared with estimated daily intakes (in g)], or differences in adjustment.

Our international study, involving a large number of mother-child pairs, comprehensively recorded a wide range of exposure that allowed us to carry out the most-detailed exploration of potential heterogeneity than, to our knowledge, has been previously reported. In populations included in the current meta-analysis, only 4 populations showed inverse associations between fish intake and birth weight, of which none of the associations was significant. The current findings underscore scientific gaps in the experimental evidence of fish intake during pregnancy, specifically the lack of studies that involve healthy populations and randomized clinical trials that target fish intake rather than using supplements, which may have different mechanistic effects.

Strengths of the current study included the population-based prospective design, large sample size, and centralized statistical analysis after a consensus protocol. We did not rely on published data, which excluded any potential publication bias. The study population included women from the follow-up of several birth cohorts, which provided us with the opportunity to account for the effect of exposures during pregnancy prospectively collected within each cohort. In addition, we adjusted for many socioeconomic and lifestyle variables known to be associated with fish intake during pregnancy and fetal growth, although some residual confounding, mainly related to socioeconomic positions, could not be completely ruled out. After we excluded cohorts one by one, effect estimates did not change importantly, which minimized the effect of single cohorts.

As in most studies on diet and health, we used self-reported dietary information during pregnancy, and therefore, an information bias could have occurred. However, in the majority of cohorts, fish intake was assessed by using a detailed food-frequency questionnaire that was developed and validated for use in pregnancy. Studies of nutrition in pregnancy have suggested that food-frequency methods could present valid and reproducible estimates of dietary intakes in pregnant women (45). Moreover, we collected detailed information on the consumption of different fish types, which enabled us to separate the analyses, although we did not have enough data to distinguish between big and small species that would be relevant in terms of toxicant exposures. Women who consume more fish may have a healthier diet and lifestyle. Although careful adjustment for potential lifestyle confounding variables did not appreciably alter the results, we did not have information on other dietary variables, fish-oil supplementation, and alcohol intake during pregnancy across all cohorts.

Because preterm birth is a rather heterogeneous entity, we did not have the possibility to distinguish between spontaneous and medically indicated preterm births because this information was not available across all cohorts.

In conclusion, available data from European birth cohort studies indicate that moderate fish intake during pregnancy is associated with lower risk of preterm birth and a small but significant increase in birth weight. Although these findings cannot establish causality, they support the need for public health advice to promote fish consumption in pregnant women in accordance with country-specific restrictions regarding fish species and items known to have high concentrations of pollutants.

The authors' responsibilities were as follows—LC: designed the research; LC, VL, and TR: conducted the research, had full access to all study data, and took responsibility for the integrity of the data and accuracy of the data analysis; TR and DM: analyzed data and performed the statistical analysis; VL and LC: wrote the manuscript: LC, MK, and MV: supervised the study; and all authors: provided essential materials (cohort-specific databases necessary for research), had primary responsibility for the final content of the manuscript, and read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES

1. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 2008;359:61–73.
2. Koletzko B, Symonds ME, Olsen SF. Programming research: where are we and where do we go from here? Am J Clin Nutr 2011;94: 2036S–43S.
3. Philibert A, Vanier C, Abdelouahab N, Chan HM, Mergler D. Fish intake and serum fatty acid profiles from freshwater fish. Am J Clin Nutr 2006;84:1299–307.
4. Mahaffey KR. Fish and shellfish as dietary sources of methylmercury and the omega-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: risks and benefits. Environ Res 2004;95:414–28.
5. Halldorsson TI, Thorsdottir I, Meltzer HM, Nielsen F, Olsen SF. Linking exposure to polychlorinated biphenyls with fatty fish consumption and reduced fetal growth among Danish pregnant women: a cause for concern? Am J Epidemiol 2008;168:958–65.
6. Brantsæter AL, Birgisdottir BE, Meltzer HM, Kvalem HE, Alexander J, Magnus P, Haugen M. Maternal seafood consumption and infant birth weight, length and head circumference in the Norwegian Mother and Child Cohort Study. Br J Nutr 2012;107:436–44.
7. Drouillet P, Kaminski M, De Lauzon-Guillain B, Forhan A, Ducimetiere P, Schweitzer M, Magnin G, Goua V, Thiebaugeorges O, Charles MA. Association between maternal seafood consumption before pregnancy and fetal growth: evidence for an association in overweight women. The EDEN mother-child cohort. Paediatr Perinat Epidemiol 2009;23:76–86.
8. Guldner L, Monfort C, Rouget F, Garlantezec R, Cordier S. Maternal fish and shellfish intake and pregnancy outcomes: a prospective cohort study in Brittany, France. Environ Health 2007;6:33.
9. Heppe DH, Steegers EA, Timmermans S, Breeijen H, Tiemeier H, Hofman A, Jaddoe VW. Maternal fish consumption, fetal growth and the risks of neonatal complications: the Generation R Study. Br J Nutr 2011;105:938–49.
10. Oken E, Kleinman KP, Olsen SF, Rich-Edwards JW, Gillman MW. Associations of seafood and elongated n−3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort. Am J Epidemiol 2004;160:774–83.
11. Olsen SF, Osterdal ML, Salvig JD, Kesmodel U, Henriksen TB, Hedegaard M, Secher NJ. Duration of pregnancy in relation to seafood intake during early and mid pregnancy: prospective cohort. Eur J Epidemiol 2006;21:749–58.
12. Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. BMJ 2002;324:447.
13. Thorsdottir I, Birgisdottir BE, Halldorsdottir S, Geirsson RT. Association of fish and fish liver oil intake in pregnancy with infant size at birth among women of normal weight before pregnancy in a fishing community. Am J Epidemiol 2004;160:460–5.
14. Olsen SF, Olsen J, Frische G. Does fish consumption during pregnancy increase fetal growth? A study of the size of the newborn, placental weight and gestational age in relation to fish consumption during pregnancy. Int J Epidemiol 1990;19:971–7.
15. Olsen SF, Grandjean P, Weihe P, Videro T. Frequency of seafood intake in pregnancy as a determinant of birth weight: evidence for a dose dependent relationship. J Epidemiol Community Health 1993;47:436–40.
16. Halldorsson TI, Meltzer HM, Thorsdottir I, Knudsen V, Olsen SF. Is high consumption of fatty fish during pregnancy a risk factor for fetal growth retardation? A study of 44,824 Danish pregnant women. Am J Epidemiol 2007;166:687–96.
17. Mendez MA, Plana E, Guxens M, Foradada Morillo CM, Albareda RM, Garcia-Esteban R, Goni F, Kogevinas M, Sunyer J. Seafood consumption in pregnancy and infant size at birth: results from a prospective Spanish cohort. J Epidemiol Community Health 2010;64:216–22.
18. Rylander L, Stromberg U, Hagmar L. Lowered birth weight among infants born to women with a high intake of fish contaminated with persistent organochlorine compounds. Chemosphere 2000;40:1255–62.
19. Makrides M, Gibson RA, McPhee AJ, Yelland L, Quinlivan J, Ryan P. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. JAMA 2010;304:1675–83.
20. Hauner H, Much D, Vollhardt C, Brunner S, Schmid D, Sedlmeier EM, Heimberg E, Schuster T, Zimmermann A, Schneider KT, et al. Effect of reducing the n−6:n−3 long-chain PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial. Am J Clin Nutr 2012;95:383–94.
21. Carlson SE, Colombo J, Gajewski BJ, Gustafson KM, Mundy D, Yeast J, Georgieff MK, Markley LA, Kerling EH, Shaddy DJ. DHA supplementation and pregnancy outcomes. Am J Clin Nutr 2013;97:808–15.
22. Makrides M, Duley L, Olsen SF. Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by preeclampsia or intrauterine growth restriction. Cochrane Database Syst Rev 2006;CD003402.
23. Salvig JD, Lamont RF. Evidence regarding an effect of marine n−3 fatty acids on preterm birth: a systematic review and meta-analysis. Acta Obstet Gynecol Scand 2011;90:825–38.
24. Szajewska H, Horvath A, Koletzko B. Effect of n−3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2006; 83:1337–44.
25. US Department of Health and Human Services, US Environmental Protection Agency. What you need to know about mercury in fish and shellfish 2004 EPA and FDA advice for: women who might become pregnant women who are pregnant nursing mothers young children. Washington, DC: US Government Printing Office, 2004.
26. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstet Gynecol 1996;87: 163–8.
27. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.
28. Huedo-Medina TB, Sanchez-Meca J, Marin-Martinez F, Botella J. Assessing heterogeneity in meta-analysis: Q statistic or I2 index? Psychol Methods 2006;11:193–206.
29. Matorras R, Ruiz JI, Perteagudo L, Barbazan MJ, Diaz A, Valladolid A, Sanjurjo P. Longitudinal study of fatty acids in plasma and erythrocyte phospholipids during pregnancy. J Perinat Med 2001;29:293–7.
30. Al MD, van Houwelingen AC, Hornstra G. Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. Am J Clin Nutr 2000;71:285S–91S.
31. Olsen SF, Hansen HS, Sorensen TI, Jensen B, Secher NJ, Sommer S, Knudsen LB. Intake of marine fat, rich in (n−3)-polyunsaturated fatty acids, may increase birthweight by prolonging gestation. Lancet 1986; 2:367–9.
32. Olsen SF. Is supplementation with marine omega-3 fatty acids during pregnancy a useful tool in the prevention of preterm birth? Clin Obstet Gynecol 2004;47:768–74, discussion 881–2.
33. Sørensen JD, Olsen SF, Pedersen AK, Boris J, Secher NJ, FitzGerald GA. Effects of fish oil supplementation in the third trimester of pregnancy on prostacyclin and thromboxane production. Am J Obstet Gynecol 1993;168:915–22.

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34. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. BMJ 2013;346:f1169.
35. Buppasiri P, Lumbiganon P, Thinkhamrop J, Ngamjarus C, Laopaiboon M. Calcium supplementation (other than for preventing or treating hypertension) for improving pregnancy and infant outcomes. Cochrane Database Syst Rev 2011;CD007079.
36. Morse NL. Benefits of docosahexaenoic acid, folic acid, vitamin D and iodine on foetal and infant brain development and function following maternal supplementation during pregnancy and lactation. Nutrients 2012;4:799–840.
37. Ramón R, Ballester F, Aguinagalde X, Amurrio A, Vioque J, Lacasana M, Rebagliato M, Murcia M, Iniguez C. Fish consumption during pregnancy, prenatal mercury exposure, and anthropometric measures at birth in a prospective mother-infant cohort study in Spain. Am J Clin Nutr 2009;90:1047–55.
38. Govarts E, Nieuwenhuijsen M, Schoeters G, Ballester F, Bloemen K, de Boer M, Chevrier C, Eggesbo M, Guxens M, Kramer U, et al. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts. Environ Health Perspect 2012;120:162–70.
39. Papadopoulou E, Caspersenb HI, Kvalem HE, Knutsen HK, Duarte-Salles T, Alexander J, Meltzer HM, Kogevinas M, Brantsæter AL, Haugen M. Maternal dietary intake of dioxins and polychlorinated biphenyls and birth size in the Norwegian Mother and Child Cohort Study (MoBa). Environ Int 2013;60:209–16.
40. Vejrup K, Brantsæter AL, Knutsen HK, Magnus P, Alexander J, Kvalem HE, Meltzer HM, Haugen M. Prenatal mercury exposure and infant birth weight in the Norwegian Mother and Child Cohort Study. Public Health Nutr (Epub ahead of print 8 October 2013).
41. Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, Niu T, Wise PH, Bauchner H, Xu X. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. JAMA 2002;287:195–202.
42. Palaniappan U, Jacobs Starkey L, O'Loughlin J, Gray-Donald K. Fruit and vegetable consumption is lower and saturated fat intake is higher among Canadians reporting smoking. J Nutr 2001;131:1952–8.
43. Drouillet P, Forhan A, De Lauzon-Guillain B, Thiebaugeorges O, Goua V, Magnin G, Schweitzer M, Kaminski M, Ducimetiere P, Charles MA. Maternal fatty acid intake and fetal growth: evidence for an association in overweight women. The 'EDEN mother-child' cohort (study of pre- and early postnatal determinants of the child's development and health). Br J Nutr 2009;101:583–91.
44. Dutta-Roy AK. Transport mechanisms for long-chain polyunsaturated fatty acids in the human placenta. Am J Clin Nutr 2000;71:315S–22S.
45. Meltzer HM, Brantsaeter AL, Ydersbond TA, Alexander J, Haugen M. Methodological challenges when monitoring the diet of pregnant women in a large study: experiences from the Norwegian Mother and Child Cohort Study (MoBa). Matern Child Nutr 2008;4:14–27.

4.2 In the Rhea mother-child birth cohort study

4.2.1 Paper 2. Breastfeeding duration and cognitive, language, and motor development at 18 months of age: Rhea mother-child cohort in Crete, Greece

Main Findings:

- Longer duration of breastfeeding duration was associated with increased scores in the scales of cognitive, language, and fine motor development at 18 months of age.
- Children who were breastfed longer than 6 months had 4.44 points increase in the scale of fine motor development compared with those never breastfed.

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Breastfeeding duration and cognitive, language and motor development at 18 months of age: Rhea mother–child cohort in Crete, Greece

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ABSTRACT

Background Breast feeding duration has been associated with improved cognitive development in children. However, few population-based prospective studies have evaluated dose–response relationships of breastfeeding duration with language and motor development at early ages, and results are discrepant.
Methods The study uses data from the prospective mother–child cohort ('Rhea' study) in Crete, Greece. 540 mother–child pairs were included in the present analysis. Information about parental and child characteristics and breastfeeding practices was obtained by interview-administered questionnaires. Trained psychologists assessed cognitive, language and motor development by using the Bayley Scales of Infant Toddler Development (3rd edition) at the age of 18 months.
Results Duration of breast feeding was linearly positively associated with all the Bayley scales, except of gross motor. The association persisted after adjustment for potential confounders with an increase of 0.28 points in the scale of cognitive development (β=0.28; 95% CI 0.01 to 0.55), 0.29 points in the scale of receptive communication (β=0.29; 95% CI 0.04 to 0.54), 0.30 points in the scale of expressive communication (β=0.30; 95% CI 0.04 to 0.57) and 0.29 points in the scale of fine motor development (β=0.29; 95% CI 0.02 to 0.56) per accumulated month of breast feeding. Children who were breast fed longer than 6 months had a 4.44-point increase in the scale of fine motor development (β=4.44; 95% CI 0.06 to 8.82) compared with those never breast fed.
Conclusions Longer duration of breast feeding was associated with increased scores in cognitive, language and motor development at 18 months of age, independently from a wide range of parental and infant characteristics. Additional longitudinal studies and trials are needed to confirm these results.

INTRODUCTION

Breast feeding is a key component of optimal infant nutrition and provides many important health benefits to both babies and mothers. It contains the ideal amounts of fatty acids, lactose, water and amino acids for human digestion, brain development and growth, as well as many bioactive ingredients such as cytokines, nucleotides, hormones and growth factors.[1 2] Its high content of docosahexaenoic acid (DHA; 22:6 n-3), a major form of n-3 long-chain polyunsaturated fatty acids (LC-PUFAs), is well known for its beneficial effects on neurotransmission, neurodevelopment and particularly visual acuity.[3–5] The WHO considers exclusive breast feeding for the first 6 months of life to be the optimal method for feeding infants, a recommendation that is also supported by the American Academy of Paediatrics.[6] Breast feeding is recommended for at least 2 years and for as long thereafter as mother and child desire it. The frequency and duration of breast feeding in Crete, Greece, is relatively low compared with WHO recommendations.[7]

Several recent systematic reviews tried to summarise the effect of breast feeding on child's cognitive development with inconsistent results.[8–10] As both breast feeding and infant neurodevelopment are heavily influenced by socioeconomic and psychosocial factors,[7 11 12] these should be taken into account in studies in order to explain its effects on infant neurodevelopment.

Many of the observational studies in this field have included participants from highly selected groups, such as those born preterm or low-birth-weight infants,[13–16] and it is not clear whether the results of such studies can be extrapolated to the general population. Only three birth cohorts have evaluated neurodevelopment using valid psychometric scales at early ages (<2 years of age).[17–19] One of them reported a significant positive association between breastfeeding duration with gross motor (GM) but not with fine motor (FM) development at the age of 9 months,[17] while a birth cohort from Spain has shown that higher cumulative breast feeding was related with an increase in mental development scores at 14 months, whereas no association was found with psychomotor development scores.[18] The 'Taiwan Birth Cohort Study' has shown that longer duration of breast feeding was positively related to children's GM, FM, language and personal/social development in early childhood (<18 months of age).[19]

The aim of the present study was to investigate the association between breastfeeding practices and child's cognitive, language and motor development at the age of 18 months, as assessed by the 3rd edition of Bayley Scales of Infant and Toddler Development (Bayley-III) in the mother–child cohort, 'Rhea' study in Crete, Greece, after adjusting for several maternal and infant characteristics from pregnancy and throughout the life course of the child.

METHODS

Study population

The 'Rhea' study is a prospective mother–child cohort conducted in Heraklion, Greece.[20] Study subjects were recruited in early pregnancy at the time of the first major ultrasound examination. The inclusion criteria for study participants were pregnant women, residents in the study area; aged 16 years or above; and no communication handicap. Interview-administered questionnaires were used to obtain information on sociodemographic, environmental and psychosocial factors during pregnancy and early childhood. The study has followed the guidelines of the Declaration of Helsinki. In addition, ethical approval for the study was provided by the ethical committee of the University Hospital in Heraklion, Crete, Greece. Written informed consent was obtained from all women participating in the study.

Of 1765 eligible women, 1610 (91%) agreed to participate and 1388 (86%) were followed up until delivery. A random sample of 828 mothers was contacted by telephone at the 18 months follow-up, and 599 (73%) agreed to participate in the neurodevelopmental assessment.[21 22] We included only women with singleton pregnancies; thus, multiple pregnancies were excluded from the present analysis (n=26). Forty-six women (7.7%) did not provide complete information on breastfeeding practices; therefore, they were excluded from the analysis. We also excluded seven infants due to incomplete examination (n=1), signs of pervasive developmental disorders (PDD) (n=1), plagiocephalus (n=1), brain tumour (n=1), microcephalus (n=2) and hydrocephalus (n=1). Hence, a cohort of 540 (88% of the study population of children with neurodevelopmental assessment) mother–child pairs was available for the present analyses.

Breast feeding
Information on breastfeeding initiation, duration, use of infant formula, complementary food and other kinds of bottle food was collected at the ninth-month postpartum, and this information was updated at the age of 18 months. All mother–child pairs included in this study have provided information at both time points. Mothers were asked if they had ever breast fed their child (or placed the child on their breast to feed). If they never breast fed, the reason was recorded. If women initiated breast feeding, further information on breastfeeding intensity and duration was asked, as well as information regarding the first time they breast fed their infant and the duration of breast feeding.[23] Duration of breast feeding was categorised as 'never' breast fed, breast fed for '1–6 months' (according to WHO recommendations) and breast fed for '>6 months'. Breast feeding was also categorised according to the WHO breastfeeding definitions as exclusive, predominant and complementary breast feeding.[24]

Neurodevelopmental assessment
The children's cognitive, language and motor development was assessed at 18 months (±6 weeks) using Bayley-III.[21 25] Neurodevelopmental assessments were conducted by three trained psychologists, who completed the formal training course in the use, administration and interpretation of Bayley-III. Bayley-III assesses infant and toddler development across three domains: (i) the cognitive scale (COG); (ii) the language scale, which is composed of the receptive communication (RC) and the expressive communication (EC) subtest; and (iii) the motor scale, which is divided into the FM and the GM subtests. All testing was done at the Medical School of the University of Crete and two public hospitals in Heraklion in the presence of the mother. Total administration time was approximately 90 min.

The examiners also noted critical comments about the difficulties or special conditions of the neurodevelopmental assessment so as to evaluate the 'quality of assessment' such as no difficulties, difficulties due to physical problems (eg, physical illness, tiredness, asleep, etc.) and difficulties due to behaviour problems (eg, nervousness, shyness, etc.).

Potential confounders
Potential confounders included characteristics that have an established or potential association with child cognitive, language and motor development and breast feeding, including the following characteristics. (A) *Parental characteristics:* maternal and paternal age at birth; education at recruitment (low level ≤6 years of school, medium level 7–12 years of school and high level university or technical college degree); origin (Greek/non-Greek); maternal working status at 18 months of child's age (yes/no); marital status at birth (married–engaged/other); maternal and paternal smoking during pregnancy (non-smoker/quit during pregnancy/smoker); maternal and paternal smoking at 9 months of child's age (yes/no); parents' relationship at 9 months of child's age (improved/same as before/more arguments); and postpartum depressive symptoms assessed at 8–10 weeks after delivery by using the Edinburgh Postpartum Depression Scale (EPDS).[26] (B) *Perinatal and infant characteristics:* gender (male/female); type of delivery (caesarean/vaginal); siblings at birth (yes/no); birth order (order number); birth weight (g); head circumference (cm); length (cm); gestational age (completed weeks); preterm birth (<37 weeks of gestation; yes/no); neonatal intensive care unit and hospitalisation (yes/no); day care attendance at 18 months of age (yes/no); hours/day spent with mother at 18 months of age (number); hours/day spent with father at 18 months of age (number); and age of introduction of solid foods (months).

Statistical analysis
Neurodevelopmental assessment raw scores were standardised for psychologist and child's age at test administration using a parametric method for the estimation of age-specific reference intervals.[21 27] Standardised residuals were then typified having a mean of 100 points with a 15 SD to homogenise the scales (parameters conventionally used in psychometrics for assessing IQs).

Bivariate associations between normally distributed continuous dependent variables (Bayley scores) and categorical independent variables were studied using either Student t test or ANOVA. Bivariate associations between non-normally continuous exposure variable (breastfeeding duration) and independent variables (predictors) were studied using non-parametric statistical methods (Mann–Whitney, Kruskal–Wallis), whereas associations of categorical exposure variables (breastfeeding initiation, exclusivity of breast feeding, categorised duration) and independent variables (predictors) were tested using Pearson's χ^2 test. Pearson's r or Spearman's rho correlation coefficient was used to estimate the strength of the association between continuous dependent and independent variables.

Multivariable linear regression models were implemented to examine the associations between Bayley scores and any breast feeding (initiation, duration and exclusivity) after adjusting for several confounders. Potential confounders related with the exposure and outcomes of interest in the bivariate models with $p<0.1$ were included in the multivariable models. All multivariable models included 520 mother–child pairs (96.3% of the study population) with available data (non-missing values) on the exposure, outcome and confounding variables. The 'quality

Table 1 Descriptive characteristics of the study population and breastfeeding duration, Rhea cohort study, Crete, Greece (n=540)

		Breastfeeding duration			
	N*	Never (n=60)	1–6 months (n=346)	>6 months (n=134)	p Value†
Parental characteristics					
Maternal age (years), mean (SD)	532	30.5 (5.1)	29.8 (4.7)	31.6 (3.9)	0.001
Paternal age (years), mean (SD)	528	33.8 (6.8)	33.8 (5.2)	35.2 (5.1)	0.032
Maternal education, n (%)					<0.001
Low	65	16 (26.7)	38 (11.2)	11 (8.3)	
Medium	257	33 (55.0)	171 (50.6)	53 (40.2)	
High	207	11 (18.3)	129 (38.2)	68 (51.5)	
Paternal education, n (%)					<0.001
Low	151	29 (49.2)	96 (28.5)	26 (19.8)	
Medium	223	19 (32.2)	155 (46.0)	50 (38.2)	
High	152	11 (18.6)	86 (25.5)	55 (42.0)	
Maternal origin, n (%)					0.864
Greek	517	58 (96.7)	332 (96.5)	128 (95.5)	
Non-Greek	20	2 (3.3)	12 (3.5)	6 (4.5)	
Paternal origin, n (%)					0.395
Greek	518	59 (98.3)	333 (98.2)	127 (96.2)	
Non-Greek	12	1 (1.7)	6 (1.8)	5 (3.8)	
Maternal working status at 18 months, n (%)					0.453
Working	335	27 (45.0)	129 (37.3)	48 (35.8)	
Not working	204	33 (55.0)	217 (62.7)	86 (64.2)	
Marital status, n (%)					0.993
Married/engaged	523	59 (98.3)	335 (98.5)	130 (98.5)	
Other	8	1 (1.7)	5 (1.5)	2 (1.5)	
Maternal smoking during pregnancy, n (%)					<0.001
Non smoker	306	36 (63.2)	199 (59.9)	111 (84.1)	
Quit during pregnancy	117	8 (14.0)	79 (23.8)	12 (9.1)	
Smoker	104	13 (22.8)	54 (16.3)	9 (6.8)	
Maternal smoking postpartum, n (%)					<0.001
Yes	149	27 (45.0)	114 (33.1)	8 (6.0)	
No	387	33 (55.0)	230 (66.9)	125 (94.0)	
Paternal smoking during pregnancy					0.020
Non-smoker	228	23 (40.4)	132 (43.9)	73 (60.8)	
Quit during pregnancy	45	6 (10.5)	32 (10.6)	7 (5.8)	
Smoker	204	28 (49.1)	137 (45.5)	40 (33.3)	
Paternal smoking postpartum, n (%)					0.015
Yes	243	33 (55.0)	164 (47.7)	47 (35.3)	
No	293	27 (45.0)	180 (52.3)	86 (64.7)	
Parents' relationship after birth, n (%)					0.131
Same as before	279	36 (61.0)	177 (51.5)	67 (50.0)	
Improved	155	14 (23.7)	108 (31.4)	33 (24.6)	
More arguments	102	9 (15.3)	59 (17.2)	34 (25.4)	
EPDS, n (%)					0.570
<13	392	45 (88.2)	246 (86.3)	101 (90.2)	
≥13	56	6 (11.8)	39 (13.7)	11 (9.8)	
Perinatal and infant characteristics					
Birth weight (kg); mean (SD)	532	3008.8 (430.2)	3181.0 (427.7)	3270.5 (413.4)	<0.001
Infant gender, n (%)					0.890
Male	290	34 (56.7)	186 (53.8)	71 (53.0)	
Female	249	26 (43.3)	160 (46.2)	63 (47.0)	
Type of delivery, n (%)					0.106
Vaginal	261	22 (36.7)	169 (49.1)	70 (53.0)	
C-section	274	38 (63.3)	175 (50.9)	62 (47.0)	
Siblings at birth, n (%)					0.032
Yes	246	18 (30.0)	167 (48.3)	62 (46.3)	
No	293	42 (70.0)	179 (51.7)	72 (53.7)	
Preterm, n (%)					0.001
Yes	56	14 (23.3)	34 (9.9)	8 (6.0)	
No	479	46 (76.7)	308 (90.1)	126 (94.0)	

Continued

Table 1 Continued

	N*	Breastfeeding duration			p Value†
		Never (n=60)	1–6 months (n=346)	>6 months (n=134)	
Neonatal intensive care unit, n (%)					0.003
Yes	77	16 (27.1)	50 (14.7)	11 (8.2)	
No	456	43 (72.9)	291 (85.3)	123 (91.8)	
Day care attendance at 18 months of age, n (%)					0.972
Yes	51	6 (10.2)	32 (9.3)	13 (9.7)	
No	486	53 (89.8)	313 (90.7)	121 (90.3)	
Introduction of solid foods, n (%)					0.305
<5 months	101	48 (80.0)	274 (79.7)	114 (85.7)	
≥5 months	436	12 (20.0)	70 (20.3)	19 (14.3)	
Hours/day with mother at 18 months of age, mean (SD)	537	9.4 (3.9)	8.5 (3.4)	9.2 (3.4)	0.044
Hours/day with father at 18 months of age, mean (SD)	537	4.0 (2.6)	3.5 (2.3)	3.5 (2.5)	0.429

*Reported frequencies do not include missing values.
†p Values obtained from Pearson's χ^2 tests of independence (for categorical variables) or ANOVA (for continuous measures).
EPDS, Edinburgh Postpartum Depression Scale.

of assessment' and child's gender were treated as a priori confounders and were included in all regression models.

Also, the influence of each confounder on the outcomes was assessed by adding those variables one by one in the models. Sensitivity analyses were performed by excluding (i) preterm and/or low-birth-weight neonates and (ii) women with high levels of postpartum depressive symptoms. Effect modification was evaluated using the likelihood ratio test. Fractional polynomials were used to evaluate the linearity of the relationship between breastfeeding duration and neurodevelopmental outcomes using graphical representation. All hypothesis testing was conducted assuming a 0.05 significance level and a two-sided alternative hypothesis. All statistical analyses were performed using SPSS Statistics 19 software (SPSS Inc, Chicago, Illinois, USA).

RESULTS

The mean duration of any breast feeding was 4.97 (SD 4.38) months and of exclusive breast feeding 0.80 months (SD 1.47). The percentage of non-breastfeeding mothers rises on average by 10% per month, reaching 68% at the sixth month. The prevalence of complementary breast feeding (breast milk and solid or semi-solid foods allowing liquid and non-human milk and formula) was 63% at the first month, dropping to 30% at the sixth month. Approximately 52% of infants were breast fed for 1–6 months (n=249), while 13% of children (n=62) were breast fed for less than 1 month and 35% of children (n=169) were breast fed six or more months postpartum, among breastfed infants (n=480). The prevalence of complementary breast feeding (breast milk and solid or semi-solid foods allowing liquid and non-human milk and formula) was 63% at the first month, dropping to 30% at the sixth month. Only 6% of infants (n=32) were exclusively or predominantly breast fed the first five months of life.

A description of the population characteristics and the distribution of the selected covariates by duration of breast feeding are shown in table 1. Mothers were more likely to breast feed their child longer if they were older, had a university education, did not smoke during pregnancy or after birth. Longer duration of breast feeding was also positively associated with some paternal characteristics: age, higher education and non-smoking during pregnancy or postpartum. Breastfeeding initiation was inversely associated with preterm birth, infant hospitalisation in neonatal care unit and low birth weight. Mothers who participated in the current analysis were significantly older, with higher education, more likely to be married and Greek compared with those who were not included in this analysis from the initial number of women followed until birth (data not shown).

Table 2 presents the crude effect of any breast feeding (initiation and duration) on Bayley-III scales at 18 months of age. Infants who were ever breast fed scored higher in the Bayley-III cognitive, RC and FM scales. Longer duration of any breast feeding (>6 months) was associated with increased scores in cognitive, RC, EC and FM scales. Additional analysis of exclusivity of breast feeding, according to WHO definitions, did not reveal any other significant results (results not shown).

Table 3 presents the adjusted coefficients of the multivariate linear regression analysis between any breast feeding (initiation and duration) and Bayley-III scales at 18 months of age. Infants who were ever breast fed had higher scores in the scales of cognitive, RC and FM development though not statistically significant. Fractional polynomials used to evaluate the form of the relationship did not indicate a significant deviation from linearity (p-gain>0.05 for all scales). Duration of any breast feeding (in months) was positively associated with higher scores in all the Bayley-III developmental scales, except the GM scale. More specifically, per each accumulated month of breast feeding, there was an estimated increase of 0.28 points in the scale of cognitive development (β=0.28; 95% CI 0.01 to 0.55), 0.29-point increase in the scale of RC (β=0.29; 95% CI 0.04 to 0.54), 0.30-point increase in the scale of EC (β=0.30; 95% CI 0.04 to 0.57) and 0.29-point increase in the scale of FM development (β=0.29; 95% CI 0.02 to 0.56) after adjusting for several confounders. Children who were breast fed longer than 6 months had a 4.44-point increase in the scale of FM development (β=4.44; 95% CI 0.06 to 8.82) compared with those never breast fed. Additional analysis with more categories of breastfeeding duration (never, 1–3 months, 4–6 months and >6 months) has shown similar results with the initial analysis. Analysis of exclusivity of breast feeding (exclusive, predominant and complementary) did not show significant differences in the effect estimates for breastfeeding categories (data not shown).

In an additional analysis, we performed a series of models by adding potential confounding variables one by one in an effort to assess the influence of different parental and infant characteristics on the relationship between breastfeeding duration and neurodevelopmental outcomes. The strongest confounder of the

Table 2 Breastfeeding and mean standardised Bayley-III scales at 18 months of age, Rhea cohort study, Crete, Greece (n=540)

			Cognitive			Receptive communication			Expressive communication			Fine motor			Gross motor		
	N	Per cent	Mean	(SD)	p Value*	Mean	(SD)	p Value*	Mean	(SD)	p Value*	Mean	(SD)	p Value*	Mean	(SD)	p Value*
Breast feeding																	
Never	60	11.1	96.8	(13.3)	0.019	96.6	(16.0)	0.035	98.8	(15.0)	0.333	96.2	(13.2)	0.005	99.6	(14.1)	0.530
Ever	480	88.9	101.2	(14.8)		101.2	(14.3)		100.8	(15.1)		101.5	(14.4)		100.8	(15.0)	
Breastfeeding duration																	
Never	60	11.1	96.8	(13.3)	0.003	96.6	(16.0)	0.009	98.8	(15.0)	0.006	96.2	(13.2)	0.010	96.2	(13.2)	0.614
1–6 months	346	64.2	100.2	(14.9)		100.4	(14.4)		99.5	(15.4)		100.9	(14.2)		100.9	(14.2)	
>6 months	134	24.7	104.0	(14.7)		103.4	(13.9)		104.1	(13.8)		103.0	(14.7)		101.7	(15.3)	

*t test and ANOVA were used for differences between continuous normally distributed variables.

Table 3 Multivariable associations between breastfeeding initiation/duration and neurodevelopmental outcomes at age 18 months, Rhea cohort study, Crete, Greece (n=520)

	Cognitive*			Receptive communication*			Expressive communication*			Fine motor*			Gross motor*		
	β	95% CI	p Value	β	95% CI	p Value	β	95% CI	p Value	β	95% CI	p Value	β	95% CI	p Value
Breast feeding															
Never	Ref			Ref			Ref			Ref			Ref		
Ever	1.16	(−2.69 to 5.01)	0.554	0.39	(−3.18 to 3.95)	0.831	−1.64	(−5.44 to 2.17)	0.398	3.33	(−0.50 to 7.17)	0.089	−0.71	(−4.79 to 3.36)	0.731
Breastfeeding duration															
Per month	0.28	(0.01 to 0.55)	0.043	0.29	(0.04 to 0.54)	0.028	0.30	(0.04 to 0.57)	0.026	0.29	(0.02 to 0.56)	0.034	0.06	(−0.23 to 0.35)	0.682
Duration categorised															
Never	Ref			Ref			Ref			Ref			Ref		
1–6 months	0.30	(−3.60 to 4.19)	0.880	−0.35	(−3.96 to 3.27)	0.851	−2.83	(−6.67 to 1.01)	0.148	2.96	(−0.95 to 6.87)	0.137	−0.48	(−4.64 to 3.69)	0.822
>6 months	3.70	(−0.66 to 8.06)	0.096	2.53	(−1.53 to 6.58)	0.221	1.85	(−2.45 to 6.15)	0.398	4.44	(0.06 to 8.82)	0.047	0.33	(−4.33 to 5.00)	0.888

*All models have been adjusted for parental characteristics (maternal and paternal education, maternal and paternal country of origin, maternal age, smoking during pregnancy and working situation) and infant characteristics (birth order, birth weight, gender and quality of assessment).

relationship between breastfeeding duration and neurodevelopmental outcomes was estimated to be maternal education (13% change in effect estimate for cognitive development), followed by maternal working status and parity (<5% decrease for each). No significant interaction was observed between breastfeeding duration and maternal education.

Finally, to elucidate whether prematurity or fetal growth restriction and maternal postpartum depressive symptoms confounded the observed results, we performed additional sensitivity analyses in which we excluded (i) all children who were preterm and/or low-birth-weight neonates (preterm births, n=56; low-birth-weight neonates, n=29) and (ii) all women with high levels of postpartum depressive symptoms (EPDS ≥ 13, n=56). Results did not differ substantially from those derived from the main analysis (see online supplementary tables 1S and 2S).

DISCUSSION

The main finding of this population-based cohort study was the positive and linear association of breastfeeding duration with increased scores in the scales of cognitive, language and FM development at 18 months of age. This is the first longitudinal analysis of breastfeeding practices in association with child cognitive, language and motor development in Greece, a country with relatively low breastfeeding levels.[7 23] The beneficial effect of breast feeding persisted even after adjustment for a large array of potential confounders.

Our findings are consistent with previous studies that have reported positive associations between breast feeding and children's cognitive[8 9 11 18 28–32] and motor development.[17 19 33–37] A recent meta-analysis has shown that ever breast feeding was associated with a 3.2-point increase in the scale of cognitive development compared with formula feeding.[8] Only few population-based studies evaluated dose–response relationships of breastfeeding duration with vocabulary development and verbal comprehension at early childhood,[19 35 36] and all of them have shown positive associations with language skills at the age of 6 months,[35] 18 months[19] and 3 years.[36] As noted in several systematic reviews,[8–10] the great majority of published articles have important methodological issues, making it difficult to fully understand the relationship between breast feeding and child mental development. The main limitations of previous studies are the study design (cross-sectional), and data quality (as a consequence of retrospective data collection on breast feeding at the time of cognitive measurements), lack of valid neuropsychological measurements, small sample sizes and insufficient adjustment for critical potential confounders.

There are several potential mechanisms linking breast feeding with enhanced neurodevelopment in infancy. Some studies have concentrated on the effects of particular components of breast milk, although it is not clear which constituent(s) of breast milk might be most beneficial in promoting brain development. Most of them indicated the beneficial role of LC-PUFAs, which are structural lipids critical for retina and cortical brain development in early life.[38] However, randomised trials with infant formulas supplemented with LC-PUFAs have generally not found any clear effects.[39 40] Infant levels of LC-PUFAs depend not only on maternal levels but also on genes such as FADS1 and FADS2 regulating PUFA metabolism and determining PUFA levels in breast milk.[41 42] Recent publications have revealed that genetic variation of FADS genes may modify the effect of breast feeding on cognitive development.[43] Breast milk also contains hormones, oligosaccharides, phospholipids and other trophic factors that are important for optimal neural function.[44] Apart from nutrient components of human milk, there are other possible mechanisms that may explain the association between breast feeding and child neurodevelopment. Breast feeding provides enhanced psychosocial experiences for children such as mother–child interaction, bonding and greater variety of daily stimulations that may contribute to the development of the infant's limbic system and its cortical connections.[45 46] In the present study, longer duration of breast feeding was still positively correlated with young children's cognitive, language and motor development after adjusting for maternal education, employment and other confounding factors.

Strengths of the present study include the population-based prospective design, the high participation rate (72%) and the assessment of a number of potential predictors of child neurodevelopment. Most of the studies evaluating breast feeding in association with cognitive development compared only the cognitive function between 'never breastfed' and 'ever breastfed' children and were not able to provide estimates of the effects of long-term breast feeding on child neurodevelopment.[10] However, in the present analysis, we have measured breast feeding primarily as a continuous variable in order to avoid misclassification bias, to maximise statistical power and to allow detection of dose–response relationships. Bayley-III, which was used for the neurodevelopmental assessment of children, is recognised internationally as one of the most comprehensive tools to identify infant and toddler strengths and competencies, as well as their weaknesses, and to provide a valid and reliable measure of a child's cognitive, language and motor abilities.[25] Deviant language development, neurocognitive functioning and impaired social interaction in the first 18 months of life, as assessed by the Bayley scales among other instruments, can predict the child having any neurodevelopmental disorder at a later age.[47] Participants were unaware of the hypothesis being tested, so misclassification of breastfeeding practices estimated by the questionnaire is likely to be random with respect to neurodevelopmental outcomes. Although the study sample in the 'Rhea' cohort included pregnant women who visited the two local public hospitals, as well as the two major local private maternity clinics, we need to consider significant differences in the population studied compared with participants not included in the present analyses when considering generalisability of results.

Several limitations need to be taken into account. We did not have data on biomarkers of environmental contaminants in breast milk, such as methyl mercury or polychlorinated biphenyls, both of which may harm child development.[48] However, preliminary results from the Rhea study have shown that prenatal concentrations of polychlorinated biphenyls as measured in maternal blood are extremely low in this population compared with other mother–child cohorts in Europe.[49] The possibility of residual confounding cannot be fully discounted since the inclusion of maternal and paternal educational level and maternal working status in the multivariate models might not have removed part of the variance in maternal intelligence, variable not available in the present analysis. Moreover, we had no information on the quality of home stimulation that has been shown as a positive predictor on neurodevelopmental outcomes in early childhood. Exposure information relies on a recall over a 9-month interval, and it could introduce recall bias on breastfeeding practices. Another limitation of this study may be that neurodevelopmental testing was performed at a single time point (18 months of age). Longitudinal data related also to children's neurodevelopment at 4 years of age will be available for analysis in due course and will help us to examine whether protective effects of breast feeding are sustained into early childhood as well. This is the first analysis of breastfeeding practices in association with child neurodevelopment in Greece indicating the association of longer duration of breast feeding during the first year of life with higher neurodevelopmental scores at 18 months. The follow-up of this birth cohort will allow us to explore if this beneficial effect of breast feeding persists at older ages.

What is already known on this subject

- Breastfeeding duration has been associated with improved cognitive development in children.
- Few population-based prospective studies have evaluated dose–response relationships of breastfeeding duration with communication skills and motor development before the age of 2 years, and results are discrepant.

What this study adds

- The study has shown a positive and linear association of breastfeeding duration with increased scores in the scales of cognitive, language and fine motor development at 18 months of age.
- Further follow-up of this population-based birth cohort will allow us to explore if this beneficial effect of breast feeding persists at older ages.

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Contributors All authors had a substantial contribution to the study, and they have all reviewed and approved the submitted manuscript VL participated in the field study coordination, participated in the statistical analysis and wrote the first draft of the paper. TR supervised the statistical analysis and helped with the data interpretation and manuscript preparation. KK supervised the neurodevelopmental assessment and contributed to the drafting of the manuscript. MV provided feedback and critical revision of the manuscript and helped with data interpretation. EM and PB participated in the design of the study and in the drafting of the paper. MK and LC conceived the study, supervised the data collection, provided critical review of the manuscript and helped with data interpretation and manuscript preparation.

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Competing interests None.

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REFERENCES

1 Picciano MF. Nutrient composition of human milk. *Pediatr Clin North Am* 2001;48:53–67.
2 Hamosh M. Bioactive factors in human milk. *Pediatr Clin North Am* 2001;48:69–86.
3 Jensen CL, Lapillonne A. Docosahexaenoic acid and lactation. *Prostaglandins Leukot Essent Fatty Acids* 2009;81:175–8.
4 McCann JC, Ames BN. Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *Am J Clin Nutr* 2005;82:281–95.
5 Koletzko B, Lien E, Agostoni C, *et al*. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *J Perinat Med* 2008;36:5–14.
6 Gartner LM, Morton J, Lawrence RA, *et al*. Breastfeeding and the use of human milk. *Pediatrics* 2005;115:496–506.
7 Ladomenou F, Kafatos A, Galanakis E. Risk factors related to intention to breastfeed, early weaning and suboptimal duration of breastfeeding. *Acta Paediatr* 2007;96:1441–4.
8 Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. *Am J Clin Nutr* 1999;70:525–35.
9 Drane DL, Logemann JA. A critical evaluation of the evidence on the association between type of infant feeding and cognitive development. *Paediatr Perinat Epidemiol* 2000;14:349–56.
10 Der G, Batty GD, Deary IJ. Effect of breast feeding on intelligence in children: prospective study, sibling pairs analysis, and meta-analysis. *BMJ* 2006;333:945.
11 Jacobson SW, Jacobson JL. Breastfeeding and IQ: evaluation of the socio-environmental confounders. *Acta Paediatr* 2002;91:258–60.
12 Kelly YJ, Watt RG. Breast-feeding initiation and exclusive duration at 6 months by social class—results from the Millennium Cohort Study. *Public Health Nutr* 2005;8:417–21.
13 Morley R, Fewtrell MS, Abbott RA, *et al*. Neurodevelopment in children born small for gestational age: a randomized trial of nutrient-enriched versus standard formula and comparison with a reference breastfed group. *Pediatrics* 2004;113:515–21.
14 Fewtrell MS, Morley R, Abbott RA, *et al*. Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. *Pediatrics* 2002;110:73–82.
15 O'Connor DL, Hall R, Adamkin D, *et al*. Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective, randomized controlled trial. *Pediatrics* 2001;108:359–71.
16 Lucas A, Fewtrell MS, Morley R, *et al*. Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. *Pediatrics* 2001;108:703–11.
17 Sacker A, Quigley MA, Kelly YJ. Breastfeeding and developmental delay: findings from the millennium cohort study. *Pediatrics* 2006;118:e682–9.
18 Guxens M, Mendez MA, Molto-Puigmarti C, *et al*. Breastfeeding, long-chain polyunsaturated fatty acids in colostrum, and infant mental development. *Pediatrics* 2011;128:e880–9.
19 Chiu WC, Liao HF, Chang PJ, *et al*. Duration of breast feeding and risk of developmental delay in Taiwanese children: a nationwide birth cohort study. *Paediatr Perinat Epidemiol* 2011;25:519–27.
20 Chatzi L, Plana E, Daraki V, *et al*. Metabolic syndrome in early pregnancy and risk of preterm birth. *Am J Epidemiol* 2009;170:829–36.
21 Koutra K, Chatzi L, Roumeliotaki T, *et al*. Socio-demographic determinants of infant neurodevelopment at 18 months of age: Mother-Child Cohort (Rhea Study) in Crete, Greece. *Infant Behav Dev* 2012;35:48–59.
22 Chatzi L, Papadopoulou E, Koutra K, *et al*. Effect of high doses of folic acid supplementation in early pregnancy on child neurodevelopment at 18 months of age: the mother-child cohort 'Rhea' study in Crete, Greece. *Public Health Nutr* 2012;15:1728–36.
23 Vassilaki M, Chatzi L, Bagkeris E, *et al*. Smoking and caesarean deliveries: major negative predictors for breastfeeding in the mother-child cohort in Crete, Greece (Rhea study). *Matern Child Nutr* 2012. doi:10.1111/j.1740-8709.2012.00420.x
24 WHO. Indicators for Assessing Breastfeeding Practices: Reprinted report of an Informal Meeting, 1991. WHO: Geneva
25 Bayley N. Bayley scales of infant and toddler development. 3rd edn. San Antonio, TX: PsychCorp, Harcourt Assessment Inc., 2006.
26 Chatzi L, Melaki V, Sarri K, *et al*. Dietary patterns during pregnancy and the risk of postpartum depression: the mother-child 'Rhea' cohort in Crete, Greece. *Public Health Nutr* 2011;14:1663–70.
27 Royston P, Wright EM. How to construct 'normal ranges' for fetal variables. *Ultrasound Obstet Gynecol* 1998;11:30–8.
28 Angelsen KN, Vik T, Jacobsen G, *et al*. Breastfeeding and cognitive development at age 1 and 5 years. *Arch Dis Child* 2001;85:183–8.
29 Gordon AG. Breast-feeding, breast-milk feeding, and intelligence quotient. *Am J Clin Nutr* 2000;72:1063–4.
30 Michaelsen KF, Lauritzen L, Mortensen EL. Effects of breast-feeding on cognitive function. *Adv Exp Med Biol* 2009;639:199–215.
31 Quigley MA, Hockley C, Carson C, *et al*. Breastfeeding is associated with improved child cognitive development: a population-based cohort study. *J Pediatr* 2012;160:25–32.
32 Andres A, Cleves MA, Bellando JB, *et al*. Developmental status of 1-year-old infants fed breast milk, cow's milk formula, or soy formula. *Pediatrics* 2012;129:1134–40.

4.2.2 Paper 3. Relative validity of an FFQ for pre-school children in the mother-child 'Rhea' birth cohort in Crete, Greece

Main Findings:

- A fair agreement was observed between the FFQ and the Food Records. The validation study indicated that the Rhea 4 years FFQ produces a realistic and relatively precise estimate of habitual intake of food groups and nutrients among preschool children in Crete, Greece.

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Relative validity of an FFQ for pre-school children in the mother–child 'Rhea' birth cohort in Crete, Greece

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Abstract

Objective: To examine the relative validity of an FFQ based on parental report for pre-school children in the mother–child 'Rhea' birth cohort.
Design: The children's mothers completed an FFQ that referred to the children's dietary intake for the previous year by telephone interview. Mothers completed also three food records, two on weekdays and one on a weekend day. Spearman correlation coefficients were calculated for the energy-adjusted values. Weighted kappa statistics (κ_w) and the Bland–Altman technique were used to test the degree of agreement between the two dietary methods.
Setting: Heraklion, Crete, Greece, 2011–2012.
Subjects: A total of ninety-nine mothers (corresponding to fifty-one boys and forty-eight girls) participated in the validation study.
Results: The mean and median values of all food group and nutrient intakes did not differ significantly between the two dietary methods. Overall, fair agreement was observed between the FFQ and the food records for ranking participants based on their intake, with κ_w ranging from 0·21 to 0·40 for most foods and nutrients. On average, 88 % of participants were classified into the same or adjacent tertiles for nutrient and food group intakes by both dietary methods. The degree of agreement was also confirmed by the visual examination of the Bland–Altman plots.
Conclusions: The study indicates that the Rhea 4 years FFQ is a relatively accurate tool for assessing habitual food group and nutrient intakes among pre-school children in Crete, Greece.

Keywords
Validation
FFQ
Pre-school children
Food records

Early-life nutrition has been established not only as a key component for the maintenance of lifelong health[1], but also as a major determinant of chronic diseases such as type 2 diabetes[2], CVD[3,4], obesity[5,6], hypertension[7,8], allergies[9,10] and certain cancers[11]. Contrary to other risk factors, diet is potentially modifiable, especially in childhood. It is therefore important to assess dietary intake with valid tools in order to establish the role early nutrition plays in chronic disease promotion or prevention.

In large-scale population studies, it is a continuous challenge to assess diet during childhood. FFQ, 24 h recalls and food records (FR) are well-known dietary assessment instruments based on parental report[12]. Although accurate, 24 h recalls and FR are time consuming and costly[13]. On the other hand, FFQ are designed to measure long-term intake and are a less expensive and lengthy option[14,15]. In order to assess the relative validity of an FFQ, 24 h recalls and FR are usually used as they do not require participant literacy and provide high levels of specificity[1,13,16,17].

The aim of the present study was to examine the relative validity of an FFQ designed for pre-school children aged 4 years in the region of Heraklion, Crete, Greece, against 3 d FR.

Methods

Validation study participants and design

The present study is a sub-project of the mother–child 'Rhea' study in Crete[18]. In summary, the Rhea study has been following a total of 1317 women and their offspring since 2007–2008. Healthy children participating in the Rhea cohort were invited to take part in the validation study when they came for their routine follow-up at 4 years (2011–2012), which included children's dietary assessment via an FFQ. The Rhea cohort has obtained dietary information for mothers and children at different time points up to 4 years: during pregnancy, at 9 and 18 months postpartum and recently at 4 years. The mothers who agreed to participate in

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the validation study were asked to keep a 3 d weighed FR for their children. Of the 204 mothers who were invited to participate in the validation study, 104 completed the FR. Five of them did not complete the FR correctly and thus were excluded from the analysis. The final number of participants included in the validation study was ninety-nine children (fifty-one boys, forty-eight girls). Information on child and maternal characteristics such as weight, height, lifestyle characteristics, parity, marital status, smoking status and maternal education were gathered at enrolment and during the 4-year follow-up.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethical committee of the University Hospital in Heraklion, Crete, Greece. Written informed consent was obtained from all women participating in the study.

Dietary assessment

The Rhea follow-up FFQ is a newly developed questionnaire designed to assess habitual dietary intake in preschool children. The Rhea follow-up FFQ was completed by telephone interview where parents were asked to recall their child's diet for the previous year. The Rhea follow-up FFQ is a semi-quantitative questionnaire that asks about the intake of 118 food items. It also includes nine supplementary questions regarding type of meals during the day (breakfast, morning snack, lunch, afternoon snack, dinner, evening snack), use of dietary supplements (brand name of product, number of daily doses, time period of intake), type of fat used for cooking, medical conditions that can affect diet (i.e. high cholesterol), frequency of meals consumed in restaurants or takeaways, and television viewing during meals. The interviewer could also add anything else consumed by the child that was not included in the existing list of the 118 food items. Mothers could choose from one or two portion sizes, and reported the child's frequency of intake (times per day, week, month or year, or never). Seasonality of consumption could also be reported in all food items.

In the validation study, the FR was self-administered by the participants who received the FR via post (including a prepaid envelope) together with instructions on how to complete it. Additional information and clarifications were provided by telephone. Parents were asked to feed their child his/her normal diet, to weigh and record all foods, beverages and dietary supplements consumed by their child during two consecutive weekdays and one weekend day, and were also asked to collect information about their child's diet from the caregiver or day nursery. Upon collection, the FR were checked for completeness by a nutritionist.

Analysis of food consumption data

In the present study, FFQ and FR data were converted into daily intakes of foods and nutrients using the UK food tables (*McCance & Widdowson's The Composition of Foods*, 6th summary edition) and standard Greek recipes for complex mixed dishes. For the FR, the mean of the three recording days was assessed. The dietary intake data were analysed by using a software program developed at the Department of Applied Information Technology and Multimedia, TEI-Crete, Heraklion, Crete, Greece.

Statistical analysis

Pearson's χ^2 test for independence was used to compare the number of participants classified according to maternal education, marital status, residence, smoking status, parity and child gender in the validation study sample and the Rhea sample, as well as in the validation study sample and those who did not send back FR (non-responders). The Mann–Whitney U test was used to compare both maternal and child age and BMI. The nutrients presented in the current study were extracted from food sources only. The differences in food group and nutrient intakes between the Rhea follow-up FFQ and the FR were tested with the Wilcoxon signed-rank test in order to identify systematic bias. The relationship between the two dietary methods was assessed by using the Spearman rank correlation coefficient.

The agreement between the two methods was analysed as proposed by Bland and Altman[19], using a graphical technique and simple calculations for all nutrient and food group intakes. This was accomplished by plotting the differences in intakes between the FFQ and FR *v.* the mean intakes of the FFQ and FR. The 95 % limits of agreement (mean difference $\pm$1·96 SD) show how well the two methods agree. This was performed for all nutrients and food groups, and some of the plots are presented herein. Following the graphical representation, when the standard error increased at higher measured values, the detection of heteroscedasticity was possible. The degree of heteroscedasticity was examined using Kendall's tau correlation (τ) between the absolute differences and the mean of the two dietary methods. When $\tau > 0{\cdot}1$ the data are heteroscedastic and when $\tau < 0{\cdot}1$ or negative the data are homoscedastic.

Furthermore, agreement on a categorical level was examined by classification of food and nutrient intakes into the same (correct classification), adjacent or opposite tertiles (gross misclassification) by the FFQ and the FR for energy-adjusted intakes. The agreement between the two dietary methods was additionally assessed with the weighted kappa statistic (κ_w) based on the Fleiss–Cohen quadratic weighting scheme and 95 % confidence intervals are presented[20]. The following ranges describe the different degrees of agreement: $\kappa_w = 0{\cdot}00$ to 0·20 (slight agreement), $\kappa_w = 0{\cdot}21$ to 0·40 (fair agreement), $\kappa_w = 0{\cdot}41$ to 0·60 (moderate agreement), $\kappa_w = 0{\cdot}61$ to 0·80 (substantial agreement) and $\kappa_w = 0{\cdot}81$ to 1·00 (almost perfect agreement)[21].

All analyses were performed with crude data and energy-adjusted data using the residual method[22].

The significance level was set at 5 % and a two-sided alternative hypothesis. All statistical analyses were performed using the statistical software packages IBM SPSS Statistics version 20·0 and STATA SE version 13·0.

Results

The validation study participants were compared with the total population of the Rhea study (Table 1). No differences were found between these two groups regarding several sociodemographic characteristics such as maternal and child age, maternal education and origin, child gender, marital status, residence, maternal and child BMI and maternal parity. Similarly, no differences were found between the validation study participants and those who, though enrolled, did not send FR (non-responders), with the exception of smoking status (data not shown).

The mean age of mothers participating in the current study was 33·6 years, 36·4 % of them were highly educated and the great majority were married and of Greek origin (87·9 % and 96·0 %, respectively; Table 1). The children participating in the validation study were 3·7 years old with a mean BMI of 16·5 kg/m^2.

Table 2 presents the mean and median daily intakes of food groups and nutrients as assessed by the FFQ and the FR. In particular, the FFQ recorded higher intakes of vegetables, fruits, milk and eggs, while lower intakes were recorded for sugar preserves and confectionery, pulses, poultry and red meat. For all nutrients and food groups, according to the Wilcoxon signed-rank test, the mean difference between the methods (systematic bias) was not statistically significant. The average energy-adjusted correlation coefficient between the two dietary assessment methods for food groups was 0·380, ranging from $r=0·109$ for poultry to $r=0·671$ for milk. The mean and median intakes of all nutrients were larger when assessed by the FFQ than by the FR (Table 2). The average correlation between the two dietary assessment methods for nutrients was 0·315, ranging from $r=0·099$ for total fat to $r=0·484$ for vitamin B_{12} and iodine. The average energy-adjusted Spearman's correlation coefficient reached statistical significance for almost all food groups and nutrients.

The differences between the FFQ and FR for absolute intakes of nutrients and foods were examined using Bland–Altman plots. The plots for all nutrients and food groups were similar to the plot for energy (Fig. 1(a)), fat (Fig. 1(d)) and carbohydrate intake (Fig. 1(e)). For all plots the 95 % limits of agreement indicated fairly good agreement between the two dietary methods, although for

Table 1 Characteristics of the participants included in the validation study of the mother–child 'Rhea' birth cohort, Heraklion, Crete, Greece, 2011–2012 and comparison with the total population of the Rhea study

	Validation study (*n* 99)		Rhea (*n* 1008)		
	n	%	*n*	%	*P* value
Maternal education					0·353
Low	13	13·1	169	17·7	
Medium	50	50·5	495	51·8	
High	36	36·4	292	30·5	
Mother's origin					0·301
Greek	95	96·0	929	93·3	
Other	4	4·0	67	6·7	
Marital status					0·748
Married/engaged	87	87·9	853	88·9	
Other	12	12·1	106	11·1	
Residence					0·982
Urban	63	71·6	555	71·7	
Rural	25	28·4	219	28·3	
Smoking status					0·003*
Smoker	19	21·6	292	12·5	
Non-smoker	69	78·4	484	87·5	
Parity					0·268
Multiparous	50	51·5	543	57·4	
Primiparous	47	48·5	403	42·6	
Child gender					0·894
Male	51	51·5	526	52·2	
Female	48	48·5	482	47·8	
	Mean	SD	Mean	SD	*P* value
Maternal age (years)	33·64	5·37	33·50	4·92	0·910
Maternal BMI (kg/m^2)	25·59	5·44	25·43	4·92	0·973
Child age (years)	3·65	0·11	3·69	0·39	0·639
Child BMI (kg/m^2)	16·48	1·87	16·43	1·98	0·845

Missing values have been excluded (pair-wise: validation study, Rhea): maternal education (*n* 0, *n* 52); Greek origin (*n* 0, *n* 12); marital status (*n* 0, *n* 49); residence (*n* 11, *n* 234); smoking status (*n* 11, *n* 232); parity (*n* 2, *n* 62); child gender (*n* 0, *n* 0).
*Statistically significant difference ($P<0·05$) based on Mann–Whitney *U* test for two independent samples and Pearson's χ^2 test for independence.

Table 2 Daily food and nutrient intakes assessed by the FFQ and the 3 d food record (FR), and differences between both methods, among pre-school children (fifty-one boys and forty-eight girls) in the validation study of the mother–child ‘Rhea’ birth cohort, Heraklion, Crete, Greece, 2011–2012

		FFQ				FR				Difference (FFQ – FR)	Spearman’s correlation *r*	
	n	Mean	SD	Median	IQR	Mean	SD	Median	IQR	*P* value†	Unadjusted	Energy adjusted
Food groups												
Sugar preserves and confectionery (g)	98	45·0	27·6	40·3	35·0	51·4	37·4	42·9	50·0	0·716	0·463*	0·437*
Vegetables (g)	99	156·0	76·9	145·1	86·2	94·6	60·0	91·2	93·9	0·936	0·362*	0·295*
Fruits (g)	94	179·1	103·3	154·1	163·0	138·9	107·2	125·8	148·5	0·998	0·505*	0·597*
Pulses (g)	51	6·4	3·6	5·5	4·1	11·2	7·4	8·5	9·0	0·866	0·318*	0·322*
Milk (g)	99	423·0	247·1	408·0	251·7	348·7	161·9	316·7	218·1	0·264	0·622*	0·671*
Poultry (g)	62	13·8	8·2	12·3	8·6	31·3	17·9	28·3	23·1	0·656	0·038	0·109
Red meat (g)	99	24·4	12·8	21·9	14·9	32·4	21·0	29·1	29·7	0·398	0·183	0·253*
Eggs (g)	88	20·2	11·5	20·8	17·7	18·7	13·9	16·7	16·3	0·591	0·384*	0·427*
Nutrients												
Protein (g)	99	60·7	13·7	58·1	17·4	54·8	11·7	53·6	13·4	0·821	0·242*	0·338*
Carbohydrate (g)	99	161·8	35·1	162·2	45·8	136·3	38·4	132·5	49·6	0·812	0·160	0·162
Total fat (g)	99	78·4	19·7	78·8	22·8	71·7	19·2	71·6	24·2	0·589	0·178	0·099
MUFA (per 100 g)	99	28·6	8·4	27·4	10·3	25·8	8·9	25·0	11·6	0·505	0·129	0·211*
PUFA (per 100 g)	99	5·2	1·5	5·2	1·6	5·0	1·9	4·8	2·5	0·834	0·156	0·352*
SFA (per 100 g)	99	33·6	8·7	32·4	11·2	29·1	7·9	29·1	11·2	0·975	0·175	0·177
Vitamin B_{12} (μg)	99	5·7	3·0	5·7	4·4	4·0	2·2	3·7	2·8	0·562	0·411*	0·484*
Vitamin C (mg)	99	110·2	46·8	96·5	77·0	71·9	41·8	69·5	52·6	0·802	0·342*	0·434*
Folate (μg)	99	222·1	56·2	218·4	70·8	163·8	57·2	159·8	70·5	0·724	0·268*	0·338*
Zn (mg)	99	7·0	1·6	6·8	2·0	6·0	1·3	5·8	1·8	0·761	0·159	0·322*
Ca (mg)	99	1184·5	360·0	1136·2	406·6	968·7	289·0	965·2	377·6	0·772	0·447*	0·379*
Fe (mg)	99	7·9	2·6	7·5	2·7	6·7	2·9	6·5	3·1	0·989	0·134	0·222*
Iodine (μg)	99	191·2	94·9	184·8	125·4	153·9	69·0	144·4	70·0	0·287	0·411*	0·484*
P (mg)	99	1255·6	311·0	1189·1	398·2	1074·0	262·3	1044·5	349·1	0·655	0·404*	0·401*

IQR, interquartile range.
*Statistically significant Spearman’s *r* ($P < 0·05$).
†Wilcoxon signed-rank test, adjusted for energy intake ($P < 0·05$ indicates significance).

energy intake the limits of agreement were wide. The Bland–Altman analysis showed a positive mean difference, indicating that the FFQ recorded higher intakes than the FR. The plots for vegetable (Fig. 1(b)) and fruit intake (Fig. 1(c)) indicate a suspicion of data being heteroscedastic. This was examined using Kendall’s τ correlation, which was found not to be statistically significant for fruits ($\tau = -0·01$; $P = 0·904$) and vegetables ($\tau = 0·08$; $P = 0·112$). Heteroscedasticity was tested for all nutrients and food groups with no significant results.

Cross-classification into tertiles showed that the percentage of participants correctly classified ranged from 37·3 % for Fe to 58·6 % for iodine, as regards nutrient intakes (Table 3). For food group intakes, the percentage of participants correctly classified ranged from 36·5 % for poultry to 58·5 % for both milk and fruits. On average, 89 % of children were classified by both methods into the same or adjacent tertiles according to their food group intakes and 87 % according to their nutrient intakes.

Overall, the agreement between the FFQ and the FR was generally moderate when tested with κ_w (Table 3) since most food groups and nutrients reached statistical significance, although few moderate agreements were observed. After adjustment for total energy intake, the κ_w values ranged from 0·12 (poultry) to 0·62 (milk) for the food groups and from 0·12 (SFA) to 0·48 (iodine) for the nutrients. Substantial agreement was observed for milk ($\kappa_w = 0·62$), while moderate agreement was observed for fruits, vitamin B_{12} and iodine ($\kappa_w = 0·57$, 0·44 and 0·48, respectively; Table 3).

Discussion

The present study is the first validation study of an FFQ addressed to pre-school children in Greece. The participants were selected from the population for which the questionnaire was designed, and there were no differences regarding sociodemographic characteristics between the study participants and the overall population of the Rhea cohort, with the exception of maternal smoking status.

The average correlations of energy-adjusted data between the FFQ and FR found in the present study ($r = 0·38$ for food groups and $r = 0·31$ for nutrients) are low to moderate but comparable to those reported in other validation studies. In a validation study conducted by Blum *et al.*[23] on pre-school children aged 1–5 years, moderate correlations were reported for nutrients ranging from $r = 0·26$ to $r = 0·63$, while the average correlation was $r = 0·52$. Similar correlations were found by Parrish *et al.*[24], with range from $r = 0·33$ to 0·42 for nutrients, in a study conducted in children aged 1–3 years. For older children aged 4–9 years, Wilson and Lewis[25] reported correlations from 0·40 to 0·55 for nutrients. Additionally, Treiber *et al.*[12] reported a mean correlation of

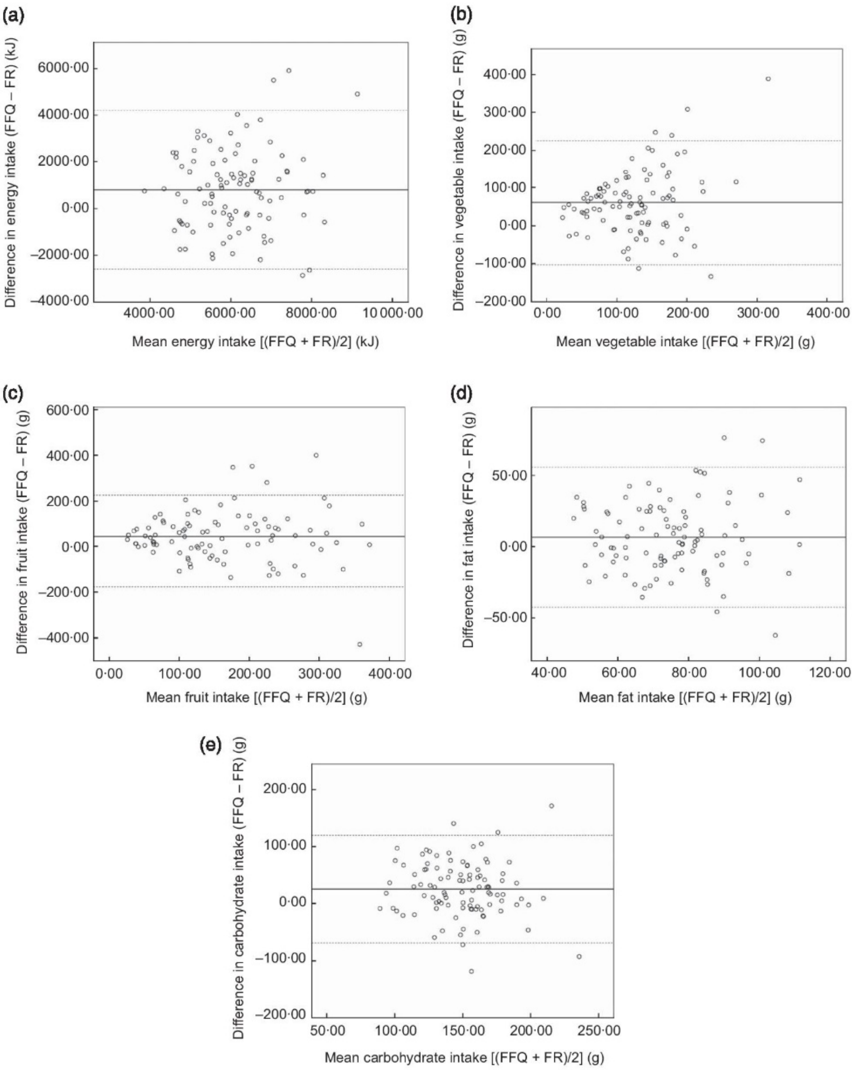

Fig. 1 Bland–Altman plots showing the agreement between the FFQ and the 3 d food record (FR) in estimating daily nutrient and food group intakes among pre-school children (fifty-one boys and forty-eight girls) in the validation study of the mother–child 'Rhea' birth cohort, Heraklion, Crete, Greece, 2011–2012. The difference between the two methods (FFQ – FR) is plotted *v.* the mean of the two methods (FFQ + FR)/2), where ——— represents the mean difference between the two dietary methods and - - - - - represent the 95 % limits of agreement (LOA; corresponding to the mean difference ± 1·96 SD), for: (a) energy intake (mean difference = 794 kJ and LOA = – 2589 kJ, 4179 kJ); (b) vegetable intake (mean difference = 61 g and LOA = – 102 g, 225 g); (c) fruit intake (mean difference = 45 g and LOA = – 176 g, 226 g); (d) fat intake (mean difference = 6 g and LOA = – 42 g, 55 g); (e) carbohydrate intake (mean difference = 25 g and LOA = – 69 g, 120 g)

0·67 (range: $r = 0·42$ to 0·83) for nutrients in a study of fifty-five children aged 3–5 years. Weaker correlations ($r = 0·48$ for total energy, $r = 0·35$ for total fat and $r = 0·37$ for saturated fat) were observed in a study performed by Stein *et al.*[26], probably due to incorrect parental reports on dietary intake when children were not under their surveillance.

In our validation study, we found moderate agreement between the FFQ and FR as regards food groups and nutrients. Calculating solely correlations is not sufficient to give credence to the FFQ. Comparisons of tertiles are more informative than a correlation coefficient when reporting the capacity of an assessment method to rank persons with regard to their intake[27]. In our analysis, in spite of the relatively modest correlations between the two dietary methods, the Rhea follow-up FFQ and FR showed relatively good agreement in ranking participants based on their food and nutrient intakes. The degree of misclassification was overall small, while the majority

Table 3 Cross-classification of participants by tertiles of calculated daily food group and nutrient intakes from the FFQ and the 3 d food record (FR) among pre-school children (fifty-one boys and forty-eight girls) in the validation study of the mother–child 'Rhea' birth cohort, Heraklion, Crete, Greece, 2011–2012

	Agreement of tertiles for food groups and nutrients				
	Correctly classified (%)	Adjacent classified (%)	Grossly misclassified (%)	Weighted kappa (κ_w)†	95 % CI
Food groups					
Sugar preserves and confectionery (g)	49·0	39·8	11·2	0·37*	0·23, 0·44
Vegetables (g)	43·4	39·5	17·1	0·21*	0·05, 0·31
Fruits (g)	58·5	36·1	5·4	0·57*	0·53, 0·63
Pulses (g)	47·0	41·2	11·8	0·31*	0·20, 0·45
Milk (g)	58·5	38·5	3·0	0·62*	0·47, 0·73
Poultry (g)	36·5	47·3	16·2	0·12	0·00, 0·20
Red meat (g)	44·7	39·7	15·6	0·17*	0·06, 0·32
Eggs (g)	45·5	44·3	10·2	0·36*	0·16, 0·42
Nutrients					
Protein (g)	38·4	48·4	13·2	0·24*	0·15, 0·32
Carbohydrate (g)	39·4	46·4	14·2	0·23*	0·10, 0·42
Total fat (g)	38·4	46·4	15·2	0·20*	0·01, 0·34
MUFA (per 100 g)	41·4	40·4	18·2	0·15	0·01, 0·32
PUFA (per 100 g)	47·5	42·4	10·1	0·38*	0·28, 0·53
SFA (per 100 g)	43·4	36·4	20·2	0·12	0·01, 0·28
Vitamin B_{12} (μg)	52·5	38·4	9·1	0·44*	0·39, 0·52
Vitamin C (mg)	39·4	50·5	10·1	0·32*	0·20, 0·40
Folate (μg)	43·4	44·4	12·2	0·30*	0·21, 0·40
Zn (mg)	43·4	42·4	14·2	0·26*	0·03, 0·44
Ca (mg)	49·5	38·4	12·1	0·35*	0·19, 0·39
Fe (mg)	37·3	46·5	16·2	0·17*	0·03, 0·24
Iodine (μg)	58·6	32·3	9·1	0·48*	0·32, 0·59
P (mg)	53·6	32·2	14·2	0·33*	0·17, 0·53

Correctly classified if classified into the same tertile and grossly misclassified if classified into opposing tertiles.
*Statistically significant κ_w ($P < 0·05$).
†κ_w analysis of the agreement between the FFQ and the FR was undertaken on energy-adjusted data, using Fleiss–Cohen quadratic weight.

of participants (88 %) were classified into the same or adjacent tertiles for both food groups and nutrients.

The moderate agreement found between the two dietary methods was also attested by the Bland–Altman technique. The plots for energy, fat and carbohydrate intakes suggest that the mean differences between the FFQ and FR are similar not only at low but also at high intakes, with the limits of agreement wide for energy intake. This was also observed in all plots for nutrients and food groups (data not shown), except for fruits and vegetables where the mean differences increased at high intake. Furthermore, the test for heteroscedasticity showed no significant correlations ($P > 0·05$). Overall, the plots showed a small positive mean difference for food groups and nutrients between the two dietary methods, indicating that the Rhea 4 years FFQ tended to slightly overestimate food group and nutrient intakes compared with the FR. Nevertheless, the overestimation of nutrients suggested by the Rhea 4 years FFQ in the present study is in agreement with previous studies[12,24,26].

The energy adjustment method was used in order to reduce dietary method error and to avoid misleading conclusions based on the differences found in total energy intake among participants. In fact, the energy-adjusted correlation coefficients were higher than the crude for almost all food groups and nutrients. In this analysis, we excluded those food groups with very low consumption (<50 % of study participants).

The degree of agreement between the FFQ and FR could also be affected by parameters such as time span covered by the dietary tool, the ability to record seasonality and day-to-day variability[28]. In particular, the great variation in dietary intake is more pronounced in children, leading to weakened agreement between the different dietary tools used[29]. Finally, a limitation of validation studies is that they are not applicable to other populations[30].

Conclusion

In summary, the present validation study indicates that the Rhea 4 years FFQ produces a realistic and relatively precise estimate of habitual intakes of food groups and nutrients among pre-school children in Crete, Greece.

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None. *Authorship:* V.L. was the field study co-coordinator, participated in the statistical analysis and wrote the first draft of the paper; V.G. supervised the statistical analysis and helped with the data interpretation and manuscript preparation; L.C. supervised the data collection and provided feedback and critical revision of the manuscript; K.S. participated in the design of the study, helped with data interpretation and manuscript preparation, and provided critical review of the manuscript. *Ethics of human subject participation:* The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethical committee of the University Hospital in Heraklion, Crete, Greece. Written informed consent was obtained from all women participating in the study.

References

1. Koletzko B, Brands B, Poston L *et al.* (2012) Early nutrition programming of long-term health. *Proc Nutr Soc* **71**, 371–378.
2. Hussain A, Claussen B, Ramachandran A *et al.* (2007) Prevention of type 2 diabetes: a review. *Diabetes Res Clin Pract* **76**, 317–326.
3. Flock MR & Kris-Etherton PM (2011) Dietary Guidelines for Americans 2010: implications for cardiovascular disease. *Curr Atheroscler Rep* **13**, 499–507.
4. Osler M (2002) Nutritional modification of cardiovascular disease risk. *Int Congr Ser* **1229**, 109–111.
5. Johnson L, Mander AP, Jones LR *et al.* (2008) Energy-dense, low-fiber, high-fat dietary pattern is associated with increased fatness in childhood. *Am J Clin Nutr* **87**, 846–854.
6. Reilly JJ, Armstrong J, Dorosty AR *et al.* (2005) Early life risk factors for obesity in childhood: cohort study. *BMJ* **330**, 1357.
7. Becton LJ, Shatat IF & Flynn JT (2012) Hypertension and obesity: epidemiology, mechanisms and clinical approach. *Indian J Pediatr* **79**, 1056–1061.
8. Labarthe DR, Eissa M & Varas C (1991) Childhood precursors of high blood pressure and elevated cholesterol. *Annu Rev Public Health* **12**, 519–541.
9. Rosenlund H, Kull I, Pershagen G *et al.* (2011) Fruit and vegetable consumption in relation to allergy: disease-related modification of consumption? *J Allergy Clin Immunol* **127**, 1219–1225.
10. Chatzi L, Torrent M, Romieu I *et al.* (2007) Diet, wheeze, and atopy in school children in Menorca, Spain. *Pediatr Allergy Immunol* **18**, 480–485.
11. Vergnaud AC, Romaguera D, Peeters PH *et al.* (2013) Adherence to the World Cancer Research Fund/American Institute for Cancer Research guidelines and risk of death in Europe: results from the European Prospective Investigation into Nutrition and Cancer cohort study. *Am J Clin Nutr* **97**, 1107–1120.
12. Treiber FA, Leonard SB, Frank G *et al.* (1990) Dietary assessment instruments for preschool children: reliability of parental responses to the 24-hour recall and a food frequency questionnaire. *J Am Diet Assoc* **90**, 814–820.
13. Watson JF, Collins CE, Sibbritt DW *et al.* (2009) Reproducibility and comparative validity of a food frequency questionnaire for Australian children and adolescents. *Int J Behav Nutr Phys Act* **6**, 62.
14. Kobayashi T, Kamimura M, Imai S *et al.* (2011) Reproducibility and validity of the food frequency questionnaire for estimating habitual dietary intake in children and adolescents. *Nutr J* **10**, 27.
15. Subar AF, Thompson FE, Kipnis V *et al.* (2001) Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. *Am J Epidemiol* **154**, 1089–1099.
16. Schatzkin A, Kipnis V, Carroll RJ *et al.* (2003) A comparison of a food frequency questionnaire with a 24-hour recall for use in an epidemiological cohort study: results from the biomarker-based Observing Protein and Energy Nutrition (OPEN) study. *Int J Epidemiol* **32**, 1054–1062.
17. Willett W (2001) Commentary: dietary diaries versus food frequency questionnaires – a case of undigestible data. *Int J Epidemiol* **30**, 317–319.
18. Chatzi L, Plana E, Daraki V *et al.* (2009) Metabolic syndrome in early pregnancy and risk of preterm birth. *Am J Epidemiol* **170**, 829–836.
19. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1**, 307–310.
20. Fleiss JL & Cohen J (1973) The equivalence of weighted kappa and the intraclass correlation coefficient as measures of reliability. *Educ Psychol Meas* **33**, 613–619.
21. Landis JR & Koch GG (1977) The measurement of observer agreement for categorical data. *Biometrics* **33**, 159–174.
22. Willett WC (1998) *Nutritional Epidemiology.* New York: Oxford University Press.
23. Blum RE, Wei EK, Rockett HR *et al.* (1999) Validation of a food frequency questionnaire in Native American and Caucasian children 1 to 5 years of age. *Matern Child Health J* **3**, 167–172.
24. Parrish LA, Marshall JA, Krebs NF *et al.* (2003) Validation of a food frequency questionnaire in preschool children. *Epidemiology* **14**, 213–217.
25. Wilson AM & Lewis RD (2004) Disagreement of energy and macronutrient intakes estimated from a food frequency questionnaire and 3-day diet record in girls 4 to 9 years of age. *J Am Diet Assoc* **104**, 373–378.
26. Stein AD, Shea S, Basch CE *et al.* (1992) Consistency of the Willett semiquantitative food frequency questionnaire and 24-hour dietary recalls in estimating nutrient intakes of preschool children. *Am J Epidemiol* **135**, 667–677.
27. Garrow JS (1995) Validation of methods for estimating habitual diet: proposed guidelines. *Eur J Clin Nutr* **49**, 231–232.
28. Cade J, Thompson R, Burley V *et al.* (2002) Development, validation and utilisation of food-frequency questionnaires – a review. *Public Health Nutr* **5**, 567–587.
29. Nelson M, Black AE, Morris JA *et al.* (1989) Between- and within-subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision. *Am J Clin Nutr* **50**, 155–167.
30. Plummer M & Kaaks R (2003) Commentary: An OPEN assessment of dietary measurement errors. *Int J Epidemiol* **32**, 1062–1063.

4.2.3 Paper 4. Early life determinants of dietary patterns in preschool children: Rhea mother-child cohort, Crete, Greece.

Main Findings:

- Three dietary patterns were identified. The 'Mediterranean' pattern was based on pulses, olive oil, vegetables, fish and fruits; the 'Snacky' pattern included potatoes and other starchy roots, salty snacks, sugar products and eggs; the 'Western' pattern contained cereals, cheese, added lipids, beverages and meat.
- Preschool attendance and increased time spent with the mother (≥ 2 hours/day) was positively associated with the 'Mediterranean' pattern, whereas TV watching was inversely associated with this pattern. Lower parental education, maternal age and earlier introduction to solid foods were positively associated with the 'Snacky' pattern. Higher scores on the 'Western' type diet were associated with exposure to passive smoking and TV watching.

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ORIGINAL ARTICLE

Early life determinants of dietary patterns in preschool children: Rhea mother–child cohort, Crete, Greece

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BACKGROUND/OBJECTIVES: The determination of dietary patterns in children examines the effects of the overall diet at early ages, instead of looking at individual foods or energy providing nutrients. The present analysis aims to identify the dietary patterns of preschool children and to examine their associations with multiple socio-economic and lifestyle characteristics.
SUBJECTS/METHODS: Dietary data were collected for 1081 children participating in the Rhea mother–child cohort in Crete, Greece. Diet was assessed using a validated food frequency questionnaire, and dietary patterns were identified with principal component analysis. Multivariable linear regression models were used to examine factors associated with each dietary pattern.
RESULTS: Three dietary patterns were identified explaining 45.8% of the total diet variation. The 'Mediterranean' pattern was based on pulses, olive oil, vegetables, fish and fruits; the 'Snacky' pattern included potatoes and other starchy roots, salty snacks, sugar products and eggs; the 'Western' pattern contained cereals, cheese, added lipids, beverages and meat. Preschool attendance and increased time spent with the mother (⩾2 h/day) were positively associated with the 'Mediterranean' pattern, whereas watching TV was inversely associated with this pattern. Lower parental education, maternal age and earlier introduction to solid foods were positively associated with the 'Snacky' pattern. Higher scores on the 'Western' type diet were associated with exposure to passive smoking and watching TV. No variation in energy providing nutrient intake was observed across tertiles of the identified dietary patterns.
CONCLUSIONS: The results from this analysis indicate the important role of socio-demographic factors on children's dietary preferences in early age.

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INTRODUCTION

Dietary food preferences that are first established at preschool age encourage children to adopt healthy eating in adult life.[1,2] Dietary patterns in the first years of life have been associated with multiple health outcomes.[3] Unhealthy dietary patterns in the first years of life, with high fat and low fiber foods, have been positively associated with greater risk of childhood obesity[4] and negatively associated with cognitive development.[5,6] Understanding early life dietary choices is of great interest in terms of developing strategies that will ensure healthy nutrition in early childhood, as dietary patterns are possibly age specific.

A number of studies have examined dietary patterns in infancy and toddlerhood,[7–13] with only a few focused at preschool age.[14–17] Although the type and number of identified patterns in preschoolers' vary among studies, three main patterns have been observed. These patterns have been described as 'healthy', 'less healthy' and 'traditional'. Several parental socio-demographic and lifestyle characteristics have been associated with the adoption of specific dietary patterns in early childhood. Lower levels of maternal education[13–16,18] and the presence of older siblings[14] have been associated with 'less healthy' diets. On the other hand, breastfeeding has been positively associated with the adoption of a 'healthy' dietary pattern in childhood.[17]

The use of dietary patterns allows the combination of foods usually eaten together and the exploration of the whole diet.[19] Principal component analysis (PCA) is a statistical method that groups individual foods and nutrients and describes specific patterns. Pattern analysis can form the basis for the development of specific dietary guidelines and recommendations.[20]

To our knowledge, this is the first birth cohort study exploring the association of dietary patterns using PCA in Greek preschool children and the level of influence by several early life socio-demographic and lifestyle factors. As PCA method is data driven, results from the literature cannot be extrapolated to other populations. Thus, understanding dietary patterns at early ages is essential, given that Greece has the highest prevalence of overweight and obese preadolescent children in Europe, based on recent reports.[21]

MATERIALS AND METHODS

Study population

The 'Rhea' birth cohort study is a prospective cohort that started in February 2007 in Crete, Greece.[22] Briefly, pregnant women, residents in the study area, aged 16 years or above, with no communication handicap were included in the study. Pregnant women (Greek and immigrant) who became pregnant within a 12-month period, starting in February 2007, were contacted and asked to participate in the study. The first contact was made before 15-week gestation, at the time of the first major ultrasound examination, and participants were invited to provide blood and urine samples and to participate in a face-to-face interview. Women were contacted again at various times during pregnancy, at birth, at 8–10 weeks after delivery and for children's follow-up at 9th, 18th months and at 4 years of age. Face-to face completed questionnaires together with self-

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administered questionnaires and medical records were used to obtain information on dietary, environmental and psychosocial exposures during pregnancy and early childhood. The study was approved by the Ethical Committee of the University Hospital of Heraklion (Crete, Greece), and all participants provided written informed consent after complete description of the study.

Dietary information was available for 1081 children participating at the 4-year follow-up by completing a food frequency questionnaire (FFQ). Children following a specific diet for health purposes ($n = 20$) were excluded from the analysis resulting to 1061 subjects. In total, 683 children with full information on dietary intake and multiple socio-demographic and lifestyle factors collected at different time points were included in this analysis.

Dietary assessment

Dietary assessment at 4 years of age was performed with the use of the 'Rhea follow-up FFQ', which is a validated semi-quantitative food frequency questionnaire designed to assess habitual dietary intake in preschool children.[23] Primary caregivers were phone-interviewed by a dietitian trained in a standard protocol to complete the questionnaire. The questionnaire contained questions on 118 food items with the following components: food frequency, type of meals during the day (breakfast, morning snack, lunch, afternoon snack, dinner and evening snack), use of dietary supplements, type of fat used for cooking, frequency of meals consumed in restaurants or take away and television viewing during meals. The questions were hierarchical from general to more specific questions. The 118 food items were aggregated into 17 food groups (cereals and cereal products, meat and meat products, fish and seafood, milk and milk products, eggs, total added lipids, olive oil, potatoes and other starchy roots, pulses, vegetables, nuts, fruits, sugar preserves and confectionery, non-alcoholic beverages, olives, salty snacks and miscellaneous). Parents could choose from one or two portion sizes and report the child's intake in terms of times per day, week, month and year or never. Seasonality of consumption was also reported in all food items.

The FFQ data were converted into daily intake of foods and nutrients using the UK food tables (McCance & Widdowson's The Composition of Foods, 6th summary edition) and standard greek recipes for complex mixed dishes. The dietary intake data were analyzed by using a software program developed at the Department of Applied Information Technology and Multimedia, TEI-Crete, Heraklion, Crete, Greece.

Parental and child characteristics

Parents provided information on several socio-demographic and lifestyle characteristics from early pregnancy up to the age of 4 years through interviewer-administered questionnaires, including the following: (A) *Parental characteristics*: maternal age at 4 years of age (<31, 31–36, $\geqslant 36$ years); maternal residence (urban, rural) at 4 years of age; maternal and paternal origin at recruitment (Greek/Non-Greek); maternal and paternal education at recruitment (low level: $\leqslant 6$ years of school, medium level: 7–12 years of school, high level: university or technical college degree); maternal working status at 4 years of age (yes/no); marital status at 4 years of age (married-engaged/other); pre-pregnancy maternal body mass index (BMI, kg/m^2) (number); type of delivery (caesarian/vaginal). (B) *Child characteristics*: gender (male/female); birth order (order number); birth weight (grams); preterm birth (<37 weeks of gestation; yes/no); breastfeeding duration (months); age of introduction to solid foods at 9 months (months); preschool attendance at 4 years of age (yes/no); hours/day spent with mother and father at 4 years of age (<1, 1–2, $\geqslant 2$); passive smoking at home at 4 years (yes/no); hours/day spending watching television at 4 years (almost never, 1–2, $\geqslant 3$); hours/day spent outside home at 4 years (<1, 1–3, $\geqslant 3$); child's BMI z-scores based on cohort-specific, gender and age-adjusted growth curves. Child's BMI cutoffs were based on the International Obesity Task Force (IOTF) criteria.[24]

Statistical analyses

Differences in distributions of normally distributed variables were tested with a *t*-test, non-normally distributed continuous variables were tested by using nonparametric tests (i.e., Mann–Whitney, Kruskal–Wallis and Spearman's non-parametric statistical tests), whereas categorical variables were tested with the chi-square test (Pearson's or Fisher's exact test with Monte–Carlo correction).

PCA was used to identify the dietary patterns using the child's daily intake (in grams) of the 17 food groups as input. This method reduces the

Table 1. Descriptive characteristics of the study population (mother–child pairs) included in the PCA, for the Rhea Cohort Study, Crete, Greece ($n = 1061$)

	N	% or Mean ± s.d.
Parental characteristics		
Maternal age (years)	1023	33.43 ± 4.96
Maternal residence, n (%)		
Urban	743	70.8
Rural	307	29.2
Maternal origin, n (%)		
Greek	972	93.5
Non-Greek	68	6.5
Paternal origin, n (%)		
Greek	960	94.4
Non-Greek	57	5.6
Maternal education, n (%)		
Low	179	17.9
Medium	511	51.1
High	310	31.0
Paternal education, n (%)		
Low	346	34.5
Medium	430	42.9
High	227	22.6
Maternal working status, n (%)		
Working	716	87.1
Not working	106	12.9
Pre-pregnancy BMI (kg/m^2)	797	24.43 ± 4.70
Type of delivery, n (%)		
Vaginal	512	49.3
Caesarian	526	50.7
Infant characteristics		
Sex, *n (%)*		
Male	551	51.9
Female	510	48.1
No. of siblings, *n (%)*		
0	153	14.4
1	438	41.3
⩾2	232	21.9
Birth weight (kg)	1026	3.17 ± 0.47
Preterm birth, n (%)		
Yes	128	12.5
No	893	87.5
Breastfeeding duration (months)	992	3.84 ± 4.20
Age of introduction to solid foods (months)	954	5.41 ± 0.82
Child life-style characteristics at 4 years		
Pre-school attendance		
No	120	14.6
Yes	703	85.4
Hours/day spent with mother, *n* (%)		
<1	108	13.0
1–2	278	33.5
⩾2	444	53.5
Hours/day spent with father, *n* (%)		
<1	252	30.5
1–2	305	36.9
⩾2	270	32.6
Passive smoking at home, *n (%)*		
No	508	61.7
Yes	315	38.3
Hours/day spent watching TV, *n (%)*		
Almost never	222	27.0
1–2	513	62.3
⩾3	88	10.7
Hours/day spent outside home, *n (%)*		
<1	329	39.6
1–3	413	49.8
⩾3	88	10.6
Child's BMI (kg/m^2),[a] *n (%)*		
No excess weight	655	78.8
Overweight	120	14.3
Obese	63	7.5
Child's BMI (kg/m^2)	838	16.44 ± 1.96

Abbreviations: BMI, body mass index; PCA, principal component analysis; s.d., standard deviation; TV, television. [a]Child's BMI cut offs were based on International Obesity Task Force (IOTF) criteria.[24]

data by forming linear combinations of the original observed variables, thereby grouping together correlated variables, which in its turn identifies any underlying dimensions in the data. The Kaiser-Mayer-Olkin measure was calculated to evaluate the level of intra-correlation between the variables (values >0.6 indicated good intra-correlation and, therefore, PCA could give interpretable results). To identify the number of components, we used the eigenvalue of >1 criterion and the visual representation by the scree plot[25] and the interpretability of the factor loadings. Varimax rotation was applied to enhance the determination of the dietary components.[26,27] Rotation redistributes the explained variance for the individual components, thereby achieving a simpler structure, increasing the number of larger and smaller loadings. Factor loadings above 0.3 on a component were considered to have a strong association with that component. A score was attributed to every child for each of the components identified. The score was calculated for each of the components retained by summing the standardized values of the food items weighted by their scoring coefficients. Tertiles of the factor score of each component were used for cut offs.

Multiple linear regression models were fitted for each of the retained components to determine which of the early life socio-demographic and lifestyle characteristics were significantly associated with the dietary components. Estimated associations were described with β-coefficients and 95% confidence interval (CI). Potential determinants related with dietary components in the bivariate models with a *P*-value of <0.10 were included in the multivariable linear models. Age, sex and pre-pregnancy maternal BMI were included *a priori* in the analyses of dietary components.

All hypotheses testing were conducted assuming a 0.05 significance level and a two-sided alternative hypothesis. Stata S.E. version 13 was used for the statistical analyses (StataCorp, College Station, TX, USA).

RESULTS

A description of the population characteristics is presented in Table 1. The mean maternal age when children were 4 years old was 33.4 (s.d.: 4.96) years. The majority of parents had Greek origin and medium education. Most mothers were working and were married and/or engaged. Dietary data were provided for 551 (51.9%) boys and 510 (48.1%) girls at the age of 4 years. Children had mean birth weight 3.17 (s.d.: 0.47) kg, the majority attended preschool ($n = 703$, 85.4%), were not exposed to passive smoking ($n = 508$, 61.7%) and spent 1–2 h/day watching TV ($n = 513$, 62.3%).

Three dietary patterns were extracted at 4 years of age (Table 2). We have chosen to give each factor a label ('Mediterranean', 'Snacky' and 'Western'); these do not perfectly describe each underlying pattern but correspond to current views on health diet and aid in the report and discussion of results. The 'Mediterranean' dietary pattern comprised mainly vegetables, fruits, pulses, olive oil, fish and seafood. The second pattern included foods that require minimum preparation such as potatoes and other starchy roots, salty snacks, sugar preserves and confectionery and eggs with the description 'Snacky' being the most suitable. The last pattern named 'Western' loaded highly for cereals and bakery products, cheese, lipids of animal and vegetable origin, sweetened beverages, (soft drinks, packed fruit juices) and meat products. The eigenvalues were 3.72, 1.48 and 1.21 for the 'Mediterranean', the 'Snacky' and the 'Western' factors, respectively. Collectively, these factors explained 45.79% of total variance.

Table 3 presents the multivariable associations between several maternal and child socio-demographic and lifestyle characteristics and the three identified dietary patterns.

The 'Mediterannean' pattern

Increased number of siblings ($\beta = 0.24$; 95% CI: 0.01, 0.47), longer breastfeeding duration ($\beta = 0.02$; 95% CI: 0.001, 0.04), preschool attendance ($\beta = 0.28$; 95% CI: 0.06, 0.50) and time spent with mother ($\beta = 0.24$; 95% CI: 0.01, 0.47) were positively associated with the adherence to the 'Mediterranean' pattern at 4 years of age, whereas watching TV at 4 years of age (hours/day) was inversely associated with this pattern ($\beta = -0.31$; 95% CI: −0.60, −0.03).

The 'Snacky' pattern

Higher scores on the 'Snacky' pattern were associated with rural residence ($\beta = 0.18$; 95% CI: 0.03, 0.34), increasing number of older siblings ($\beta = 0.28$; 95% CI: 0.07, 0.49) and hours spent with the mother ($\beta = 0.22$; 95% CI: 0.01, 0.43). Children who were later introduced to solid foods and whose mothers were older and with higher pre-pregnancy BMI scored lower on this pattern. Lower scores were also observed for children of the more educated parents.

The 'Western' pattern

Higher scores on the 'Western' pattern were observed for children with increasing number of siblings, exposed to passive smoking and for those spent more than 3 h per day watching TV and less than 1 h per day outside home. Negative associations were found between maternal Greek origin ($\beta = -0.39$; 95% CI: −0.70, −0.08),

Table 2. Dietary patterns identified by PCA and factor loadings of food groups/items[a] included in each dietary pattern for preschool children in the Rhea cohort, Crete, Greece ($n = 1061$)

Food items[b]	*Dietary pattern*		
	'Mediterranean' type	*'Snacky' type*	*'Western' type*
Pulses	0.730		
Olive oil	0.712		
Vegetables	0.629		
Fish and seafood	0.609		
Fruits	0.491		
Potatoes and other starchy roots		0.689	
Salty snacks		0.575	
Sugar preserves and confectionary		0.503	
Eggs		0.434	
Cereals and cereal products			0.638
Cheese			0.608
Total added lipids			0.570
No alcoholic beverages			0.461
Meat and meat products			0.384
Variance explained (%)	26.562	10.552	8.681

Abbreviation: PCA, principal component analysis. [a]Only food groups/items with factor loadings >0.30 were retained for each factor. [b]Food variables included in PCA as g/day.

Table 3. Multivariable associations between the three dietary patterns and socio-demographic and lifestyle characteristics for preschool children in the Rhea cohort, Crete, Greece ($n=683$)

Characteristics	*'Mediterranean'*[a] β (95% CI)	*'Snacky'*[a] β (95% CI)	*'Western'*[a] β (95% CI)
Parental characteristics			
Maternal age (years)	−0.01 (−0.02, 0.01)	**−0.02 (−0.03, −0.002)**	−0.004 (−0.02, 0.01)
P-value	0.383	**0.023**	0.611
Maternal residence			
Urban	Ref	Ref	Ref
Rural	0.09 (−0.08, 0.26)	**0.18 (0.03, 0.34)**	−0.005 (−0.16, 0.15)
P-value for trend	0.279	**0.021**	0.950
Maternal origin			
Non-Greek	Ref	Ref	Ref
Greek	−0.12 (−0.45, 0.22)	−0.04 (−0.34, 0.27)	**−0.39 (−0.70, −0.08)**
P-value for trend	0.578	0.801	**0.014**
Maternal education			
Low	Ref	Ref	Ref
Medium	−0.06 (−0.27, 0.17)	**−0.25 (−0.45, −0.05)**	0.03 (−0.18, 0.23)
High	−0.12 (−0.38, 0.14)	**−0.34 (−0.58, −0.11)**	−0.03 (−0.28, 0.21)
P-value for trend	0.654	**0.015**	0.793
Paternal education			
Low	Ref	Ref	Ref
Medium	0.14 (−0.04, 0.32)	**−0.25 (−0.42, −0.09)**	0.08 (−0.09, 0.25)
High	0.17 (−0.07, 0.40)	**−0.29 (−0.51, −0.08)**	0.01 (−0.21, 0.23)
P-value for trend	0.242	**0.005**	0.600
Maternal working status			
Not working	Ref	Ref	Ref
Working	0.04 (−0.18, 0.26)	0.10 (−0.09, 0.31)	0.01 (−0.20, 0.21)
P-value for trend	0.704	0.312	0.965
Pre-pregnancy BMI (kg/m^2)	0.007 (−0.10, 0.02)	**−0.02 (−0.03, −0.002)**	−0.01 (−0.02, 0.01)
P-value	0.430	**0.023**	0.392
Type of delivery			
Vaginal	Ref	Ref	Ref
Caesarian	0.08 (−0.08, 0.23)	0.03 (−0.11, 0.17)	0.14 (−0.002, 0.28)
P-value for trend	0.321	0.650	0.054
Infant characteristics			
Sex			
Male	Ref	Ref	Ref
Female	−0.05 (−0.20, 0.09)	−0.01 (−0.15, 0.13)	−0.03 (−0.17, 0.11)
P-value for trend	0.498	0.870	0.697
No. of siblings			
0	Ref	Ref	Ref
1	0.17 (−0.04, 0.37)	0.09 (−0.10, 0.27)	**0.28 (0.09, 0.47)**
⩾2	**0.24 (0.01, 0.47)**	**0.28 (0.07, 0.49)**	**0.22 (0.003, 0.43)**
P-value for trend	0.123	**0.017**	**0.016**
Birth weight (kg)	−0.18 (−0.36, 0.01)	−0.08 (−0.25, 0.09)	0.01 (−0.16, 0.18)
P-value	0.060	0.352	0.889
Preterm birth			
No	Ref	Ref	Ref
Yes	0.04 (−0.20, 0.28)	0.05 (−0.17, 0.26)	0.21 (−0.02, 0.43)
P-value for trend	0.758	0.686	0.070
Breastfeeding duration (months)	**0.02 (0.001, 0.04)**	−0.002 (−0.02, 0.02)	−0.01 (−0.02, 0.01)
P-value	**0.039**	0.805	0.436
Age of introduction to solid foods (months)	−0.02 (−0.12, 0.08)	**−0.11 (−0.20, −0.01)**	−0.03 (−0.13, 0.06)
P-value	0.769	**0.025**	0.496
Child lifestyle characteristics at 4 years			
Pre-school attendance			
No	Ref	Ref	Ref
Yes	**0.28 (0.06, 0.50)**	0.13 (−0.07, 0.34)	**−0.23 (−0.44, −0.03)**
P-value for trend	**0.013**	0.195	**0.027**
Hours/day spent with mother			
<1	Ref	Ref	Ref
1–2	0.13 (−0.11, 0.36)	0.15 (−0.07, 0.37)	−0.15 (−0.37, 0.07)
⩾2	**0.24 (0.005, 0.47)**	**0.22 (0.01, 0.43)**	−0.07 (−0.28, 0.15)
P-value for trend	0.105	0.126	0.350
Passive smoking at home			
No	Ref	Ref	Ref
Yes	−0.01 (−0.17, 0.16)	0.12 (−0.03, 0.27)	**0.23 (0.08, 0.38)**
P-value for trend	0.948	0.127	**0.003**

Table 3. (Continued)

Characteristics	'Mediterranean'[a] β (95% CI)	'Snacky'[a] β (95% CI)	'Western'[a] β (95% CI)
Hours/day spent watching TV			
Almost never	Ref	Ref	Ref
1–2	−0.09 (−0.26, 0.07)	0.14 (−0.01, 0.29)	0.08 (−0.08, 0.23)
⩾3	**−0.31 (−0.60, −0.03)**	0.22 (−0.04, 0.48)	**0.29 (0.02, 0.56)**
P-value for trend	0.100	0.130	0.104
Hours/day spent outside home			
<1	Ref	Ref	Ref
1–3	0.06 (−0.10, 0.22)	0.12 (−0.02, 0.27)	**0.16 (0.01, 0.31)**
⩾3	0.04 (−0.22, 0.30)	0.13 (−0.11, 0.37)	0.22 (−0.03, 0.46)
P-value for trend	0.774	0.221	0.063
Child's BMI z-score[b]	0.04 (−0.04, 0.12)	−0.03 (−0.10, 0.04)	−0.01 (−0.08, 0.06)
P-value	0.278	0.384	0.792

Abbreviations: BMI, body mass index; CI, confidence interval; PCA, principal component analysis; s.d., standard deviation; TV, television. All models are adjusted for energy intake (kcal/day). [a]Bold writing indicates statistical significance (*P*-value < 0.05). [b]Child's BMI z-score based on cohort-specific, gender and age-adjusted growth curves.

preschool attendance ($\beta = -0.23$; 95% CI: −0.44, −0.03) and the 'Western' pattern.

Analyses of associations between mean energy and energy-adjusted nutrient intake showed no overall variation among the three identified dietary patterns (Supplementary Table 1).

DISCUSSION

The aim of the present study was to identify the dietary patterns in Greek preschool children and to examine their associations with multiple socio-demographic and lifestyle factors.

The total variance (45.8%), explained by the three dietary patterns identified in the present analysis was higher compared with other studies in the Mediterranean region. Studies in Greece and Spain have identified dietary patterns with a lot smaller variance 12.5 and 22.5%, respectively,[16,28] whereas in a Portuguese study where 48% of variance was explained by a total of eight dietary patterns only 9.9% of the variance was explained by the first dietary pattern.[18]

In the present analysis, the 'Mediterranean' dietary component was the principal pattern that explained the higher percentage of variability, 26.6%. Even though traditional Greek diet has undergone many changes the last years,[29–32] it seems that the Mediterranean pattern still reflects the main dietary choice for children. At the same time, it is important to highlight that the other two 'less healthy' patterns also explain a high percentage of the children's dietary variation (19.2%).

Regarding our findings on the determinants of dietary patterns, other studies have also found an association between lower parental educational levels,[14,16–18,33] the presence of older siblings, exposure to passive smoking[8,14] and earlier introduction to solid foods[9,12] with less 'healthy' patterns. Similarly, higher scores on 'healthier' patterns have been observed for children with longer breastfeeding duration[9,11,12–17] and for those attending preschool.[34] It has been supported that attending pre- and elementary school lunch is associated with healthier eating habits and lower sedentary behavior.[34] When giving children the option to a single meal proposed at school may encourage them to taste more foods and therefore improve their dietary diversity.[35]

Overall, there is little evidence on the underlying mechanisms of the previously mentioned determinants on the configuration of preschoolers' dietary patterns. However, the observed associations of earlier or later introduction to solid foods and breastfeeding duration with dietary patterns can be possibly explained by early feeding practices. There are mechanisms involving genetic determinants that modulate the perception of flavor, the food acceptance and taste preferences.[36,37] Flavors can be transmitted prenatally through amniotic fluid and postnatally through breast milk, depending on maternal diet.[38] Therefore, maternal choices are essential for children's future eating habits.

Comparing our findings with other studies is rather complex mainly because of multiple methodological approaches including study design, dietary assessment tools, food variability across countries and different statistical techniques used. Factor analysis is a sample-specific method, and its results cannot be extrapolated to the general population.[39] The main reservation of PCA is that it introduces researcher's subjectivity in the analysis, when determining the number of components and their interpretation. However, these subjective decisions derive from previous scientific knowledge on nutritional epidemiologic research.

Strengths of the present study include the population-based prospective design and the high participation rate (82%). Diet was assessed using a validated FFQ for this population,[23] although parental misreporting should always be counted in.[40] PCA is also a validated method for the determination of dietary patterns.[41] The use of this method gives the opportunity to gain deep understanding of the whole diet instead of isolated foods and nutrients and thus design valuable interventions and health policies.[42] A limitation of our analysis is that we found no variation in nutrient intake underpinning each dietary pattern, which could have provided us with useful information on the association between diet and health.

The findings of the present study clearly indicate that dietary patterns followed from preschoolers' are associated with multiple socio-demographic factors and lifestyle choices. As dietary and other lifestyle choices are formed early in life, it is essential to enforce policies targeting the whole family and encouraging healthy behaviors from pregnancy onward.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Ventura AK, Worobey J. Early influences on the development of food preferences. *Curr Biol* 2013; **23**: R401–R408.
2 Birch L, Savage JS, Ventura A. Influences on the development of children's eating behaviours: from infancy to adolescence. *Can J Diet Pract Res* 2007; **68**: s1–s56.
3 Smithers LG, Golley RK, Brazionis L, Lynch JW. Characterizing whole diets of young children from developed countries and the association between diet and health: a systematic review. *Nutr Rev* 2011; **69**: 449–467.
4 Ambrosini GL. Childhood dietary patterns and later obesity: a review of the evidence. *Proc Nutr Soc* 2014; **73**: 137–146.
5 Smithers LG, Golley RK, Mittinty MN, Brazionis L, Nortstone K, Emmett P *et al.* Do dietary trajectories between infancy and toddlerhood influence IQ in childhood and adolescence? Results from a prospective birth cohort study. *PLoS One* 2013; **8**: e58904.
6 Northstone K, Joinson C, Emmett P, Ness A, Paus T. Are dietary patterns in childhood associated with IQ at 8 years of age? A population-based cohort study. *J Epidemiol Community Health* 2012; **66**: 624–628.
7 Ystrom E, Niegel S, Vollrath ME. The impact of maternal negative affectivity on dietary patterns of 18-month-old children in the Norwegian Mother and Child Cohort Study. *Matern Child Nutr* 2009; **5**: 234–242.
8 Smithers LG, Brazionis L, Golley RK, Mittinty MN, Nortstone K, Emmett P *et al.* Associations between dietary patterns at 6 and 15 months of age and socio-demographic factors. *Eur J Clin Nutr* 2012; **66**: 658–666.
9 Robinson S, Marriott L, Poole J, Crozier S, Borland S, Lawrence W *et al.* Dietary patterns in infancy: the importance of maternal and family influences on feeding practice. *Br J Nutr* 2007; **98**: 1029–1037.
10 Okubo H, Miyake Y, Sasaki S, Tanaka K, Murakami K, Hirota Y. Dietary patterns in infancy and their associations with maternal socio-economic and lifestyle factors among 758 Japanese mother-child pairs: the Osaka Maternal and Child Health Study. *Matern Child Nutr* 2014; **10**: 213–225.
11 Kiefte-de Jong JC, de Vries JH, Bleeker SE, Jaddoe VW, Hofman A, Raat H *et al.* Socio-demographic and lifestyle determinants of 'Western-like' and 'Health conscious' dietary patterns in toddlers. *Br J Nutr* 2013; **109**: 137–147.
12 Bell LK, Golley RK, Daniels L, Magarey AM. Dietary patterns of Australian children aged 14 and 24 months, and associations with socio-demographic factors and adiposity. *Eur J Clin Nutr* 2013; **67**: 638–645.
13 Northstone K, Emmett P. The associations between feeding difficulties and behaviours and dietary patterns at 2 years of age: the ALSPAC cohort. *Matern Child Nutr* 2013; **9**: 533–542.
14 North K, Emmett P. Multivariate analysis of diet among three-year-old children and associations with socio-demographic characteristics. The Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) Study Team. *Eur J Clin Nutr* 2000; **54**: 73–80.
15 Wall CR, Thompson JM, Robinson E, Mitchell EA. Dietary patterns of children at 3.5 and 7 years of age: a New Zealand birth cohort study. *Acta Paediatr* 2013; **102**: 137–142.
16 Aranceta J, Perez-Rodrigo C, Ribas L, Serra-Majem L. Sociodemographic and lifestyle determinants of food patterns in Spanish children and adolescents: the enKid study. *Eur J Clin Nutr* 2003; **57**: S40–S44.
17 Grieger JA, Scott J, Cobiac L. Dietary patterns and breast-feeding in Australian children. *Public Health Nutr* 2011; **14**: 1939–1947.
18 Moreira P, Santos S, Padrao P, Cordeiro T, Bessa M, Valente H *et al.* Food patterns according to sociodemographics, physical activity, sleeping and obesity in Portuguese children. *Int J Environ Res Public Health* 2010; **7**: 1121–1138.
19 Smith AD, Emmett PM, Newby PK, Northstone K. Dietary patterns obtained through principal components analysis: the effect of input variable quantification. *Br J Nutr* 2013; **109**: 1881–1891.
20 Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ *et al.* AHA Dietary Guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation* 2000; **102**: 2284–2299.
21 Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012; **7**: 284–294.
22 Chatzi L, Plana E, Daraki V, Karakosta P, Alegkakis D, Tsatsanis C *et al.* Metabolic syndrome in early pregnancy and risk of preterm birth. *Am J Epidemiol* 2009; **170**: 829–836.
23 Leventakou V, Georgiou V, Chatzi L, Sarri K. Relative validity of an FFQ for pre-school children in the mother-child 'Rhea' birth cohort in Crete, Greece. *Public Health Nutr* 2014; **18**: 421–427.
24 Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000; **320**: 1240–1243.
25 Cattell RB. The scree test for the number of factors. *Multivariate Behav Res* 1966; **1**: 245–276.
26 Gorsuch RL. *Factor Analysis*. WB Saunders Company: Philadelphia, PA, USA, 1974.
27 Kline P. *An Easy Guide to Factor Analysis*. Routledge: London, UK; New York, USA, 1994.
28 Manios Y, Kourlaba G, Grammatikaki E, Androutsos O, Ioannou E, Roma-Giannikou E. Comparison of two methods for identifying dietary patterns associated with obesity in preschool children: the GENESIS study. *Eur J Clin Nutr* 2010; **64**: 1407–1414.
29 Lazarou C, Panagiotakos DB, Matalas AL. Level of adherence to the Mediterranean diet among children from Cyprus: the CYKIDS study. *Public Health Nutr* 2009; **12**: 991–1000.
30 Kontogianni MD, Vidra N, Farmaki AE, Koinaki S, Belogianni K, Sofrona S *et al.* Adherence rates to the Mediterranean diet are low in a representative sample of Greek children and adolescents. *J Nutr* 2008; **138**: 1951–1956.
31 Antonogeorgos G, Panagiotakos DB, Grigoropoulou D, Papadimitriou A, Anthracopoulos M, Nicolaidou P *et al.* The mediating effect of parents' educational status on the association between adherence to the Mediterranean diet and childhood obesity: the PANACEA study. *Int J Public Health* 2013; **58**: 401–408.
32 Farajian P, Risvas G, Karasouli K, Pounis GD, Kastorini CM, Panagiotakos DB *et al.* Very high childhood obesity prevalence and low adherence rates to the Mediterranean diet in Greek children: the GRECO study. *Atherosclerosis* 2011; **217**: 525–530.
33 Nobre LN, Lamounier JA, Franceschini SC. Preschool children dietary patterns and associated factors. *J Pediatr (Rio J)* 2012; **88**: 129–136.
34 Dubuisson C, Lioret S, Dufour A, Volatier JL, Lafay L, Turck D. Associations between usual school lunch attendance and eating habits and sedentary behaviour in French children and adolescents. *Eur J Clin Nutr* 2012; **66**: 1335–1341.
35 Nelson M, Lowes K, Hwang V. The contribution of school meals to food consumption and nutrient intakes of young people aged 4-18 years in England. *Public Health Nutr* 2007; **10**: 652–662.
36 Beauchamp GK, Mennella JA. Early flavor learning and its impact on later feeding behavior. *J Pediatr Gastroenterol Nutr* 2009; **48**: S25–S30.
37 Harris G. Development of taste and food preferences in children. *Curr Opin Clin Nutr Metab Care* 2008; **11**: 315–319.
38 Mennella JA, Jagnow CP, Beauchamp GK. Prenatal and postnatal flavor learning by human infants. *Pediatrics* 2001; **107**: E88.
39 Schulze MB, Hoffmann K, Kroke A, Boeing H. An approach to construct simplified measures of dietary patterns from exploratory factor analysis. *Br J Nutr* 2003; **89**: 409–419.
40 Treiber FA, Leonard SB, Frank G, Musante L, Davis H, Strong WB *et al.* Dietary assessment instruments for preschool children: reliability of parental responses to the 24-hour recall and a food frequency questionnaire. *J Am Diet Assoc* 1990; **90**: 814–820.
41 Hu FB, Rimm E, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A *et al.* Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr* 1999; **69**: 243–249.
42 Newby PK, Tucker KL. Empirically derived eating patterns using factor or cluster analysis: a review. *Nutr Rev* 2004; **62**: 177–203.

Supplementary Information accompanies this paper on European Journal of Clinical Nutrition website (http://www.nature.com/ejcn)

4.2.4 Paper 5. Dietary patterns in early childhood and child cognitive and psychomotor development: the Rhea mother-child cohort study in Crete

Main Findings:

- Children who followed the ‘Snacky’ dietary pattern had lower scores in the scale of verbal ability, general cognitive ability, and cognitive functions of posterior cortex. Poorer food choices at preschool age characterized by foods high in fat, salt, and sugar are associated with reduced scores in verbal and cognitive ability
- The ‘Western’ and the ‘Mediterranean’ dietary patterns were not associated with child neurodevelopmental scales.

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Dietary patterns in early childhood and child cognitive and psychomotor development: the Rhea mother-child cohort study in Crete.

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Abstract

Early life nutrition is critical for optimal brain development, however only few studies have evaluated the impact of diet as whole in early childhood on neurological development with inconsistent results. The present study aims to examine the association of dietary patterns with child cognitive and psychomotor development in 804 preschool children from the RHEA pregnancy cohort in Crete, Greece. Parents completed a validated food-frequency questionnaire at preschool age (mean age 4·2 years). Dietary patterns were identified using principal component analysis. Child cognitive and psychomotor development was assessed by the McCarthy Scales of Children's Abilities (MSCA). Multivariable linear regression models were used to investigate the associations of dietary patterns with the MSCA scales. The dietary patterns identified included the 'mediterranean' pattern based on pulses, olive oil, vegetables, fish and fruits; the 'snacky' pattern included potatoes and other starchy roots, salty snacks, sugar products and eggs; the 'western' pattern contained cereals, cheese, added lipids, beverages and meat. Children who followed the 'snacky' dietary pattern had lower scores in the scale of verbal ability (β=-1·31; 95% CI: -2·47, -0·16), general cognitive ability (β=-1·13; 95% CI: -2·25, -0·02) and cognitive functions of posterior cortex (β=-1·20; 95% CI: -2·34, -0·07). The 'western' and the 'mediterranean' dietary patterns were not associated with child neurodevelopmental scales. The present findings suggest that poorer food choices at preschool age characterized by foods high in fat, salt, and sugar are associated with reduced scores in verbal and cognitive ability.

Introduction

Early-life nutrition could influence cognitive development as demonstrated in several studies carried out in humans and experimental animals(1-3). However, existing literature has mainly focused on infant nutrition and its association with cognitive and psychomotor development by highlighting the positive effect of prolonged breastfeeding(4-7).

Early childhood is a significant period for the establishment of dietary habits, since dietary preferences are first established, laying the formation of adult eating habits(8,9). Only a few studies have investigated the role of child diet as a whole on cognitive development(10-13). Birth cohort studies in infants aged 6, 12, 15 and 24 months reported that those who followed the 'breastfeeding' and the 'home made' pattern had increased Intelligence Quotient (IQ) scores at 4 and 8 years of age(10,11). A cross sectional study performed by Theodore and colleagues observed that children with high consumption of breads and cereals and fish at 3.5 and 7 years of age had higher IQ scores(12). Half of the children included in this study were born small for gestational age and generalizability of the results to other populations is rather difficult. On the other hand, a 'processed' (high fat and sugar content) pattern of diet at 3 years of age was negatively associated with IQ assessed at 8.5 years of age in the ALSPAC cohort in UK(13).

In this study, we aimed to examine for the first time the impact of children's dietary patterns as obtained by Principal Component Analysis (PCA) on cognitive and psychomotor development at preschool age, as assessed by valid and complete versions of age appropriate psychometric tests in a prospective pregnancy cohort in Crete, Greece, after controlling for several confounding and mediator factors.

Materials and methods

Study population

The "Rhea" study is an ongoing prospective birth cohort based in southern Greece, in the island of Crete. The population included in the study are women living in the Prefecture of Heraklion, aged 16 years or above, with no communication handicap, who were pregnant during 2007-2008. Starting in February 2007, women in early pregnancy (before 15th week of gestation), were asked to participate in the study. They were contacted again various times during pregnancy, at birth, at 8-10 weeks after delivery, and for children's follow-up at 9 months, 18 months, and at 4 years of age. The initial recruitment and the subsequent follow ups of the mothers and their children have been approved by the Ethical Committee of the University Hospital of Heraklion (Crete, Greece). All participants provided written informed consent.

During the 4 year follow up of the children, we obtained dietary information for a total of 1081 children with the use of a validated food frequency questionnaire (FFQ)[(14)]. Children who followed a specific diet for health purposes (n=20) were excluded from the analysis. During the same follow up cognitive and neuropsychological development was assessed for 925 children by means of the MSCA test. Twenty-six children with a neurodevelopmental disorder diagnosis, or other diagnosed medical conditions (i.e. plagiocephalus, microcephalus, hydrocephalus and brain tumour) and/or incomplete examination were excluded. We also excluded 25 pairs of twins. In total, 804 singleton children with full information on dietary intake and neurodevelopmental assessment were included in the present analysis.

Dietary assessment

Dietary intake information was collected using the "Rhea 4 year follow-up FFQ", a validated semi-quantitative FFQ designed to assess habitual dietary intake in preschool children[(14)]. The FFQ was administered to primary caregivers by a well-trained dietician through telephone interview. The questionnaire included questions on 118 food items with the following components: food frequency, type of meals during the day (breakfast, morning snack, lunch, afternoon snack, dinner, evening snack), use of dietary supplements, type of fat used for cooking, frequency of meals consumed in restaurants or take away, and television viewing during meals). Parents could choose from one or two portion sizes and report the child's intake in terms of times per day, week, month, year or never. Seasonality of consumption was also reported in all food items. Daily intake of foods and nutrients was calculated using the UK food tables (McCance & Widdowson’s The Composition of Foods, 6th summary edition) and standard Greek recipes for complex mixed dishes. The dietary intake data were analyzed by using a software program developed at the Department of Applied Information Technology and Multimedia, TEI-Crete, Heraklion, Crete, Greece.

Assessment of neurodevelopment

Children’s neurodevelopment was assessed by two trained psychologists, with the age appropriate instrument MSCA, developed for children aged 2½ - 8½. In brief, MSCA test aims to identify possible developmental delay in different skills with the use of six scales: the Verbal scale, the Perceptual-Performance scale, the Quantitative scale, the

General Cognitive scale the Memory and the Motor scale[15]. Executive function and cognitive functions of posterior cortex are two additional scales derived from the MSCA test[16,17]. Each child received a score for every scale based on its performance and at the end of the neurodevelopmental assessment the examiners evaluated the "quality of assessment".

Potential confounders

Maternal and child socio-demographic and lifestyle characteristics were obtained through face-to face or self-administered questionnaires and medical records from early pregnancy up to children's 4 years of age. Maternal education (low level: ≤6 years of school, medium level: 7 to 12 years of school, high level: university or technical college degree) and maternal age were available at recruitment and were updated during the 4 year follow up. At birth, we collected details on child's sex (male/female), parity (only child/ first child/ other), birth weight (kg), preterm birth (<37 weeks of gestation; yes/no). Breastfeeding duration (months) was reported at two time points, at 9 and 18 months of child's age. Birth weight z-scores were adjusted for gestational age and gender. During the 4 year follow up we collected information on maternal residence (urban, rural), maternal working status (yes/no), marital status (married-engaged /other), pre-school attendance (yes/no), passive smoking at home (yes/no), hours/day spent watching television (almost never, 1, ≥2). Maternal intelligence was measured using the Raven's Standard Progressive Matrices test[18].

Statistical analysis

The PCA method was used to reduce the data from the child's daily intake (in grams) of the 17 food groups and to identify the dietary patterns. The Kaiser-Mayer-Olkin measure (KMO) was calculated to evaluate the sample adequacy. In order to identify the number of components, we used the eigenvalue of >1 criterion, the visual representation by the scree plot[19] and the interpretability of the factor loadings. Varimax rotation was applied to enhance the determination of the dietary components[20-21]. Factor loadings above 0.3 on a component were considered to have a strong association with that component. A score was attributed to every child for each of the components identified. The score was calculated for each of the components retained by summing the standardized values of the food items weighted by their scoring coefficients.

Raw scores of the neurodevelopmental assessment scales were standardized for child's age at test administration using a method for the estimation of age-specific reference intervals based on fractional polynomials[22]. Standardized residuals were then typified having a mean of 100 points with a 15 SD to homogenize the scales (parameters conventionally used in psychometrics for IQ assessment).

The possibility of nonlinear associations was tested by generalized additive models (GAMs) indicating linear relationships between children's dietary patterns and the neurodevelopmental outcome variables (P>0·1). Therefore, multivariable linear regression models were fitted to examine the associations after adjusting for confounders that modified any of the exposure coefficients by 10% or more. The 'quality of assessment', child's sex and examiner were included as *a priori* confounders in the present analysis. Estimated associations were described with β-coefficients and 95% CI.

We examined the role of confounders on the associations of dietary patterns with children's' neurodevelopment at 4 years of age in 4 models. The Model 1-basic model-was adjusted for quality of assessment, child sex, and examiner. Model 2 was adjusted for model 1 plus infant characteristics like birth weight z-scores and breastfeeding duration. Model 3 was adjusted for model 2 plus maternal characteristics including maternal age, maternal education, parity and marital status. Model 4 was adjusted for model 3 plus child lifestyle characteristics such as day care attendance, passive smoking and hours per day spent watching television. Cases with missing values were excluded from multivariable models. An extra adjustment for maternal intelligence was performed for a subsample of the study population with available information as a sensitivity analysis, and model fit was tested using likelihood ratio test. An additional sensitivity analysis was carried out by excluding preterm births.

All hypotheses testing were conducted assuming a 0.05 significance level and a 2-sided alternative hypothesis. Stata S.E. version 13 was used for the statistical analyses (StataCorp, Texas, USA).

Results

A description of the population characteristics is presented in Table 1. The majority of mothers had Greek origin (n=751, 94·4%), medium education (n=394, 51·4%), and were married (n=783, 98·0%). 412 (51·2%) boys and 392 (48·8%) girls participated in the present analysis, had mean birth weight 3·20 (SD: 0·45) kg and breastfed for 4·12 (SD: 4·31) months. Most of the children attended pre-school (n=685, 85·7%), and almost half of them were exposed to passive smoking (n=371, 46·3%) at 4 years of age. A higher proportion of the mothers participating in the analysis breastfed longer (*P*<0·001) their children and were of higher education (*P*<0·001) as compared to the non-participants (Supplementary Table 1S).

Three dietary patterns were determined with the use of Principal Component Analysis (PCA): the 'mediterranean', 'snacky' and 'western' patterns[23]. The 'mediterranean' pattern was characterized by high intake of vegetables, fruits, pulses, olive oil, fish and seafood. The 'snacky' pattern was characterized by foods that require minimum preparation such as potatoes and other starchy roots, salty snacks, sugar preserves, confectionery and eggs. The 'western' pattern was comprised of cereals and bakery products, cheese, lipids of animal and vegetable origin, sweetened beverages (soft drinks, packed fruit juices), and meat products.

Table 2 presents the multivariable associations between the three dietary patterns and McCarthy scores at 4 years of age. In the minimally adjusted model, the western and the snacky dietary patterns were associated with lower scores in verbal, quantitative, general cognitive ability, memory, executive function and cognitive functions of posterior cortex scales. Most of these associations remained after additional adjustment for birth weight and breastfeeding duration (model 2). However, further adjustment for maternal socio-demographic characteristics (model 3) attenuated the associations and only the 'snacky' pattern remained negatively associated with verbal (β=-1·33; 95% CI: -2·49, -0·17, *P*<0·05), general cognitive ability (β=-1·23; 95% CI: -2·37, -0·10, *P*<0·05) and cognitive functions of posterior cortex (β=-1·29; 95% CI: -2·43, -0·14, *P*<0·05). Further adjustment of child's life style characteristics (model 4) did not attenuate the observed associations.

We further adjusted for maternal intelligence in a subsample of 339 mother-child pairs with available information on maternal cognition (Raven's test) (Supplementary Table 2S). The direction of associations did not change in these models, although significance levels were attenuated, probably due to small sample size. We also repeated our analysis after excluding preterm births and results remained unchanged (Supplementary Table 3S).

Discussion

In the present analysis, we found that children who had unhealthy food choices at preschool age, characterized by processed and high in sugar foods, scored lower in verbal ability, general cognitive ability and cognitive functions of posterior cortex.

These associations persisted after the sequential adjustment of multiple confounding and mediation factors.

Comparison with other studies is rather complex mainly due to different methodological approaches including study design, food variability across countries, control of confounding factors and use of valid psychometric tests. However, these results are in line with two other cohort studies which reported lower IQ scores for children who adhere to less healthy patterns, characterized by products high in fat and sugar(11,13). Similarly to our findings, these studies reported stronger associations in minimally adjusted models, but results attenuated after adjusting for a large array of confounders.

The underlying mechanisms affecting verbal and cognitive child development because of poorer food choices characterized by higher intakes of fat and sugar at preschool age are rather complex. Animal studies have demonstrated detrimental effects of a high-fat/high cholesterol diet on cognitive performance in rats, associated with reduced hippocampal dendritic integrity and activation of microglial cells in the hippocampus(24). Adult epidemiological studies have also shown that intake of a high-fat diet that includes mostly omega-6 and saturated fatty acids is associated with worse performance on cognitive tasks(25). These associations may be even more profound at early life as the brain grows at its fastest rate during the first 3 years of life, and it is possible that poorer food choices during this early period may discourage optimal head and brain growth(26).

The associations seen with the 'snacky' dietary pattern were stronger for verbal than for other cognitive scales (i.e. perceptual-performance and quantitative scales of MSCA). This is in line with the study by Northstone et al showing that a poor diet associated with high fat, sugar and processed food content in early childhood was associated with lower scores in verbal ability at 8 years of age(13). Although genetic and environmental influences on the development of general intelligence are well-documented, less is known on the development of specific cognitive abilities (i.e. verbal vs. non-verbal abilities) and the potential mechanism underlying the association of poor diet during early childhood with children's verbal abilities remain unclear. However, there is some evidence that shared family environmental factors are greater for verbal abilities than for non-verbal abilities, with the latter being related to individual's inherent mentality(27).

Advantages of the present study include the assessment of maternal diet during pregnancy with a validated dietary questionnaire, well-established outcome measures, and control for several mother and child characteristics. Dietary patterns were determined with PCA which is a method that enables deep understanding of the diet as a whole, instead of isolated foods and nutrients, and thus design valuable interventions and health policies. However, the PCA method introduces also researcher's subjectivity in the analysis, while dietary patterns derived from PCA are population-specific, and therefore cannot be reproduced in other populations. Neurodevelopment assessment at preschool age was performed with the use of MCSA test which is a valid psychometric test(15), it provides both a general level of child's intellectual functioning and an assessment of separate neurodevelopmental domains (verbal ability, perceptual performance, number aptitude, memory, motor and executive functions), and thus, detects which domain is mostly affected. We decided to exclude children with a neurodevelopmental disorder diagnosis as neurological impairment in children is associated with an increased risk of feeding and nutritional problems.

The study has also some limitations. The cross sectional design of the study does not permit the conclusion of any causal associations. Children included in the present analysis who had complete data were more socially advantaged than the remainder of the cohort and this could lead to underestimation of the observed associations. Although we incorporated extensive information on potential social and environmental factors that are associated with child neurodevelopment, we acknowledge that residual confounding because of other unmeasured confounders such as home environment may still occur. Finally, even though maternal intelligence was available only for a subset of children, further adjustment for maternal intelligence did not change the direction of the observed associations.

Overall, the present findings suggest that preschool children, who follow a 'less healthy' diet that includes salty snacks and high in fat and sugar products, might have lower scores in verbal and cognitive ability. Future research is needed to examine the long-lasting effects of early life diet on child neurodevelopment.

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Conflict of Interest: None

Authorship: All authors had a substantial contribution to the study and they have all reviewed and approved the submitted manuscript. Specifically: VL was the field study co-coordinator, participated in the statistical analysis, and wrote the first draft of the paper, TR supervised the statistical analysis and helped with the data interpretation and manuscript preparation, KS participated in the design of the study and in the drafting of the paper, KK supervised the neurodevelopmental assessment and contributed to the drafting of the manuscript, MK and AK had the responsibility of the neurodevelopmental assessment and helped with data interpretation, MV provided feedback and critical revision of the manuscript, MK and LC conceived the study, supervised the data collection, provided critical review of the manuscript, and helped with data interpretation and manuscript preparation.

Table 1. Descriptive characteristics of the study population

	N	% or Mean	SD
Maternal Characteristics			
Maternal age (years)	795	33·48	4·99
Maternal origin, *n (%)*			
Greek	751	94·4	
Non Greek	45	5·6	
Maternal education, *n (%)*			
Low	119	15·5	
Medium	394	51·4	
High	253	33·0	
Maternal working status, *n (%)*			
Working	472	59·0	
Not working	328	41·0	
Maternal marital status, *n (%)*			
Married	783	98·0	
Other	16	2·0	
Infant Characteristics			
Sex, *n (%)*			
Male	412	51·2	
Female	392	48·8	
Parity, *n (%)*			
Only child	147	18·4	
First child	226	28·3	
Other	426	53·3	
Preterm birth, *n (%)*			
Yes	93	11·9	
No	689	88·1	
Birth weight (kg)	779	3·2	0·45
Breastfeeding duration (months)	763	4·1	4·31
Child characteristics at 4 yrs			

Pre-school attendance, *n (%)*			
No	114	14·3	
Yes	685	85·7	
Passive smoking at home, *n (%)*			
No	430	53·7	
Yes	371	46·3	
Hours/day spent watching TV, *n (%)*			
Almost never	218	27·3	
1	298	37·3	
≥ 2	283	35·4	
General Cognitive Ability (score)	804	97·9	16·5

Abbreviations: SD, standard deviation; BMI, body TV, television.

Table 2. Multivariable associations between dietary patterns and neurodevelopmental test scores at 4 years of age in Rhea Cohort Study, Crete, Greece.

Dietary patterns	Verbal Ability		Perceptual performance		Quantitative ability		General cognitive ability		Memory		Motor ability		Executive function		Cognitive functions of posterior cortex	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Model 1[†] (n=804)																
Mediterranean	-0·01	-1·04, 1·02	-0·39	-1·41, 0.64	-0·55	-1·11, 1·00	-0·21	-1·23, 0·82	-0·16	-1·20, 0·88	-0·79	-1·88, 0·29	0·04	-1·01, 1·09	-0·42	-1·44, 0·59
Western	-1·53**	-2·59, -0·48	-0·10	-2·04, -0.06	-1·63**	-2·71, -0·55	-1·61**	-2·66, -0·57	-1·60**	-2·66, -0·53	-1·01	-2·11, 0·09	-1·42**	-2·49, -0·35	-1·53**	-2·57, -0·49
Snacky	-2·62***	-3·74, -1·51	-2·20***	-3·31, -1.09	-1·24*	-2·39, -0·10	-2·60***	-3·70, -1·49	-1·82**	-2·95, -0·70	-0·69	-1·85, 0·48	-2·44***	-3·57, -1·31	-2·42***	-3·52, -1·33
Model 2[‡] (n=754)																
Mediterranean	-0·34	-1·42, 0·75	-0·46	-1·54, 0.62	-0·26	-1·37, 0·85	-0·49	-1·56, 0·58	-0·45	-1·55, 0·64	-0·82	-1·96, 0·33	-0·29	-1·38, 0·81	-0·63	-1·69, 0·43
Western	-1·19*	-2·29, -0·09	-0·65	-1·75, 0.45	-1·25*	-2·38, -0·12	-1·18*	-2·27, -0·10	-1·35*	-2·45, -0·24	-0·63	-1·79, 0·53	-1·04	-2·15, 0·07	-1·11*	-2·19, -0·03
Snacky	-2·54***	-3·70, -1·38	-2·38***	-3·53, -1.22	-1·08	-2·27, 0·11	-2·59***	-3·73, -1·44	-1·82**	-2·98, -0·65	-0·94	-2·16, 0·28	-2·34***	-3·51, -1·17	-2·50***	-3·64, -1·36
Model 3[§] (n=723)																
Mediterranean	-0·06	-1·12, 0·99	-0·60	-1·66, 0.46	-0·11	-1·21, 0·99	-0·35	-1·38, 0·68	-0·21	-1·29, 0·88	-0·78	-1·93, 0·37	-0·18	-1·23, 0·88	-0·49	-1·52, 0·55
Western	-0·66	-1·73, 0·41	-0·21	-1·29, 0.87	-0·72	-1·84, 0·40	-0·59	-1·64, 0·46	-0·85	-1·95, 0·26	-0·36	-1·54, 0·81	-0·34	-1·41, 0·73	-0·65	-1·71, 0·41
Snacky	-1·33*	-2·49, -0·17	-1·16	-2·33, 0.01	-0·25	-1·46, 0·96	-1·23*	-2·37, -0·10	-0·80	-1·99, 0·39	-0·27	-1·54, 1·00	-0·97	-2·13, 0·18	-1·29*	-2·43, -0·14
Model 4[¶] (n=722)																
Mediterranean	-0·17	-1·22, 0·88	-0·85	-1·87, 0.19	-0·36	-1·44, 0·72	-0·56	-1·58, 0·45	-0·28	-1·36, 0·80	-1·00	-2·14, 0·14	-0·38	-1·42, 0·65	-0·68	-1·71, 0·35
Western	-0·40	-1·48, 0·67	0·24	-0·82, 1.31	-0·31	-1·42, 0·79	-0·19	-1·23, 0·85	-0·63	-1·74, 0·47	-0·04	-1·13, 1·20	0·06	-0·99, 1·12	-0·29	-1·34 0·77
Snacky	-1·31*	-2·47, -0·16	-1·01	-2·15, 0.13	-0·12	-1·31, 1·06	-1·13*	-2·25, -0·02	-0·82	-2·01, 0·37	-0·13	-1·38, 1·13	-0·87	-2·01, 0·26	-1·20*	-2·34 -0·07

[†]Model 1: adjusted for quality of assessment, child sex, and examiner
[‡]Model 2: plus birth weight z-scores, breastfeeding duration
[§]Model 3: plus maternal age, maternal education, parity, marital status
[¶]Model 4: plus day care attendance, passive smoking, tv watching
* *P*<0.05, ** *P*<0.01, *** *P*<0.05

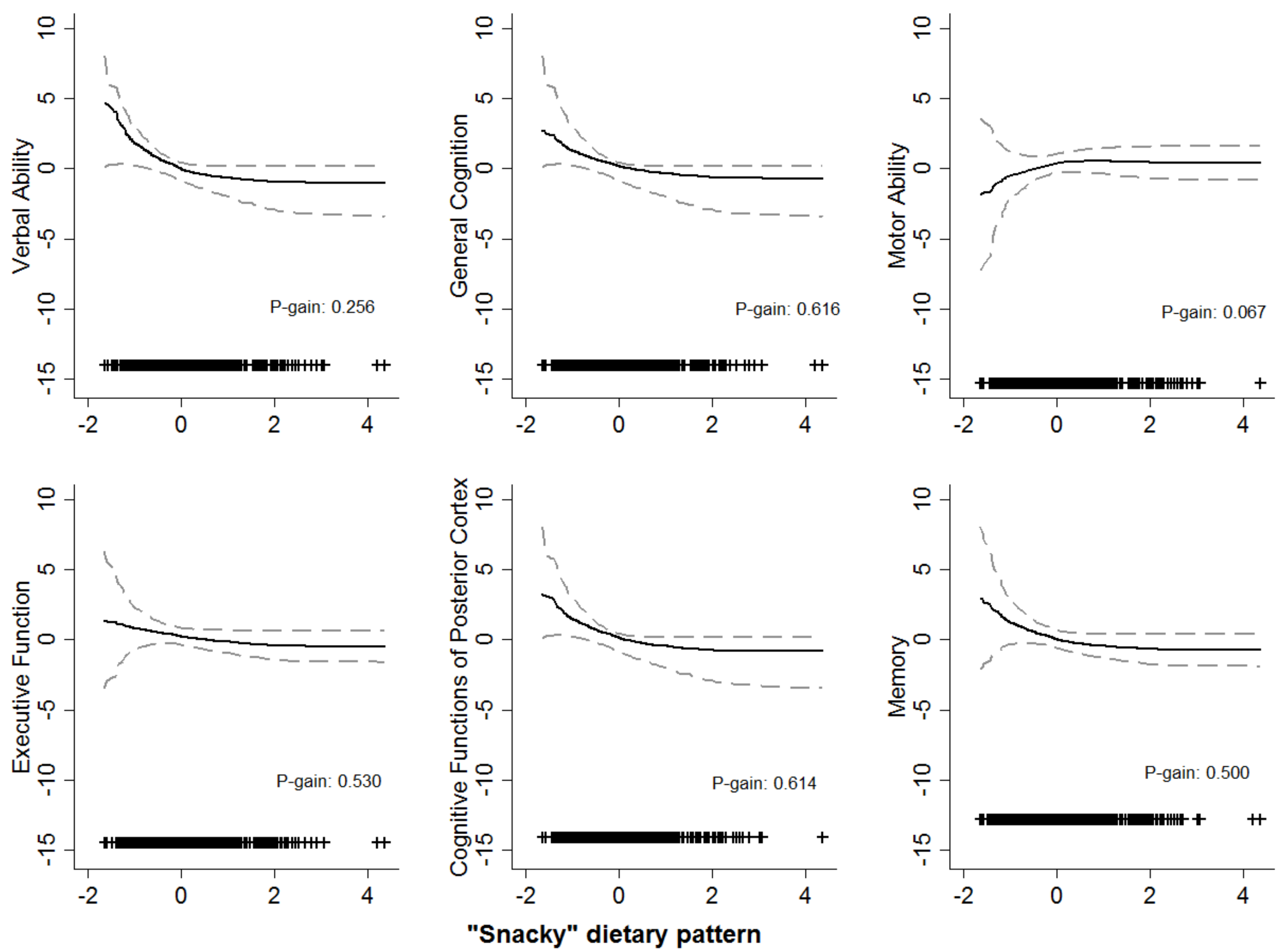

Figure 1. GAMs adjusted associations (95% CIs) of the 'snacky' dietary pattern with the verbal ability, the general cognitive ability, the motor ability, the executive function, the cognitive functions of posterior cortex and the memory scale. Adjusted for quality of assessment, child sex, examiner, birth weight z-scores, breastfeeding duration, maternal age, maternal education, parity, marital status, day care attendance, passive smoking, and tv watching.

References

1. Smithers LG, Golley RK, Brazionis L *et al.* (2011) Characterizing whole diets of young children from developed countries and the association between diet and health: a systematic review. *Nutr Rev* **69**,449-467.
2. Lucas A. (1998) Programming by early nutrition: an experimental approach. *J Nutr* **128**, 401S-6S.
3. Laus MF, Vales LD, Costa TM *et al.* (2011) Early postnatal protein-calorie malnutrition and cognition: a review of human and animal studies. *Int J Environ Res Public Health* **8**, 590-612.
4. Anderson JW, Johnstone BM, Remley DT (1999) Breast-feeding and cognitive development: a meta-analysis. *Am J Clin Nutr* **70**, 525-35.
5. Leventakou V, Roumeliotaki T, Koutra K *et al*. (2015) Breastfeeding duration and cognitive, language and motor development at 18 months of age: Rhea mother-child cohort in Crete, Greece. *J Epidemiol Community Health* **69**, 232-9.
6. Bernard JY, De Agostini M, Forhan A *et al*. (2013) Breastfeeding duration and cognitive development at 2 and 3 years of age in the EDEN mother-child cohort. *J Pediatr* **163**,36-42 e1.
7. Belfort MB, Rifas-Shiman SL, Kleinman KP *et al*. (2013) Infant feeding and childhood cognition at ages 3 and 7 years: Effects of breastfeeding duration and exclusivity. *JAMA Pediatr* **167**, 836-44.
8. Ventura AK, Worobey J. (2013) Early influences on the development of food preferences. *Curr Biol* **23**, R401-8.
9. Birch L, Savage JS, Ventura A. (2007) Influences on the Development of Children's Eating Behaviours: From Infancy to Adolescence. *Can J Diet Pract Res* **68**, s1-s56.
10. Gale CR, Martyn CN, Marriott LD *et al.* (2009) Dietary patterns in infancy and cognitive and neuropsychological function in childhood. *J Child Psychol Psychiatry* **50**, 816-23.
11. Smithers LG, Golley RK, Mittinty MN *et al.* (2012) Dietary patterns at 6, 15 and 24 months of age are associated with IQ at 8 years of age. *Eur J Epidemiol* **27**, 525-35.
12. Theodore RF, Thompson JMD, Waldie KE *et al.* (2009) Dietary patterns and intelligence in early and middle childhood. *Intelligence* **37**, 506-13.

13. Northstone K, Joinson C, Emmett P, *et al.* (2012) Are dietary patterns in childhood associated with IQ at 8 years of age? A population-based cohort study. *J Epidemiol Community Health* **66**, 624-8.
14. Leventakou V, Georgiou V, Chatzi L *et al.* (2015) Relative validity of an FFQ for pre-school children in the mother-child 'Rhea' birth cohort in Crete, Greece. *Public Health Nutr* **18**, 421-7.
15. McCarthy D (editor) (1972) *Manual for the McCarthy scales of children's abilities*. New York: Psychological Corp.
16. Julvez J, Ribas-Fito N, Torrent M *et al.* (2007) Maternal smoking habits and cognitive development of children at age 4 years in a population-based birth cohort. *Int J Epidemiol* **36**, 825-32.
17. Barkley RA (2001) The executive functions and self-regulation: an evolutionary neuropsychological perspective. *Neuropsychol Rev* **11**, 1-29.
18. Raven JC, Court JH (1996) *Manual for Raven's Progressive Matrices and Vocabulary Scales.* Oxford: Oxford Psychologists Press.
19. Cattell RB (1966) The scree test for the number of factors. *Multivariate Behav Res* **1**, 245-76.
20. Gorsuch RL (1974) *Factor Analyses*. Philadelphia: W.B. Saunders.
21. Kline P (1994) *An Easy Guide to Factor Analysis.* London: Routledge.
22. Royston P, Wright EM (1998) A method for estimating age-specific reference intervals ("normal ranges") based on fractional polynomials and exponential transformation. *J R Statist Soc* **161**, 79-101.
23. Leventakou V, Sarri K, Georgiou V et al. (2015) Early life determinants of dietary patterns in preschool children: Rhea mother-child cohort, Crete, Greece. *European Journal of Clinical Nutrition*, Accepted for publication.
24. Granholm AC, Bimonte-Nelson HA, Moore AB et al. (2008) Effects of a saturated fat and high cholesterol diet on memory and hippocampal morphology in the middle-aged rat. *J Alzheimers Dis* **14**, 133-45.
25. Freeman LR, Haley-Zitlin V, Rosenberger DS et al. (2014) Damaging effects of a high-fat diet to the brain and cognition: a review of proposed mechanisms. *Nutr Neurosci* **17**, 241-51.
26. Gale CR, O'Callaghan FJ, Godfrey KM et al. (2004) Critical periods of brain growth and cognitive function in children. *Brain* **127**, 321-9.

27. Kaufman A (editor) (1994). *Intelligent Testing with the WISC-III*. New York: John Wiley & Sons.

4.2.5 Paper 6. Is there an association between eating behaviour and ADHD symptoms in preschool children?

Main Findings:

- Children’s food approach eating behaviours, like food responsiveness and emotional overeating were associated with children’s ADHD symptoms, including impulsivity, inattention and hyperactivity at 4 years of age. Furthermore, a food avoidant behaviour, such as food fussiness, was associated with impulsivity symptoms.

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Is there an association between eating behaviour and ADHD symptoms in preschool children?

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Abstract

Background: There is some evidence that aberrant eating behaviours and obesity co-occur with Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms. The present study is the first that aims to investigate the association between eating behaviours and ADHD symptoms in early childhood in a population-based cohort. **Methods:** We included 471 preschool children from the RHEA mother-child cohort in Crete, Greece. Parents completed the Children's Eating Behaviour Questionnaire (CEBQ) to assess children's eating behaviour and the 36-item ADHD Test (ADHDT) to evaluate ADHD symptoms at 4 years of age. Multivariable linear regression models were used to examine the association of eating behaviours with ADHD symptoms. **Results:** Regarding children's food approach eating behaviours we observed a positive association between food responsiveness and total ADHD index, as well as impulsivity, inattention and hyperactivity subscale, separately. Similarly, there was a significant positive association between emotional overeating and ADHD symptoms. With regard to children's food avoidant behaviours, food fussiness was found to be significantly associated with the impulsivity subscale. A dose response association between the food approach behaviours and ADHD symptoms was also observed. Children on the medium and highest tertile of the food responsiveness subscale had increased scores on the ADHD total scale, as compared to those on the lowest tertile. As regards emotional overeating, children in the highest tertile of the scale had higher scores on ADHD total and hyperactivity. **Conclusions:** Our findings provide evidence that food approach eating behaviours like food responsiveness and emotional overeating are associated with increased ADHD symptoms in preschool children. Future studies to better understand this overlap will enhance potential interventions.

Keywords: Eating behavior, child development, ADHD

Abbreviations: ADHD: Attention-Deficit/Hyperactivity Disorder; CEBQ: Children's Eating Behaviour Questionnaire; ADHDT: Attention Deficit Hyperactivity Disorder Test.

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a highly prevalent disorder worldwide in particular amongst children (Polanczyk, Salum, Sugaya, Caye, & Rohde, 2015; Thomas, Sanders, Doust, Beller, & Glasziou, 2015). Inattention, impulsivity and hyperactivity are well known symptoms of ADHD, predominantly detected in early childhood, that are likely to persist over time, into adolescence and adulthood. According to a recent meta-analysis the prevalence of ADHD in childhood has risen to 7.2% (Thomas, et al., 2015) worldwide, with boys being more affected than girls (Silva, Colvin, Hagemann, & Bower, 2014). The etiology of ADHD is multidimensional and still remains unclear. Genetic (Nikolas & Burt, 2010) and environmental factors (Froehlich et al., 2011; Silva, et al., 2014) such as diet (Heilskov Rytter et al., 2015; Millichap & Yee, 2012), although highly controversial, are likely to be relevant.

For the last decade, a novel focus has been on shedding light on the association between ADHD symptoms and obesity and potential mediators of this association (Cortese & Castellanos, 2014). Several studies have pointed out the potential contributing role of disturbed eating behaviours such as overeating, binge eating (overeating with loss of control), and bulimic behaviours to the association between obesity and ADHD (Cortese & Vincenzi, 2012; Docet, Larranaga, Perez Mendez, & Garcia-Mayor, 2012; Nazar et al., 2014). Existing findings have also highlighted the association between ADHD symptoms, overeating behaviours and adverse outcomes like excess weight gain (Davis, Levitan, Smith, Tweed, & Curtis, 2006; van den Berg et al., 2011). Although difficult to fully decipher the direction of the association between ADHD, eating patterns and obesity, in depth understanding of this association can not only help preventative efforts for weight gain and obesity but can also help us understand risk mechanisms. The present study aims to add to current literature by exploring the possible overlap between abnormal eating behaviours and traits of ADHD in early childhood that could lead to increased risk for obesogenic behaviours in later life.

The investigation of children's behavioural characteristics is also very challenging, since early life behavioural styles are strong predictors of individual's psychopathology in later life (Caspi et al., 2003). Longitudinal studies focusing on early life behavioural characteristics are essential in order to determine whether specific individual characteristics are associated with the development of disturbed eating patterns in childhood and their maintenance into adolescence and adulthood

(Hartmann, Czaja, Rief, & Hilbert, 2010). An in depth understanding of the association between ADHD and its core features and disturbed eating patterns in children is lacking, despite evidence of this association. A very recent population study, including male participants aged 6-10 years old, reported that children with ADHD had irregular meals, ate more than 5 times per day and consumed a lot of sweetened drinks during the day (Ptacek et al., 2014). A positive association between ADHD and binge eating has been shown cross-sectionally in children aged 10 years old (Cortese, Bernardina, & Mouren, 2007; Reinblatt et al., 2014) and longitudinally (Sonneville et al., 2015). One study evaluated the association between eating behaviours with childhood psychopathology which included emotional, behavioural and pervasive developmental disorders. This study showed that picky eating was positively associated with all domains of psychopathology among children aged 5 to 7 years, but unfortunately ADHD was not studied in depth (Micali et al., 2011). To our knowledge no study to date has investigated ADHD symptoms in detail and eating behaviour in childhood.

The timely recognition of the association between ADHD symptoms with eating behaviour at early ages could help tailor useful interventions and prevention strategies for clinical practice in order to mitigate long term negative outcomes, such as obesity. Thus, the present study aims to investigate in detail for the first time the association of eating behaviours with ADHD symptoms in a population-based sample of Greek preschool children. We hypothesized that ADHD symptoms would be positively associated with children's food approach eating behaviours, such as food responsiveness, enjoyment of food, desire to drink and emotional overeating, and negatively associated with children's food avoidant eating behaviours, such as satiety responsiveness, emotional under eating, slowness in eating and food fussiness.

Methods

Study population

The Rhea study is an ongoing birth cohort that started in February 2007 in Crete, Greece. Pregnant women, aged 16 years old and above, of Greek or immigrant origin (fluent in Greek), were eligible to participate in the study. Starting in February 2007 and within a 12-month period, these women were contacted (before 15th week of gestation) and asked to participate in the study. Participants were contacted again at various times during pregnancy, at birth, and for children's follow-up at 9, 18 months, at 4 years and 6 years of age. Face-to face completed questionnaires together with self-administered questionnaires and medical records were used to obtain information on dietary, environmental, and psychosocial exposures during pregnancy and early childhood. The study was approved by the Ethical Committee of the University Hospital of Heraklion (Crete, Greece), and all participants provided written informed consent after complete description of the study.

Of 1363 singleton live births, 879 children were followed up at 4 years of age. Twenty six children with an established diagnosis of neurodevelopmental disorder (children diagnosed with Pervasive Developmental Disorder), or other diagnosed medical conditions (i.e. plagiocephalus, microcephalus, hydrocephalus and brain tumour) and/or incomplete examination were excluded from the present analysis. Information on eating behaviour was available for 597 (67.9%) and a total of 471 children had information on both eating behaviour and symptoms of ADHD.

Eating behaviour

In order to assess children's eating behaviour in preschool age we used the Children's Eating Behaviour Questionnaire (CEBQ) (Wardle, Guthrie, Sanderson, & Rapoport, 2001). This instrument was designed to be completed by parents referring to children's eating behaviour and it assesses behaviour in 8 different areas. Food approach behaviours consist of food responsiveness, enjoyment of food, desire to drink, emotional overeating and food avoidant behaviours consist of satiety responsiveness, emotional under eating, slowness in eating and food fussiness. All 8 eating behaviours, as identified by factor analysis performed by Wardle et al (2001), were included in the present analysis. This 35 item questionnaire was used to assess eating behaviour, though one redundant item was deleted. The 34 items were included in the following subscales (number of items): food responsiveness (5); enjoyment of food (4); desire to drink (2); emotional overeating (4); satiety responsiveness (5); emotional under eating (4); slowness in eating (4); food fussiness (6).The CEBQ scale had good internal consistency with Cronbach's alphas ranging from 0.57 to 0.82 for the present study. The total Cronbach alpha of all subscales was 0.60. The cross-cultural adaption of the 34-item version of the CEBQ was performed according to internationally recommended methodology, using the following guidelines: forward translation, backward translation, cognitive debriefing process, and pretesting.

Primary caregivers/parents were interviewed on the phone by a dietitian following a standard protocol in order to complete the CEBQ questionnaire.

Attention Deficit Hyperactivity Disorder Test

In this study the ADHD symptoms in preschool children were assessed with the 36-item ADHD interview test (ADHDT) (Gilliam, 1995). Mothers completed the interview, which is based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for ADHD. The instrument is designed to identify and evaluate ADHD in ages 3-23 years, and contains three subscales; (i) Hyperactivity, (ii) Inattention, and (iii) Impulsivity. It, also, provides an index for total ADHD difficulties. The ADHDT was translated and adapted to the Greek population (Maniadaki & Kakouros, 2002).

Covariates

Data on socio-demographic and lifestyle characteristics were provided by the parents from early pregnancy up to the age of 4 years via interviewer-administered questionnaires, including: A. *Parental characteristics*: maternal age at birth (years); maternal and paternal education at recruitment (low level: ≤6 years of school, medium level: 7 to 12 years of school, high level: university or technical college degree); maternal working status at 4 years of age (yes/no); marital status at 4 years of age (married-engaged/other); pre-pregnancy maternal Body Mass Index (BMI, kg/m^2) (number). B. *Infant characteristics*: gender (male/female); parity (primiparous/ multiparous); preterm birth (<37 weeks of gestation; yes/no); birth weight (gr); breastfeeding duration (months). C. *Child characteristics*: preschool attendance at 4 years of age (yes/no); passive smoking at home at 4 years (yes/no); hours/day spending watching television at 4 years (almost never, 1-2, ≥3); General cognitive ability as assessed using the McCarthy Scales of Children's Abilities (MSCA) (score)(McCarthy, 1972) at 4 years of age; Executive function (EF), which refers to the higher-level cognitive skills the individual use to control and coordinate other cognitive abilities and behaviours, as an additional scale derived from the MSCA test (score) (Barkley, 2001; Julvez et al., 2007) at 4 years of age. There is emerging literature indicating the possible role of EF as a mediator in the association between eating behaviours and ADHD. Current evidence supports that deficits in EF, a core feature of ADHD, is likely to be associated to abnormal eating behaviors, although causal relationships need to be fully tested (Dempsey, Dyehouse, & Schafer, 2011). A significant correlation between impaired EF and eating in the absence of hunger has been recently reported in a small sample of preschool children.(Pieper & Laugero, 2013); Child's BMI z-scores based on cohort-specific (Rhea mother-child cohort study), gender and age adjusted growth curves. Child's BMI cut offs were based on IOTF criteria (Cole, Bellizzi, Flegal, & Dietz, 2000) which apply to 2-18 year olds.

Statistical analyses

Associations between normally distributed variables were tested with t-test and ANOVA. Correlations were tested using the Pearson correlation coefficient. Associations between non-normally distributed continuous variables were tested using non parametric tests (i.e., Mann-Whitney, Kruskal-Wallis, and Spearman non parametric statistical tests). Correlations among subscales of the CEBQ were in the expected direction and significant at the 0.05 level.

The possibility of nonlinear associations was tested by generalized additive models (GAMs) indicating linear relationships between children's eating behaviour and ADHD symptoms (p-gain >0.1). Multivariable linear regression models were implemented to examine the associations between eating behaviour styles and ADHD symptoms, after adjusting for relevant confounders. Potential confounders/mediators related with eating behaviour items and/or ADHD symptoms in the bivariate models with a p value <0.05 were included in the multivariable linear models (maternal age, maternal education, child's executive function, child's age, child's sex, parity, child's BMI z-score, child's energy intake (kcals) and TV watching). Child's age, sex, and BMI z-score were included as *a priori* confounders in the present analyses. Estimated associations were described with β-coefficients and 95% CI. We were also able to examine effect modification stratifying by gender and child's BMI. For interaction terms we considered p value <0.1 as nominally significant.

All other hypotheses testing were conducted assuming a 0.05 significance level and a 2-sided alternative hypothesis, with Benjamini-Hochberg procedure for multiple testing correction Stata S.E. version 13 was used for statistical analyses (StataCorp, Texas, USA).

Results

A description of the population characteristics are presented in Table 1. The majority of mothers had Greek origin, medium education, and was married. We included 287 (51.4%) boys and 271 (48.6%) girls in the present analysis, mean birth weight was 3.20 kg (SD: 0.46) and average length of breastfeeding 3.97 (SD: 4.15) months. At the age of 4 years most of the children attended preschool (n=478, 86.0%), spent one hour per day watching television (n=216, 38.9%), while 77 children (13.9%) were overweight and 35 (6.3%) were obese. Most of the mothers included in the analysis had Greek origin, were more educated and were slightly older as compared to those who did not participate (data not shown).

Table 2 shows the scoring in the CEBQ subscales. Children's mean scores in the different subscales ranged from 1.69 (SD: 0.56) for the emotional overeating subscale to 3.52 (SD: 0.98) for the desire to drink. As expected, food responsiveness, emotional overeating and, enjoyment of food were positively correlated and negatively correlated with satiety responsiveness, slowness in eating, emotional under eating and food fussiness .

Multivariable associations of the eating behaviours with the ADHD subscales scores are presented in Table 3. Regarding food approach eating behaviours, food responsiveness was associated with higher scores in the total ADHD index (β=2.54; 95% CI: 1.09, 3.99, p=0.001), as well as higher scores in the impulsivity (β=0.76; 95% CI: 0.27, 1.24, p=0.002), inattention (β=0.90; 95% CI: 0.40, 1.39, p<0.001) and hyperactivity (β=0.95; 95% CI: 0.35, 1.55, p=0.002) subscales. Similarly, there was a positive association between emotional overeating and total ADHD index (β=3.71; 95% CI: 1.60, 5.82, p=0.001), impulsivity (β=0.90; 95% CI: 0.17, 1.61, p=0.016), inattention (β=0.88; 95% CI: 0.14, 1.62, p=0.020) and hyperactivity (β=1.58; 95% CI: 0.74, 2.44, p<0.001) subscales. With regard to children's food avoidant behaviours, food fussiness was found to be significantly associated with the impulsivity (β=0.51; 95% CI: 0.03, 0.98, p=0.036) subscale.

We observed a dose response association between the food responsiveness and emotional over eating subscales and ADHD symptoms (Figure 1). Children on the medium and highest tertile of the food responsiveness subscale had increased scores by 3.79 (95% CI: 0.99, 6.60) points and 5.78 (95% CI: 2.78, 8.78, p for trend <0.001) respectively on the ADHD total scale, as compared to those on the lowest tertile. Similar associations were observed between food responsiveness and impulsivity, inattention and hyperactivity. As regards emotional overeating, children in the highest tertile of the scale had higher scores on ADHD total (β=3.71; 95% CI: 1.00, 6.41, p for trend=0.011) and hyperactivity (β=1.46; 95% CI: 0.33, 2.59, p for trend=0.013).

Food approach, and emotional overeating showed a stronger association with hyperactivity for boys than girls (p for interaction <0.1) (Table 4). Although there was no indication for effect modification (p for interaction >0.1), stronger associations were observed for food responsiveness with total ADHD index, inattention, and hyperactivity and for emotional overeating with total ADHD index, in boys compared to girls. In contrast the association between food responsiveness and impulsivity was more pronounced in girls, although again there was no indication for effect modification. There was no evidence of effect modification by child BMI (data not shown). In order to examine whether the executive function was mediator in the association we compared two regression models. All previous findings persisted when adjusting for executive function among other confounding factors.

Discussion

To our knowledge, the present study is the first to specifically focus on the association between eating behaviours and ADHD symptoms in a population-based sample of preschool children. We found that children's food approach eating behaviours, like food responsiveness and emotional overeating were associated with children's ADHD symptoms, including impulsivity, inattention and hyperactivity at 4 years of age. Food fussiness, a food avoidant behavior was associated with increased impulsivity symptoms.

Our findings are consistent with previous studies which have related ADHD symptoms with food approach behaviours in adults. Current literature supports the higher prevalence of abnormal eating behaviours such as binge eating in individuals with ADHD (Cortese, et al., 2007; Docet, et al., 2012; Ptacek, et al., 2014; Seitz et al., 2013) and also the positive association of ADHD symptoms such as hyperactivity and impulsivity with eating difficulties (Blinder, Cumella, & Sanathara, 2006; Nazar, et al., 2014; Wentz et al., 2005; Wonderlich, Connolly, & Stice, 2004). Only few population-based studies have supported the association between disturbed eating patterns and ADHD symptoms in children with no consistent results (Cortese, et al., 2007; Hartmann, et al., 2010; Khalife et al., 2014; Micali, et al., 2011; Ptacek, et al., 2014; Reinblatt, et al., 2014). In a case control study by Hartmann and colleagues, school aged children with loss of control (LOC) eating compared to those without LOC, demonstrated higher impulsivity among other personality traits (Hartmann, et al., 2010). However, Khalife and colleagues recently reported that there was no association between ADHD symptoms in children aged 8 years and binge eating when children were 16 years old (Khalife, et al., 2014).

We found that the association of emotional overeating with increased ADHD symptoms was more pronounced in boys than in girls. Aligned to our findings is the existing research indicating that both boys and girls with ADHD are in higher risk of developing disturbed eating behaviours in adolescence(Reinblatt, et al., 2014) (Mikami et al., 2010) whilst others have reported that girls -

more than boys with ADHD symptoms- are likely to develop disordered eating behaviours such as binge eating (Cortese, Faraone, Bernardi, Wang, & Blanco, 2013) and bulimia nervosa (Biederman et al., 2010; Mikami, Hinshaw, Patterson, & Lee, 2008) later in life. Although incidence of disturbed eating behaviours is higher in older ages(Swanson, Crow, Le Grange, Swendsen, & Merikangas, 2011) our findings add to the existing literature by supporting that there are signs, even in preschool age, that children with ADHD symptoms and possibly boys more than girls are at higher risk to become eating disordered (or obese) in the future. Although several studies have revealed the association between ADHD symptoms, overeating behaviours and higher BMI and obesity(Davis, 2010; van den Berg, et al., 2011), a longitudinal investigation of this association is required to fully understand temporal relationships aiding early identification of youth at risk and thus effective management of the eating behaviours that lead to obesity.

The observed associations between eating behaviours and ADHD symptoms could be explained by several mechanisms. We found a positive association between overeating and ADHD symptoms. Recent genome wide association studies have supported that children with the fat and obesity-associated transcript gene (FTO) minor allele at rs9939609 were less likely to develop ADHD symptoms (Choudhry et al., 2013; Velders et al., 2012). Independent of ADHD symptoms, this gene was also positively associated with food responsiveness. In our population we were not able to control for this gene that could possibly explain some of the observed associations. Dopamine pathways involved in ADHD pathophysiology could explain this association. It is possible that individuals with ADHD-which is associated with dopamine deficiency- prefer 'palatable' foods due to an altered reward system (Davis, 2010). Other plausible mechanisms proposed by Fleming and Levy refer to the fact that individuals with ADHD may not be able respond to internal signals of hunger and satiety and thus forget to eat when necessary and overeat at a later point(Fleming & Levy, 2002). Additionally, other co-morbidity factors such as depression may intervene in the association between ADHD and overeating with 'loss of control' (Cortese, et al., 2007).

The present study has several strengths. The population-based prospective design of the study provided us the opportunity to account for the effect of exposures prospectively within the cohort. Children's eating behaviour patterns were obtained using a validated instrument (Carnell & Wardle, 2007). The ADHDT is a psychometric instrument that assesses all ADHD domains (total

ADHD, impulsivity, inattention, hyperactivity) enabling a more detailed evaluation. In the present analysis we were also able to adjust for EF as there is strong evidence that impaired executive function is a well- known feature of ADHD, likely to cause overeating behaviours (Cortese et al., 2008; Dempsey, et al., 2011). The fact that the statistical significant associations persisted after adjustment for executive function strengthens our findings between eating behaviours and ADHD symptoms. Finally, we excluded children with a neurodevelopmental disorder diagnosis since neurological impairment in children is associated with an increased risk of feeding problems and ADHD symptoms.

We acknowledge that there are also some limitations in our study. The high percentage of non-respondents should be taken into account as a possible source of bias. Participating mothers were more likely to be of Greek origin and more educated making difficult to extrapolate the present findings to general population. Another possible limitation in our study is that although the ADHDT has been translated and adapted in the Greek population, it has not been validated. However, ADHDT is a widely accepted, reliable instrument (Gilliam, 1995) based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) definition for ADHD, which has been previously used in the Greek population (Skounti, Philalithis, Mpitzaraki, Vamvoukas, & Galanakis, 2006). In addition, there are no available data with regard to ADHD in later age groups and this is also acknowledged as a possible limitation of this study. Given the cross sectional design of the study, we are not able to establish the direction of the observed associations. Although in our multivariate models we were able to adjust for a large number of confounding factors, due to the observational study design, residual confounding of other unmeasured confounders such as home environment and maternal intelligence may still occur. Finally, we assessed children's eating behaviour and ADHD symptoms using parent reported measures, which could be different from assessments made by a health care professional. However, this an epidemiological study assessing associations between eating behaviours and ADHD symptoms at population level, and both CEBQ and ADHDT are established and widely used screening tools with high specificity and sensitivity.

Conclusion

In summary, this is the first study to provide evidence that food approach eating behaviours like food responsiveness and emotional overeating are associated with increased ADHD symptoms in preschool children. The early recognition of abnormal eating behaviours can provide an avenue for intervention and thus lead to more effective management of ADHD symptoms in early childhood and prevention of other adverse outcomes that may occur, such as obesity. Moreover our findings add to the existing literature on the overlap between eating behaviours and ADHD, future studies should aim to understand the biological basis of this overlap.

What's known?

- Existing evidence has highlighted the association between ADHD symptoms, overeating behaviours and adverse outcomes like weight gain and obesity. An in depth understanding of the association between ADHD and its core features and abnormal eating patterns in young children at a population-level is lacking.

What's new?

- This is the first study to evaluate the association between eating behaviours and ADHD traits in early childhood.

What's clinically relevant?

- The timely recognition of the association between ADHD symptoms and eating behaviour at early ages could help tailor useful clinical interventions and prevention strategies in order to mitigate long term negative outcomes, such as obesity.

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References

Barkley, R. A. (2001). The executive functions and self-regulation: an evolutionary neuropsychological perspective. *Neuropsychol Rev, 11*, 1-29.

Biederman, J., Petty, C. R., Monuteaux, M. C., Fried, R., Byrne, D., Mirto, T., ... & Faraone, S. V.(2010). Adult psychiatric outcomes of girls with attention deficit hyperactivity disorder: 11-year follow-up in a longitudinal case-control study. *Am J Psychiatry, 167*, 409-417.

Blinder, B. J., Cumella, E. J., & Sanathara, V. A. (2006). Psychiatric comorbidities of female inpatients with eating disorders. *Psychosom Med, 68*, 454-462.

Carnell, S., & Wardle, J. (2007). Measuring behavioural susceptibility to obesity: validation of the child eating behaviour questionnaire. *Appetite, 48*, 104-113.

Caspi, A., Harrington, H., Milne, B., Amell, J. W., Theodore, R. F., & Moffitt, T. E. (2003). Children's behavioral styles at age 3 are linked to their adult personality traits at age 26. *J Pers, 71*, 495-513.

Choudhry, Z., Sengupta, S. M., Grizenko, N., Thakur, G. A., Fortier, M. E., Schmitz, N., & Joober, R. (2013). Association between obesity-related gene FTO and ADHD. *Obesity (Silver Spring), 21*, E738-744.

Cole, T. J., Bellizzi, M. C., Flegal, K. M., & Dietz, W. H. (2000). Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ, 320*, 1240-1243.

Cortese, S., Angriman, M., Maffeis, C., Isnard, P., Konofal, E., Lecendreux, M., . . . & Mouren, M. C. (2008). Attention-deficit/hyperactivity disorder (ADHD) and obesity: a systematic review of the literature. *Crit Rev Food Sci Nutr, 48*, 524-537.

Cortese, S., Bernardina, B. D., & Mouren, M. C. (2007). Attention-deficit/hyperactivity disorder (ADHD) and binge eating. *Nutr Rev, 65*, 404-411.

Cortese, S., & Castellanos, F. X. (2014). The relationship between ADHD and obesity: implications for therapy. *Expert Rev Neurother, 14*, 473-479.

Cortese, S., Faraone, S. V., Bernardi, S., Wang, S., & Blanco, C. (2013). Adult attention-deficit hyperactivity disorder and obesity: epidemiological study. *Br J Psychiatry, 203*, 24-34.

Cortese, S., & Vincenzi, B. (2012). Obesity and ADHD: clinical and neurobiological implications. *Curr Top Behav Neurosci, 9*, 199-218.

Davis, C. (2010). Attention-deficit/hyperactivity disorder: associations with overeating and obesity. *Curr Psychiatry Rep, 12*, 389-395.

Davis, C., Levitan, R. D., Smith, M., Tweed, S., & Curtis, C. (2006). Associations among overeating, overweight, and attention deficit/hyperactivity disorder: a structural equation modelling approach. *Eat Behav, 7*, 266-274.

Dempsey, A., Dyehouse, J., & Schafer, J. (2011). The relationship between executive function, AD/HD, overeating, and obesity. *West J Nurs Res, 33*, 609-629.

Docet, M. F., Larranaga, A., Perez Mendez, L. F., & Garcia-Mayor, R. V. (2012). Attention deficit hyperactivity disorder increases the risk of having abnormal eating behaviours in obese adults. *Eat Weight Disord, 17*, e132-136.

Fleming, J., & Levy, L. (Eds.). (2002). *Eating disorders in women with AD/HD*.

Froehlich, T. E., Anixt, J. S., Loe, I. M., Chirdkiatgumchai, V., Kuan, L., & Gilman, R. C. (2011). Update on environmental risk factors for attention-deficit/hyperactivity disorder. *Curr Psychiatry Rep, 13*, 333-344.

Gilliam, J. E. (1995). *Attention deficit/hyperactivity disorder test examiner's manual.* Austin, TX: Pro-Ed.

Hartmann, A. S., Czaja, J., Rief, W., & Hilbert, A. (2010). Personality and psychopathology in children with and without loss of control over eating. *Compr Psychiatry, 51*, 572-578.

Heilskov Rytter, M. J., Andersen, L. B., Houmann, T., Bilenberg, N., Hvolby, A., Molgaard, C., . . . & Lauritzen, L. (2015). Diet in the treatment of ADHD in children - a systematic review of the literature. *Nord J Psychiatry, 69*, 1-18.

Julvez, J., Ribas-Fito, N., Torrent, M., Forns, M., Garcia-Esteban, R., & Sunyer, J. (2007). Maternal smoking habits and cognitive development of children at age 4 years in a population-based birth cohort. *Int J Epidemiol, 36*, 825-832.

Khalife, N., Kantomaa, M., Glover, V., Tammelin, T., Laitinen, J., Ebeling, H., . . . & Rodriguez, A. (2014). Childhood attention-deficit/hyperactivity disorder symptoms are risk factors for obesity and physical inactivity in adolescence. *J Am Acad Child Adolesc Psychiatry, 53*, 425-436.

Maniadaki, K., & Kakouros, E. (2002). *Translation and Adaptation of the Attention Deficit Hyperactivity Disorder Test (ADHDT; Giliam, 1995).* In: Stalikas, A., Triliva, S., & Roussi, P. (eds) Psychometric scales in Greece. Ellinika Grammata Publications.

McCarthy, D. (1972). *Manual for the McCarthy scales of children's abilities*. New York: Psychological Corp.

Micali, N., Simonoff, E., Elberling, H., Rask, C. U., Olsen, E. M., & Skovgaard, A. M. (2011). Eating patterns in a population-based sample of children aged 5 to 7 years: association with psychopathology and parentally perceived impairment. *J Dev Behav Pediatr, 32*, 572-580.

Mikami, A. Y., Hinshaw, S. P., Arnold, L. E., Hoza, B., Hechtman, L., Newcorn, J. H., & Abikoff, H. B. (2010). Bulimia nervosa symptoms in the multimodal treatment study of children with ADHD. *Int J Eat Disord, 43*, 248-259.

Mikami, A. Y., Hinshaw, S. P., Patterson, K. A., & Lee, J. C. (2008). Eating pathology among adolescent girls with attention-deficit/hyperactivity disorder. *J Abnorm Psychol, 117*, 225-235.

Millichap, J. G., & Yee, M. M. (2012). The diet factor in attention-deficit/hyperactivity disorder. *Pediatrics, 129*, 330-337.

Nazar, B. P., Suwwan, R., de Sousa Pinna, C. M., Duchesne, M., Freitas, S. R., Sergeant, J., & Mattos, P. (2014). Influence of attention-deficit/hyperactivity disorder on binge eating behaviors and psychiatric comorbidity profile of obese women. *Compr Psychiatry, 55*, 572-578.

Nikolas, M. A., & Burt, S. A. (2010). Genetic and environmental influences on ADHD symptom dimensions of inattention and hyperactivity: a meta-analysis. *J Abnorm Psychol, 119*, 1-17.

Pieper, J. R., & Laugero, K. D. (2013). Preschool children with lower executive function may be more vulnerable to emotional-based eating in the absence of hunger. *Appetite, 62*, 103-109.

Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A., & Rohde, L. A. (2015). Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *J Child Psychol Psychiatry, 56*, 345-365.

Ptacek, R., Kuzelova, H., Stefano, G. B., Raboch, J., Sadkova, T., Goetz, M., & Kream, R. M. (2014). Disruptive patterns of eating behaviors and associated lifestyles in males with ADHD. *Med Sci Monit, 20*, 608-613.

Reinblatt, S. P., Leoutsakos, J. M., Mahone, E. M., Forrester, S., Wilcox, H. C., & Riddle, M. A. (2014). Association between binge eating and attention-deficit/hyperactivity disorder in two pediatric community mental health clinics. *Int J Eat Disord*. doi: 10.1002/eat.22342

Seitz, J., Kahraman-Lanzerath, B., Legenbauer, T., Sarrar, L., Herpertz, S., Salbach-Andrae, H., . . . & Herpertz-Dahlmann, B. (2013). The role of impulsivity, inattention and comorbid ADHD in patients with bulimia nervosa. *PLoS One, 8*, e63891.

Silva, D., Colvin, L., Hagemann, E., & Bower, C. (2014). Environmental risk factors by gender associated with attention-deficit/hyperactivity disorder. *Pediatrics, 133*, e14-22.

Skounti, M., Philalithis, A., Mpitzaraki, K., Vamvoukas, M., & Galanakis, E. (2006). Attention-deficit/hyperactivity disorder in schoolchildren in Crete. *Acta Paediatr, 95*, 658-663.

Sonneville, K. R., Calzo, J. P., Horton, N. J., Field, A. E., Crosby, R. D., Solmi, F., & Micali, N. (2015). Childhood hyperactivity/inattention and eating disturbances predict binge eating in adolescence. *Psychol Med*, 1-10.

Swanson, S. A., Crow, S. J., Le Grange, D., Swendsen, J., & Merikangas, K. R. (2011). Prevalence and correlates of eating disorders in adolescents. Results from the national comorbidity survey replication adolescent supplement. *Arch Gen Psychiatry, 68*, 714-723.

Thomas, R., Sanders, S., Doust, J., Beller, E., & Glasziou, P. (2015). Prevalence of Attention-Deficit/Hyperactivity Disorder: A Systematic Review and Meta-analysis. *Pediatrics, 135*, e994-1001.

van den Berg, L., Pietersae, K., Malik, J. A., Luman, M., Willems van Dijk, K., Oosterlaan, J., & Delemarre-van de Waal, H. A. (2011). Association between impulsivity, reward responsiveness and body mass index in children. *Int J Obes (Lond), 35*, 1301-1307.

Velders, F. P., De Wit, J. E., Jansen, P. W., Jaddoe, V. W., Hofman, A., Verhulst, F. C., & Tiemeier, H. (2012). FTO at rs9939609, food responsiveness, emotional control and symptoms of ADHD in preschool children. *PLoS One, 7*, e49131.

Wardle, J., Guthrie, C. A., Sanderson, S., & Rapoport, L. (2001). Development of the Children's Eating Behaviour Questionnaire. *J Child Psychol Psychiatry, 42*, 963-970.

Wentz, E., Lacey, J. H., Waller, G., Rastam, M., Turk, J., & Gillberg, C. (2005). Childhood onset neuropsychiatric disorders in adult eating disorder patients. A pilot study. *Eur Child Adolesc Psychiatry, 14*, 431-437.

onderlich, S. A., Connolly, K. M., & Stice, E. (2004). Impulsivity as a risk factor for eating disorder behavior: assessment implications with adolescents. *Int J Eat Disord, 36*, 172-182.

Table 1. Descriptive characteristics of the study population

	N	% or Mean± SD
Parental Characteristics		
Maternal age (years)	543	29.86±4.97
Maternal origin, *n (%)*		
Greek	516	93.5
Non Greek	36	6.5
Maternal education, *n (%)*		
Low	94	17.4
Medium	257	47.5
High	190	35.1
Paternal education, *n (%)*		
Low	189	35.1
Medium	206	38.3
High	143	26.6
Maternal working status, *n (%)*		
Working	312	43.9
Not working	244	56.1
Maternal marital status, *n (%)*		
Married	477	88.2
Other	64	11.8
Pre-pregnancy BMI (kg/m^2)	541	24.48±4.90
Infant Characteristics		
Sex, *n (%)*		
Male	287	51.4
Female	271	48.6
Parity, *n (%)*		
Primiparous	228	42.9
Multiparous	303	57.1
Preterm birth, *n (%)*		

Yes	73	13.4
No	473	86.7
Birth weight (kg)	543	3.20±0.46
Breastfeeding duration (months)	543	3.97±4.15
Child characteristics at 4 yrs		
ADHDT Total	404	14.26±12.14
Preschool attendance, *n (%)*		
No	78	14.0
Yes	478	86.0
Passive smoking at home, *n (%)*		
No	353	63.5
Yes	203	36.5
Hours/day spent watching TV, *n (%)*		
Almost never	143	25.7
1	216	38.9
≥ 2	197	35.4
Cognitive Ability (score), McCarthy	545	98.16±17.46
Child's BMI (kg/m^2), *n (%)*		
No excess weight	443	79.8
Overweight	77	13.9
Obese	35	6.3
Child's BMI (kg/m^2)	555	16.40±1.89
Child's energy intake (kcals)	531	1585.70±453.46

Abbreviations: SD: standard deviation; BMI, body mass index; TV, television

Table 2. Subscale scores of the CEBQ (n=558).

	Mean	SD	Cronbach a
Eating Behaviours			
Food Responsiveness	2.14	0.84	0.769
Emotional overeating	1.69	0.56	0.626
Enjoyment of Food	3.44	0.84	0.797
Desire to Drink	3.52	0.98	0.592
Satiety Responsiveness	3.36	0.64	0.602
Slowness in Eating	2.98	0.78	0.575
Emotional under-eating	3.12	0.94	0.712
Food Fussiness	2.41	0.86	0.820
Food approach behaviours	2.41	0.59	0.825
Food avoidant behaviours	3.11	0.53	0.796

Total scoring: 0.601

Table 3. Multivariable associations between eating behaviours and ADHD scores at 4 years of age.

ADHD	Total (n=355)		Impulsivity (n=417)		Inattention (n=412)		Hyperactivity (n=378)	
	β-coeff	**95% CI**	**β-coeff**	**95% CI**	**β-coeff**	**95% CI**	**β-coeff**	**95% CI**
Eating Behaviours								
Food Approach	**0.63**	**(0.07, 1.20)**	0.18	(-0.01, 0.37)	0.18	(-0.01, 0.38)	**0.29**	**(0.06, 0.52)**
Food Responsiveness	**2.54**	**(1.09, 3.99)**	**0.76**	**(0.27, 1.24)**	**0.90**	**(0.40, 1.39)**	**0.95**	**(0.35, 1.55)**
Emotional overeating	**3.71**	**(1.60, 5.82)**	**0.90**	**(0.17, 1.61)**	**0.88**	**(0.14, 1.62)**	**1.58**	**(0.74, 2.44)**
Enjoyment of Food	-0.72	(-2.21, 0.76)	-0.09	(-0.58, 0.40)	-0.26	(-0.77, 0.25)	-0.04	(-0.65, 0.57)
Desire to Drink	0.51	(-0.77, 1.79)	0.10	(-0.33, 0.52)	0.12	(-0.32, 0.55)	0.24	(-0.29, 0.77)
Food Avoidant	0.03	(-0.54, 0.61)	0.08	(-0.11, 0.27)	0.02	(-0.17, 0.22)	-0.05	(-0.29, 0.19)
Satiety Responsiveness	-0.20	(-2.18, 1.77)	-0.02	(-0.67, 0.63)	-0.32	(-0.99, 0.35)	-0.03	(-0.82 0.77)
Slowness in Eating	-0.39	(-1.97, 1.19)	-2.78	(-0.80, 0.24)	-0.25	(-0.79, 0.29)	0.02	(-0.64, 0.68)
Emotional under-eating	0.29	(-1.03, 1.61)	0.18	(-0.26, 0.61)	0.21	(-0.24, 0.66)	-0.13	(-0.68, 0.41)
Food Fussiness	0.30	(-1.16, 1.76)	**0.51**	**(0.03, 0.98)**	0.26	(-0.22, 0.75)	-0.09	(-0.70, 0.51)

Models are adjusted for executive function at 4 years, child's age, child's sex, maternal age, maternal education, parity, child's BMI z- score, child's energy intake (kcals), TV watching.

Bolds indicated statistically significant differences at $p<0.05$.

Study population varies across ADHD subscales due to missing values of included covariates.

Table 4. Multivariable associations between eating behaviours and ADHD test scores stratified by gender at 4 years of age in Rhea Cohort Study, Crete, Greece.

ADHD	**Total**			**Impulsivity**			**Inattention**			**Hyperactivity**		
	β-coeff	**95% CI**		**β-coeff**	**95% CI**		**β-coeff**	**95% CI**		**β-coeff**	**95% CI**	
Eating Behaviours												
Food Approach												
Boys	0.58	(-0.34,	1.49)	0.07	(-0.24,	0.39)	0.12	(-0.21,	0.46)	0.34	(-0.02,	0.71)
Girls	0.15	(-0.08,	0.39)	0.71	(-0.16,	1.59)	0.13	(-0.09,	0.35)	0.06	(-0.22,	0.34)
p for interaction	0.160			0.983			0.475			**0.051**		
Food Responsiveness												
Boys	**2.39**	**(0.09,**	**4.70)**	0.54	(-0.23,	1.31)	**1.01**	**(0.20,**	**1.82)**	**0.97**	**(0.04,**	**1.90)**
Girls	**1.59**	**(0.17,**	**3.35)**	**0.65**	**(0.02,**	**1.28)**	0.49	(-0.11,	1.09)	0.42	(-0.35,	1.19)
p for interaction	0.295			0.955			0.174			0.224		
Emotional overeating												
Boys	**4.43**	**(0.96,**	**7.90)**	0.98	(-0.20,	2.17)	0.88	(-0.40,	2.15)	**2.17**	**(0.83,**	**3.50)**
Girls	2.32	(-0.16,	4.81)	0.64	(-0.25,	1.53)	0.63	(-0.20,	1.46)	0.63	(-0.42,	1.69)
p for interaction	0.129			0.512			0.436			**0.037**		
Enjoyment of Food												
Boys	-1.87	(-4.23,	0.48)	-0.68	(-1.47,	0.10)	-0.75	(1.59,	0.09)	-0.14	(-1.08,	0.80)

Girls	-0.83	(-2.58,	0.93)	0.13	(-0.48,	0.74)	-0.05	(-0.64,	0.54)	-0.58	(-1.35,	1.19)
p for interaction	0.712			0.177			0.292			0.368		
Desire to Drink												
Boys	0.94	(-1.11,	3.01)	0.13	(-0.55,	0.82)	0.08	(-0.64,	0.81)	0.32	(-0.52,	1.15)
Girls	0.29	(-1.18,	1.76)	-0.01	(-0.52,	0.51)	0.10	(-0.38,	0.58)	0.18	(-0.47,	0.83)
p for interaction	**0.096**			0.498			0.411			0.195		

ADHD Total: boys, n=179, girls, n=176, Impulsivity: boys, n=211, girls, n= 206, Inattention: boys, n=212, girls, n= 200, Hyperactivity: boys, n=188, girls, n=190.

Bolds indicated statistically significant differences at p<0.05.

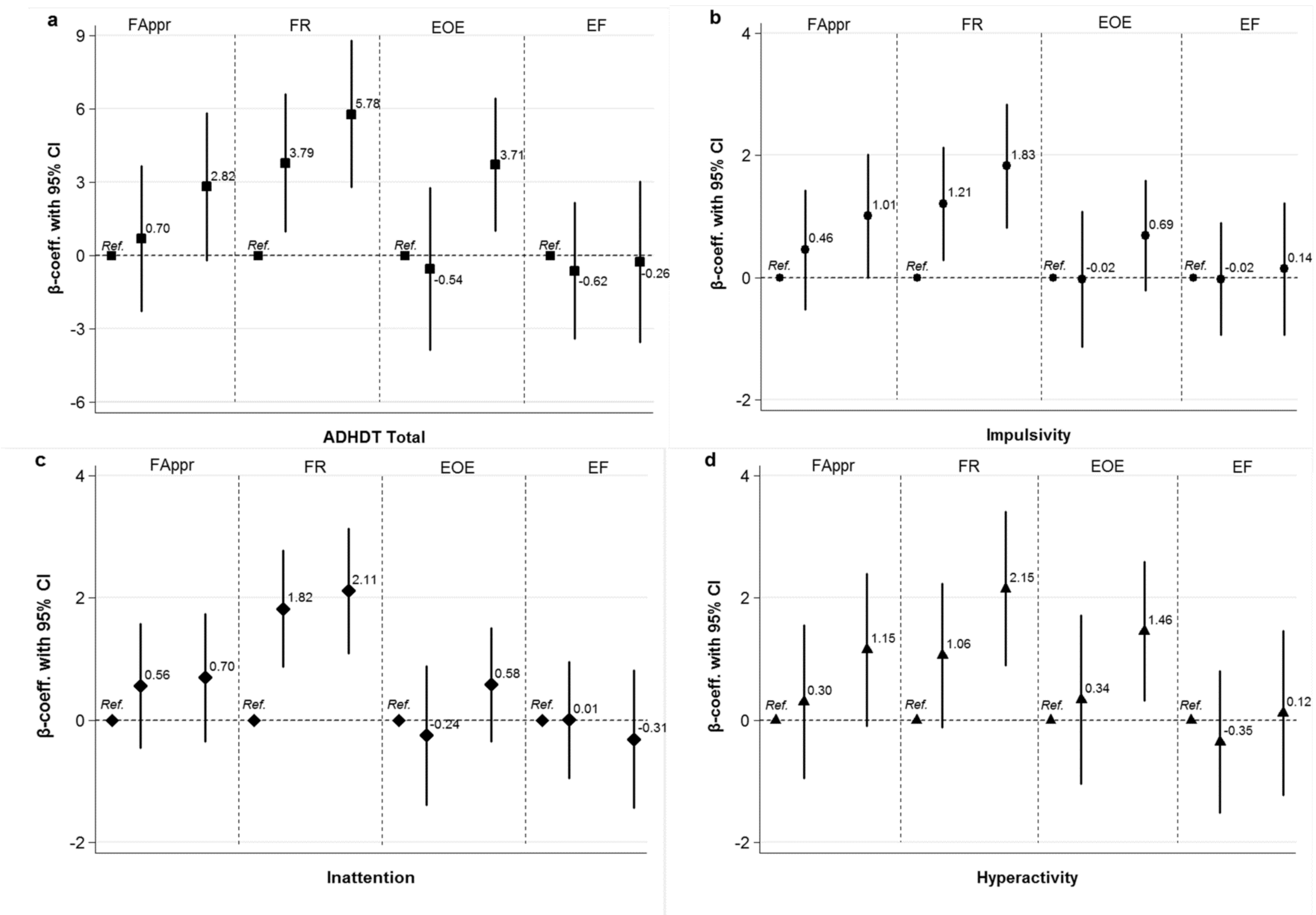
a
FAppr
FR
EOE
EF
β-coeff. with 95% CI
Ref.
0.70
2.82
3.79
5.78
-0.54
3.71
-0.62
-0.26
ADHDT Total
b
0.46
1.01
1.21
1.83
-0.02
0.69
-0.02
0.14
Impulsivity
c
0.56
0.70
1.82
2.11
-0.24
0.58
0.01
-0.31
Inattention
d
0.30
1.15
1.06
2.15
0.34
1.46
-0.35
0.12
Hyperactivity

Figure 1. Associations of eating behaviours (in tertiles) with ADHD test scores at 4 years of age, (a) Food Approach (FAppr), (b) Food Responsiveness (FR), (c) Emotional overeating (EOE) and (d) Enjoyment of Food (EF). All models are adjusted for executive function at 4 years, child's age, child's sex, maternal age, maternal education, parity, child's BMI z- score, child's energy intake (kcals) and TV watching.

5. Discussion

Findings from the present thesis indicate the important effect of diet, during pregnancy, infancy and childhood on children's neurodevelopment. To our knowledge, this is the first study that assesses in depth the impact of diet at different time points on later neurodevelopment in the Greek childhood population. This section provides a general discussion and a broader interpretation of the entire research study. It summarizes the detailed discussions of the individual articles presented in the results section of this thesis.

5.1 General discussion of the present findings

Fish intake during pregnancy and fetal growth and gestational length

The association of fish intake during pregnancy with birth weight and length of gestation including over 150,000 mother-child pairs, is described in detail in paper 1. Individual data from 19 European birth cohorts were pooled and harmonized. Findings from this study support the existing evidence for a beneficial role of moderate fish intake during pregnancy in the risk of preterm birth, and a small but significant increase in birth weight. In line with randomized controlled trials, the fatty fish types, with higher content in n-3 LCPUFAs, had the most pronounced effect on birth weight. According to these studies, maternal intake of n-3 LCPUFA during pregnancy resulted in a slightly longer gestation period and somewhat higher birth size (Szajewska, Horvath et al. 2006; Salvig and Lamont 2011). Findings from prospective birth cohort studies on the relationship between fish intake during pregnancy and fetal growth are discrepant, with reports of either positive/null effects (Olsen, Olsen et al. 1990; Olsen, Grandjean et al. 1993; Olsen and Secher 2002; Oken, Kleinman et al. 2004; Thorsdottir, Birgisdottir et al. 2004; Olsen, Osterdal et al. 2006; Guldner, Monfort et al. 2007; Drouillet, Kaminski et al. 2009; Brantsaeter, Birgisdottir et al. 2011; Heppe, Steegers et al. 2011), or negative effects (Rylander, Stromberg et al. 2000; Halldorsson, Meltzer et al. 2007; Halldorsson, Thorsdottir et al. 2008; Mendez, Plana et al. 2010). Several methodological factors may account for these inconsistencies such as inadequate sample sizes, exposure misclassification, exposure profile heterogeneity (i.e. consumption frequencies versus estimated daily intakes in grams), or differences in adjustment.

This international study, involving a large number of mother-child pairs, recorded thoroughly a wide range of exposure levels, and thus allowed us to carry out the most detailed exploration of potential heterogeneity than previously reported in the literature. The present analysis included mother-child pairs from several birth cohorts including populations with low and high fish intake, as well as regions, with low and high levels of contamination in fish species. However, we did not have the possibility of collecting accurate information on contaminants that have been negatively associated with birth size and gestational length such as PCBs, mercury, and dioxins levels (Govarts, Nieuwenhuijsen et al. 2012). We would expect confounding from pollutants bioaccumulating in fish to bias the association between fish intake and birth weight towards the null. Therefore, any true effect size might be larger than the one reported here in the absence of correction for fish pollutants.

Breastfeeding and cognitive, language and motor development in infancy

Greece is a country with low levels of breastfeeding, and the impact of breastfeeding on later neurodevelopment remains a challenging research area. Paper 2 is the first study to examine the longitudinal effect of breastfeeding on neurodevelopment early in infancy in a Greek population. In line with the existing literature, results from this study showed a positive and linear association between breastfeeding duration and increased scores in the scales of cognitive, language, and fine motor development at 18 months of age. Children who were breastfed for longer than 6 months scored higher in the scale of fine motor development as compared with those that were never breastfed. Most published articles have important methodological issues, such as the cross sectional study design, the data quality (as a consequence of retrospective data collection on breastfeeding at the time of cognitive measurements), the lack of valid neuropsychological measurements, the small sample sizes, and the insufficient adjustment for critical potential confounders, making it difficult to fully understand the relationship between breastfeeding and child mental development. In the present study we managed to overcome these methodological issues, and therefore provide reliable data. One of the defects of this analysis is that we were not able to take into account maternal intelligence and the quality of home environment, as important predictors of later neurodevelopment, since these variables were not available at the time.

It is possible that our observations could also be explained by other factors/mechanisms linking breastfeeding with enhanced neurodevelopment in infancy. To start with, gene-nutrition studies have provided evidence investigating the interaction between breastfeeding and genotype effects. Infant levels of LCPUFAs depend not only on maternal levels but also on genes such as FADS1 and FADS2 that regulate PUFA metabolism, and determining PUFA levels in breast milk (Glaser, Heinrich et al. 2010). These studies have shown that breastfeeding infants had higher IQ scores when compared to those fed with formula that was not enriched with LCPUFA. In addition, breastfed infants with the FADS genotype but with low synthesis of LCPUFA, had an additional benefit in IQ when compared to infants with a genotype supporting a more active formation of LCPUFA (Steer, Davey Smith et al. 2010; Morales, Bustamante et al. 2011). Other possible mechanisms that may explain the association between breastfeeding and child neurodevelopment include essential nutrient components in breast milk that may also contribute to optimal neural function such as hormones, oligosaccharides, phospholipids and (Innis 2007). Finally, enhanced psychosocial experiences for children provided by the breastfeeding process, including mother–child interaction, bonding, and greater variety of daily stimulations may contribute to the infant's optimal neurodevelopment (Morrow-Tlucak, Haude et al. 1988; Anand and Scalzo 2000).

Dietary patterns in preschoolers and associations with socio-demographic factors

One main aim of the present thesis was the identification of dietary patterns among Greek preschool children. For this purpose we developed and validated a semi-quantitative FFQ (paper 3) addressed to this specific population group. The FFQ was validated before use in a group of representative subjects of the study population. Since validity is the degree to which the instrument actually assesses the habitual intake of subjects, our validation study indicated that the Rhea 4 years FFQ is a relatively accurate tool in assessing habitual food group and nutrient intake for our study population. After the validation process was completed, we identified the dietary patterns using factor analysis and investigated in detail their associations with multiple socio-demographic and lifestyle factors (paper 4). The 'Mediterranean' dietary component, comprised mainly of vegetables, fruits, pulses, olive oil, fish and seafood was the principal pattern that explained the higher percentage of variability. Even though traditional Greek diet has undergone many changes the last years it seems that the 'Mediterranean' pattern still reflects the main dietary choice for children. In line with our findings, other studies have also reported positive associations between lower parental educational levels, presence of older siblings, exposure to passive smoking, and earlier introduction to solid food with 'less healthy' patterns. Higher scores on 'healthier' patterns have been observed for children with longer breastfeeding duration and for those attending pre-school. However, comparing our findings with other studies is rather complex mainly due to multiple methodological approaches including study design, dietary assessment tools, food variability across countries and different statistical techniques used. Factor analysis is also a sample specific method and its results cannot be extrapolated to the general population(Schulze, Hoffmann et al. 2003).

Dietary patterns and neurodevelopment in preschool age

The impact of children's dietary patterns on cognitive and psychomotor development at preschool age is described in paper 5 (currently under revision). Children who had unhealthy food choices at preschool age, characterized by processed and high in sugar foods, scored lower in verbal ability, general cognitive ability and cognitive functions of posterior cortex. In this analysis we investigated step by step the role of confounders with multiple models adjusting each time for additional confounders. Similarly to other studies, we observed stronger associations in minimally adjusted models, but results attenuated after adjusting for a large array of confounders with only the 'snacky' pattern (potatoes and other starchy roots, salty snacks, sugar products and eggs) remaining negatively associated with children's neurodevelopment.

Underlying mechanisms affecting this association remain inconclusive. Animal studies have reported detrimental effects of a high-fat/high cholesterol diet on cognitive performance and hippocampal morphology (Granholm, Bimonte-Nelson et al. 2008; Freeman, Haley-Zitlin et al. 2014). Adult epidemiological studies have also shown that intake of a high-fat diet that includes mostly omega-6 and saturated fatty acids is associated with worse performance on cognitive tasks(Freeman, Haley-Zitlin et al. 2014). These associations may be even more profound at early life as the brain grows at its fastest rate during the first 3 years of life, and it is possible that poorer food choices during this early period may discourage optimal head and brain growth(Gale, Martyn et al. 2009). There is also evidence that shared family environmental factors are greater for verbal abilities than for non-verbal abilities, with the latter being related to individual's inherent mentality.

Eating behavior and ADHD symptoms in preschool children

Finally, within this thesis we performed the first study (paper 6, currently under revision) to investigate the association between eating behaviours and ADHD symptoms in Greek preschool children. We found that children's food approach eating behaviours, like food responsiveness and emotional overeating were associated with children's ADHD symptoms, including impulsivity, inattention and hyperactivity at 4 years of age. Although incidence of disturbed eating behaviours is higher in older ages (Swanson, Crow et al. 2011) our findings add to the existing literature by supporting that there are signs, even in preschool age, that children with ADHD symptoms are at higher risk to become eating disordered (or obese) in the future. Childhood eating behaviours have been shown to predict later negative outcomes. In particular, there is evidence that food approach/overeating is associated with high body mass index and later obesity (Viana, Sinde et al. 2008). Therefore, it will be very interesting to determine how the eating behaviours identified develop across time. In this analysis we also had the opportunity to adjust for executive function as there is strong evidence that impaired executive function is a well-known feature of ADHD, likely to cause overeating behaviours (Cortese, Angriman et al. 2008; Dempsey, Dyehouse et al. 2011). The fact that the statistical significant associations persisted after adjustment for executive function strengthens our findings between eating behaviours and ADHD symptoms.

Dopamine pathways involved in ADHD pathophysiology could explain the positive association between overeating and ADHD symptoms that we observed. It is possible that individuals with ADHD, which is associated with dopamine deficiency- prefer 'palatable' foods due to an altered reward system (Davis 2010). Other plausible mechanisms proposed by Fleming and Levy refer to the fact that individuals with ADHD may not be able respond to internal signals of hunger and satiety and thus forget to eat when necessary and overeat at a later point(Fleming and Levy 2002). Additionally, other co-morbidity factors such as depression may intervene in the association between ADHD and overeating with 'loss of control' (Cortese, Bernardina et al. 2007).

5.2 Methodological issues

This thesis includes studies that aimed to investigate the role of nutrition from fetal life up to preschool age, on children's neurodevelopment. The data of these studies were mainly derived from the Rhea mother-child cohort, a prospective population based birth cohort study in Greece. One of the studies included a meta-analysis and was performed in collaboration with other birth cohort studies in the framework of a European project (CHICOS project). A number of methodological issues deserve to be acknowledged in the following section which provides a general description of selection and information bias.

Selection and Information bias

A major concern in epidemiological studies is to select the study participants from the source population. Selection bias include any bias arising from the procedures followed to select the study participants from the source population at the stage of the recruitment and/or during the procedure of retaining them in the study (follow ups)(Ellenberg 1994). At recruitment those individuals who volunteer to participate in a study have generally different baseline characteristics than the non-responders. Many studies have observed that the non-responders have a lower socioeconomic status and a less favorable lifestyle. Sample attrition (withdrawals or loss to follow up) is also a very common source of bias in cohort studies. Overall, attrition in the Rhea study was inevitable and this selection may have biased our observations in either direction (i.e. overestimation or underestimation) but this bias cannot be quantified.

Bias that occur during the data collection may also be introduced in epidemiological studies, known as information bias. In the present thesis the dietary data described in paper 4 and 5 were collected with the use of FFQs. Although we validated the FFQ for our population (paper 3), parental misreporting and recall bias cannot be discounted. FFQ is a dietary instrument appropriate for ranking participants in large population studies based on their food and nutrients intake. However, it is not recommended for evaluating reliable estimates of absolute intake. Other source of information bias in the present thesis could be the interviewer bias that may occur if the interviewer helped the respondent in different ways, such as gestures or put emphasis on different questions. In order to overcome these issues, trained staff and standardized data collection procedures were used. Fieldworkers and parents were also blind to the main hypotheses of the research. Underreporting bias is also common with socially undesirable lifestyle behaviours such as smoking, low socioeconomic status or unhealthy eating habits. Participants tend to under-report behaviours deemed inappropriate by researchers and over-report behaviors viewed as appropriate. Thus, the data which rely on self-report is likely to be biased.

5.3 Strengths and limitations

This section provides a general description of strengths and limitations of the present thesis that have been extensively discussed in the papers. Regarding pregnancy, the study included in the present thesis is the largest study conducted so far to assess the association of fish intake during pregnancy with birth weight and length of gestation including over 150,000 mother-child pairs. Strengths of this thesis also include the population-based prospective design of the 'Rhea' study which provided us the opportunity to account for the effect of exposures prospectively within the cohort and for a number of potential predictors of child neurodevelopment. Regarding infancy, breastfeeding was primarily measured as a continuous variable in order to avoid misclassification bias, to maximize statistical power, and to allow detection of dose-response relationships. We were able to provide estimates of long-term effects of breastfeeding on child neurodevelopment. In early childhood, diet was assessed with a validated FFQ for our population (paper 3). In addition, the use of PCA for the determination of the dietary patterns, gave us the opportunity to gain deep understanding of the whole diet instead of isolated foods and nutrients. Children's eating behavior patterns were also obtained using a validated instrument (Carnell and Wardle 2007). The Bayley Scale of Infant and Toddler Development (3rd edition) and the MCSA test were used to assess the neurodevelopment of toddlers and preschoolers, respectively. These instruments are valid psychometric tests able to provide a reliable measure of a toddler's and child's separate neurodevelopmental domains (cognition, language and motor abilities) and thus, detect which domain is mostly affected. The ADHDT used is also a valid psychometric instrument, appropriate for preschool age. It assesses all ADHD domains (total ADHD, impulsivity, inattention, hyperactivity) enabling a more detailed evaluation. Finally, in paper 2, 5 and 6, where we assessed neurodevelopment, we decided to exclude children with a neurodevelopmental impairment as it may be associated with an increased risk of feeding and nutritional problems.

We acknowledge that there are also some limitations in the present thesis. Although in our multivariate models we were able to adjust for a large number of confounding factors, due to the observational study design, residual confounding of other unmeasured confounders may still occur. Given the cross sectional design of the study design in paper 4, 5 and 6, we were not able to establish the direction of the observed associations.

5.4 Public health implications and future research

Measuring the effect of dietary intake in large populations remains challenging. Nutritional epidemiology has mainly focused on the role of diet during adulthood and only few studies to date have considered early life diet. The influence of nutrition during periods of growth is important, the effect of diet may be cumulative and the long term impact may only translate into disease decades later. The idea that early nutrition may 'program' health outcomes has obvious biological and public health implications. Public health practice is underpinned by research that establishes outcome benefit. The possibility that nutrition in early life may have major effects on later health may lead to targeted nutritional policies or recommendations in order to promote optimal future health. The identification and application of nutritional recommendations is of broad social significance for the general population in terms of how it affects education, work potential and mental illness in any age group.

Our findings further highlight the importance of diet and eating behavior at early life with children's neurodevelopment. We observed the positive and linear association of breastfeeding duration with increased scores in the scales of cognitive, language, and fine motor development at 18 months of age (paper 2). Breastfeeding longer than 6 months enhanced children's fine motor development compared to those never breastfed. These findings support national and international recommendations to promote breastfeeding through at least the age of 6 months. We also found that children's dietary patterns are clearly associated with social and demographic variables. Preschool children are at the age where they are learning to eat properly, setting the ground rules for future eating patterns. Results from this study (paper 4) highlight the importance of enhancing school-based and community-based actions to promote healthy eating addressed to children and young people. Specific actions involving families and increased opportunities to access healthy food choices are required.

As described in paper 5, it is also likely that children's diet, beyond the type of milk consumed in infancy (breast milk or formula), plays an important role in cognitive development. Pre-school children following a poor diet, with increased intake of processed foods, had lower scores in cognitive performance, even after adjusting for a large array of confounding factors. This study has extended this area of research by linking children's diet before entry into formal education with neurodevelopmental outcomes. This highlights a challenge, from a public health perspective, for establishing the location of responsibility for school outcomes related to aspects of health. The question rising is whether educational outcomes partly depend on nutritional intake before the start of school. Improving the nutritional intake of children requires for a well-coordinated effort between schools, families, government departments to improve diets inside and out of school.

Although we were only able to explore traits of ADHD and eating behaviors, not clinical conditions, paper 6 describes the only prospective study reporting the positive association between children's food approach eating behaviors with ADHD symptoms, including impulsivity, inattention and hyperactivity at 4 years of age. The assessment of eating pathology provides good grounds to explore predictors of eating disorders such as binge eating in adolescence. Considering the escalating prevalence of mental disorders such ADHD at early ages it is urgent to tailor useful interventions and prevention strategies for clinical practice in order to mitigate long term negative outcomes. Heath care providers should examine whether efforts to prevent ADHD symptoms could include measurement of abnormal eating behaviors and the opposite. If ADHD symptoms does in fact contribute to abnormal eating behaviors then the treatment of the symptoms might improve eating behaviors. Potentially effective therapeutic strategies should also explore treatment implications for co-occurrence of these two conditions.

Childhood health measures that have predictive value for adult health need to be collected at various time points in well-designed studies. For example, in a cohort study designed to assess neurodevelopment, follow up at 18 months may provide suggestive evidence, but follow ups in older ages up to adult life, would provide compelling evidence. Thus, the long term follow up is needed to better understanding the relation between nutrition and neurodevelopment. Future research should examine whether efforts to prevent neurodevelopmental impairment could include dietary interventions by health care providers for children at higher risk.

The interaction of clinical and epidemiological science is essential in evaluation of study outcomes and in defining future directions. Multiple groups need to work together in large national and international level to better understand and solve the problems. Researches in the field of nutrition, pediatrics, psychology, psychiatry and genetics should collaborate in order to improve knowledge of how nutrition affects mental health in childhood. Highly innovative tools and techniques are being developed that will, in the near future, identify novel genes and epigenetic mechanisms involved in the development of neuropsychiatric diseases. The concept of 'exposome' is an attempt to describe the co-occurrence of multiple exposures (exposure to air pollution, environmental hazards, nutrition, biomarkers of exposure) of an individual and their association with various health outcomes (growth, obesity, neurodevelopment, immune system)(Wild 2005). Results from these studies will provide us a great opportunity to unravel some existing research questions and underlying mechanisms.

6. Conclusions

In summary, findings in the present thesis support that nutrition in early life may affect neurodevelopment in infancy and childhood. Fetal development and birth outcomes are significant predictors of optimal long term neurodevelopment. More specifically, women who ate fish more than once a week during pregnancy had a lower risk of preterm birth compared with women who rarely ate fish (once a week or less). Pregnant women with a higher intake of fish during pregnancy gave birth to neonates with higher birth weight. Longer duration of breastfeeding in infancy was positively associated with neurodevelopment at the age of 18 months. In preschool age, children who followed a less 'healthy' dietary pattern, high in fat and sugar, were more likely to score lower in the neurodevelopmental scales. Dietary patterns of children at the age of 4 years may be influenced by multiple socio-demographic and lifestyle factors such as parental education, parity, day care attendance or hours watching television during the day.

7. References

Ambrosini, G. L. (2014). "Childhood dietary patterns and later obesity: a review of the evidence." Proc Nutr Soc **73**(1): 137-146.

Anand, K. J. and F. M. Scalzo (2000). "Can adverse neonatal experiences alter brain development and subsequent behavior?" Biol Neonate **77**(2): 69-82.

Anderson, J. W., B. M. Johnstone, et al. (1999). "Breast-feeding and cognitive development: a meta-analysis." Am J Clin Nutr **70**(4): 525-535.

Anjos, T., S. Altmae, et al. (2013). "Nutrition and neurodevelopment in children: focus on NUTRIMENTHE project." Eur J Nutr **52**(8): 1825-1842.

Aranceta, J., C. Perez-Rodrigo, et al. (2003). "Sociodemographic and lifestyle determinants of food patterns in Spanish children and adolescents: the enKid study." Eur J Clin Nutr **57 Suppl 1**: S40-44.

Arcangeli, T., B. Thilaganathan, et al. (2012). "Neurodevelopmental delay in small babies at term: a systematic review." Ultrasound Obstet Gynecol **40**(3): 267-275.

Bale, T. L., T. Z. Baram, et al. (2010). "Early life programming and neurodevelopmental disorders." Biol Psychiatry **68**(4): 314-319.

Banaschewski, T., K. Becker, et al. (2010). "Molecular genetics of attention-deficit/hyperactivity disorder: an overview." Eur Child Adolesc Psychiatry **19**(3): 237-257.

BBarker, D. J. (1995). "The Wellcome Foundation Lecture, 1994. The fetal origins of adult disease." Proc Biol Sci **262**(1363): 37-43.

Barkley, R. A. (2001). "The executive functions and self-regulation: an evolutionary neuropsychological perspective." Neuropsychol Rev **11**(1): 1-29.

Bayley, N. (2006). Bayley scales of infant and toddler development. San Antonio, TX, PsychCorp, Harcourt Assessment Inc.

Bell, L. K., R. K. Golley, et al. (2013). "Dietary patterns of Australian children aged 14 and 24 months, and associations with socio-demographic factors and adiposity." Eur J Clin Nutr **67**(6): 638-645.

Bellisle, F. (2004). "Effects of diet on behaviour and cognition in children." Br J Nutr **92 Suppl 2**: S227-232.

Birch, L., J. S. Savage, et al. (2007). "Influences on the Development of Children's Eating Behaviours: From Infancy to Adolescence." Can J Diet Pract Res **68**(1): s1-s56.

Brantsaeter, A. L., B. E. Birgisdottir, et al. (2011). "Maternal seafood consumption and infant birth weight, length and head circumference in the Norwegian Mother and Child Cohort Study." Br J Nutr **107**(3): 436-444.

Brown, A. S. and E. S. Susser (2008). "Prenatal nutritional deficiency and risk of adult schizophrenia." Schizophr Bull **34**(6): 1054-1063.

Bryan, J., S. Osendarp, et al. (2004). "Nutrients for cognitive development in school-aged children." Nutr Rev **62**(8): 295-306.

Campoy, C., M. V. Escolano-Margarit, et al. (2011). "Effects of prenatal fish-oil and 5-methyltetrahydrofolate supplementation on cognitive development of children at 6.5 y of age." Am J Clin Nutr **94**(6 Suppl): 1880S-1888S.

Carlson, S. E., J. Colombo, et al. (2013). "DHA supplementation and pregnancy outcomes." Am J Clin Nutr **97**(4): 808-815.

Carnell, S. and J. Wardle (2007). "Measuring behavioural susceptibility to obesity: validation of the child eating behaviour questionnaire." Appetite **48**(1): 104-113.

Caspi, A., H. Harrington, et al. (2003). "Children's behavioral styles at age 3 are linked to their adult personality traits at age 26." J Pers **71**(4): 495-513.

Chatzi, L., E. Papadopoulou, et al. (2012). "Effect of high doses of folic acid supplementation in early pregnancy on child neurodevelopment at 18 months of age: the mother-child cohort 'Rhea' study in Crete, Greece." Public Health Nutr **15**(9): 1728-1736.

Chiu, W. C., H. F. Liao, et al. (2011). "Duration of breast feeding and risk of developmental delay in Taiwanese children: a nationwide birth cohort study." Paediatr Perinat Epidemiol **25**(6): 519-527.

Cortese, S., M. Angriman, et al. (2008). "Attention-deficit/hyperactivity disorder (ADHD) and obesity: a systematic review of the literature." Crit Rev Food Sci Nutr **48**(6): 524-537.

Cortese, S., B. D. Bernardina, et al. (2007). "Attention-deficit/hyperactivity disorder (ADHD) and binge eating." Nutr Rev **65**(9): 404-411.

Cortese, S. and F. X. Castellanos (2014). "The relationship between ADHD and obesity: implications for therapy." Expert Rev Neurother **14**(5): 473-479.

Cortese, S. and B. Vincenzi (2012). "Obesity and ADHD: clinical and neurobiological implications." Curr Top Behav Neurosci **9**: 199-218.

Daniels, J. L., M. P. Longnecker, et al. (2004). "Fish intake during pregnancy and early cognitive development of offspring." Epidemiology **15**(4): 394-402.

Davis, C. (2010). "Attention-deficit/hyperactivity disorder: associations with overeating and obesity." Curr Psychiatry Rep **12**(5): 389-395.

Davis, C., R. D. Levitan, et al. (2006). "Associations among overeating, overweight, and attention deficit/hyperactivity disorder: a structural equation modelling approach." Eat Behav **7**(3): 266-274.

Dee, D. L., R. Li, et al. (2007). "Associations between breastfeeding practices and young children's language and motor skill development." Pediatrics **119 Suppl 1**: S92-98.

Demmelmair, H., J. von Rosen, et al. (2006). "Long-term consequences of early nutrition." Early Hum Dev **82**(8): 567-574.

Dempsey, A., J. Dyehouse, et al. (2011). "The relationship between executive function, AD/HD, overeating, and obesity." West J Nurs Res **33**(5): 609-629.

Der, G., G. D. Batty, et al. (2006). "Effect of breast feeding on intelligence in children: prospective study, sibling pairs analysis, and meta-analysis." BMJ **333**(7575): 945.

Docet, M. F., A. Larranaga, et al. (2012). "Attention deficit hyperactivity disorder increases the risk of having abnormal eating behaviours in obese adults." Eat Weight Disord **17**(2): e132-136.

Drane, D. L. and J. A. Logemann (2000). "A critical evaluation of the evidence on the association between type of infant feeding and cognitive development." Paediatr Perinat Epidemiol **14**(4): 349-356.

Drouillet, P., M. Kaminski, et al. (2009). "Association between maternal seafood consumption before pregnancy and fetal growth: evidence for an association in overweight women. The EDEN mother-child cohort." Paediatr Perinat Epidemiol **23**(1): 76-86.

Dziechciarz, P., A. Horvath, et al. (2010). "Effects of n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on neurodevelopment and visual function in children: a systematic review of randomized controlled trials." J Am Coll Nutr **29**(5): 443-454.

Ellenberg, J. H. (1994). "Selection bias in observational and experimental studies." Stat Med **13**(5-7): 557-567.

Escolano-Margarit, M. V., R. Ramos, et al. (2011). "Prenatal DHA status and neurological outcome in children at age 5.5 years are positively associated." J Nutr **141**(6): 1216-1223.

Fernandes, C., H. Grayton, et al. (2012). "Prenatal exposure to maternal obesity leads to hyperactivity in offspring." Mol Psychiatry **17**(12): 1159-1160.

Fleming, J. and L. Levy, Eds. (2002). Eating disorders in women with AD/HD.

Freeman, L. R., V. Haley-Zitlin, et al. (2014). "Damaging effects of a high-fat diet to the brain and cognition: a review of proposed mechanisms." Nutr Neurosci **17**(6): 241-251.

Froehlich, T. E., J. S. Anixt, et al. (2011). "Update on environmental risk factors for attention-deficit/hyperactivity disorder." Curr Psychiatry Rep **13**(5): 333-344.

Gale, C. R., C. N. Martyn, et al. (2009). "Dietary patterns in infancy and cognitive and neuropsychological function in childhood." J Child Psychol Psychiatry **50**(7): 816-823.

Gartner, L. M., J. Morton, et al. (2005). "Breastfeeding and the use of human milk." Pediatrics **115**(2): 496-506.

Gilliam, J. E. (1995). "Attention deficit/hyperactivity disorder test examiner's manual

" Austin, TX: Pro-Ed.

Glaser, C., J. Heinrich, et al. (2010). "Role of FADS1 and FADS2 polymorphisms in polyunsaturated fatty acid metabolism." Metabolism **59**(7): 993-999.

Gluckman, P. D., M. A. Hanson, et al. (2010). "A conceptual framework for the developmental origins of health and disease." J Dev Orig Health Dis **1**(1): 6-18.

Gluckman, P. D., M. A. Hanson, et al. (2008). "Effect of in utero and early-life conditions on adult health and disease." N Engl J Med **359**(1): 61-73.

Godfrey, K. M. and D. J. Barker (2000). "Fetal nutrition and adult disease." Am J Clin Nutr **71**(5 Suppl): 1344S-1352S.

Gould, J. F., L. G. Smithers, et al. (2013). "The effect of maternal omega-3 (n-3) LCPUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials." Am J Clin Nutr **97**(3): 531-544.

Govarts, E., M. Nieuwenhuijsen, et al. (2012). "Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts." Environ Health Perspect **120**(2): 162-170.

Granholm, A. C., H. A. Bimonte-Nelson, et al. (2008). "Effects of a saturated fat and high cholesterol diet on memory and hippocampal morphology in the middle-aged rat." J Alzheimers Dis **14**(2): 133-145.

Grantham-McGregor, S. and H. Baker-Henningham (2005). "Review of the evidence linking protein and energy to mental development." Public Health Nutr **8**(7A): 1191-1201.

Grieger, J. A., J. Scott, et al. (2011). "Dietary patterns and breast-feeding in Australian children." Public Health Nutr **14**(11): 1939-1947.

Guldner, L., C. Monfort, et al. (2007). "Maternal fish and shellfish intake and pregnancy outcomes: a prospective cohort study in Brittany, France." Environ Health **6**: 33.

Guxens, M., M. A. Mendez, et al. (2011). "Breastfeeding, long-chain polyunsaturated fatty acids in colostrum, and infant mental development." Pediatrics **128**(4): e880-889.

Hadders-Algra, M., H. Bouwstra, et al. (2007). "Prenatal and early postnatal fatty acid status and neurodevelopmental outcome." J Perinat Med **35 Suppl 1**: S28-34.

Halldorsson, T. I., H. M. Meltzer, et al. (2007). "Is high consumption of fatty fish during pregnancy a risk factor for fetal growth retardation? A study of 44,824 Danish pregnant women." Am J Epidemiol **166**(6): 687-696.

Halldorsson, T. I., I. Thorsdottir, et al. (2008). "Linking exposure to polychlorinated biphenyls with fatty fish consumption and reduced fetal growth among Danish pregnant women: a cause for concern?" Am J Epidemiol **168**(8): 958-965.

Hamosh, M. (2001). "Bioactive factors in human milk." Pediatr Clin North Am **48**(1): 69-86.

Hartmann, A. S., J. Czaja, et al. (2010). "Personality and psychopathology in children with and without loss of control over eating." Compr Psychiatry **51**(6): 572-578.

Hauner, H., D. Much, et al. (2012). "Effect of reducing the n-6:n-3 long-chain PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial." Am J Clin Nutr **95**(2): 383-394.

Heijmans, B. T., E. W. Tobi, et al. (2008). "Persistent epigenetic differences associated with prenatal exposure to famine in humans." Proc Natl Acad Sci U S A **105**(44): 17046-17049.

Heilskov Rytter, M. J., L. B. Andersen, et al. (2015). "Diet in the treatment of ADHD in children - a systematic review of the literature." Nord J Psychiatry **69**(1): 1-18.

Heppe, D. H., E. A. Steegers, et al. (2011). "Maternal fish consumption, fetal growth and the risks of neonatal complications: the Generation R Study." Br J Nutr **105**(6): 938-949.

Hibbeln, J. R., J. M. Davis, et al. (2007). "Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study." Lancet **369**(9561): 578-585.

Hoefer, C. and M. Hardy (1929). "Later development of breastfed and artificially fed infants: comparison of physical and mental growth." J Am Med Assoc **92**: 615-620.

Hoyland, A., L. Dye, et al. (2009). "A systematic review of the effect of breakfast on the cognitive performance of children and adolescents." Nutr Res Rev **22**(2): 220-243.

Innis, S. M. (2007). "Dietary (n-3) fatty acids and brain development." J Nutr **137**(4): 855-859.

Innis, S. M. (2007). "Human milk: maternal dietary lipids and infant development." Proc Nutr Soc **66**(3): 397-404.

Jacka, F. N., E. Ystrom, et al. (2013). "Maternal and early postnatal nutrition and mental health of offspring by age 5 years: a prospective cohort study." J Am Acad Child Adolesc Psychiatry **52**(10): 1038-1047.

Jensen, C. L. and A. Lapillonne (2009). "Docosahexaenoic acid and lactation." Prostaglandins Leukot Essent Fatty Acids **81**(2-3): 175-178.

Julvez, J., N. Ribas-Fito, et al. (2007). "Maternal smoking habits and cognitive development of children at age 4 years in a population-based birth cohort." Int J Epidemiol **36**(4): 825-832.

Kiefte-de Jong, J. C., J. H. de Vries, et al. (2013). "Socio-demographic and lifestyle determinants of 'Western-like' and 'Health conscious' dietary patterns in toddlers." Br J Nutr **109**(1): 137-147.

Koletzko, B., E. Lien, et al. (2008). "The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations." J Perinat Med **36**(1): 5-14.

Kramer, M. S., F. Aboud, et al. (2008). "Breastfeeding and child cognitive development: new evidence from a large randomized trial." Arch Gen Psychiatry **65**(5): 578-584.

Ladomenou, F., A. Kafatos, et al. (2007). "Risk factors related to intention to breastfeed, early weaning and suboptimal duration of breastfeeding." Acta Paediatr **96**(10): 1441-1444.

Liu, J. and A. Raine (2006). "The effect of childhood malnutrition on externalizing behavior." Curr Opin Pediatr **18**(5): 565-570.

Lozoff, B., J. Beard, et al. (2006). "Long-lasting neural and behavioral effects of iron deficiency in infancy." Nutr Rev **64**(5 Pt 2): S34-43; discussion S72-91.

Lucas, A. (2005). "Long-term programming effects of early nutrition -- implications for the preterm infant." J Perinatol **25 Suppl 2**: S2-6.

Maniadaki, K. and E. Kakouros, Eds. (2002). Translation and Adaptation of the Attention Deficit Hyperactivity Disorder Test (ADHDT; Giliam, 1995). The psychometric Instruments in Greece Ellinika Grammata.

Martinez, J. A., P. Cordero, et al. (2012). "Interplay of early-life nutritional programming on obesity, inflammation and epigenetic outcomes." Proc Nutr Soc **71**(2): 276-283.

McAfee, A. J., M. S. Mulhern, et al. (2012). "Intakes and adequacy of potentially important nutrients for cognitive development among 5-year-old children in the Seychelles Child Development and Nutrition Study." Public Health Nutr **15**(9): 1670-1677.

McCann, J. C. and B. N. Ames (2005). "Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals." Am J Clin Nutr **82**(2): 281-295.

McCarthy, D., Ed. (1972). Manual for the McCarthy scales of children's abilities. New York, Psychological Corp.

Mendez, M. A., E. Plana, et al. (2010). "Seafood consumption in pregnancy and infant size at birth: results from a prospective Spanish cohort." J Epidemiol Community Health **64**(3): 216-222.

Mendez, M. A., M. Torrent, et al. (2009). "Maternal fish and other seafood intakes during pregnancy and child neurodevelopment at age 4 years." Public Health Nutr **12**(10): 1702-1710.

Micali, N., E. Simonoff, et al. (2011). "Eating patterns in a population-based sample of children aged 5 to 7 years: association with psychopathology and parentally perceived impairment." J Dev Behav Pediatr **32**(8): 572-580.

Millichap, J. G. and M. M. Yee (2012). "The diet factor in attention-deficit/hyperactivity disorder." Pediatrics **129**(2): 330-337.

Morales, E., M. Bustamante, et al. (2011). "Genetic variants of the FADS gene cluster and ELOVL gene family, colostrums LC-PUFA levels, breastfeeding, and child cognition." PLoS One **6**(2): e17181.

Morrow-Tlucak, M., R. H. Haude, et al. (1988). "Breastfeeding and cognitive development in the first 2 years of life." Soc Sci Med **26**(6): 635-639.

Myers, G. J., P. W. Davidson, et al. (2003). "Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study." Lancet **361**(9370): 1686-1692.

Nazar, B. P., R. Suwwan, et al. (2014). "Influence of attention-deficit/hyperactivity disorder on binge eating behaviors and psychiatric comorbidity profile of obese women." Compr Psychiatry **55**(3): 572-578.

Niculescu, M. D. and D. S. Lupu (2009). "High fat diet-induced maternal obesity alters fetal hippocampal development." Int J Dev Neurosci **27**(7): 627-633.

North, K. and P. Emmett (2000). "Multivariate analysis of diet among three-year-old children and associations with socio-demographic characteristics. The Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) Study Team." Eur J Clin Nutr **54**(1): 73-80.

Northstone, K. and P. Emmett (2013). "The associations between feeding difficulties and behaviours and dietary patterns at 2 years of age: the ALSPAC cohort." Matern Child Nutr **9**(4): 533-542.

Northstone, K., C. Joinson, et al. (2012). "Are dietary patterns in childhood associated with IQ at 8 years of age? A population-based cohort study." J Epidemiol Community Health **66**(7): 624-628.

Oken, E., K. P. Kleinman, et al. (2004). "Associations of seafood and elongated n-3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort." Am J Epidemiol **160**(8): 774-783.

Oken, E., M. L. Osterdal, et al. (2008). "Associations of maternal fish intake during pregnancy and breastfeeding duration with attainment of developmental milestones in early childhood: a study from the Danish National Birth Cohort." Am J Clin Nutr **88**(3): 789-796.

Oken, E., J. S. Radesky, et al. (2008). "Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort." Am J Epidemiol **167**(10): 1171-1181.

Oken, E., R. O. Wright, et al. (2005). "Maternal fish consumption, hair mercury, and infant cognition in a U.S. Cohort." Environ Health Perspect **113**(10): 1376-1380.

Okubo, H., Y. Miyake, et al. (2014). "Dietary patterns in infancy and their associations with maternal socio-economic and lifestyle factors among 758 Japanese mother-child pairs: the Osaka Maternal and Child Health Study." Matern Child Nutr **10**(2): 213-225.

Olsen, S. F., P. Grandjean, et al. (1993). "Frequency of seafood intake in pregnancy as a determinant of birth weight: evidence for a dose dependent relationship." J Epidemiol Community Health **47**(6): 436-440.

Olsen, S. F., J. Olsen, et al. (1990). "Does fish consumption during pregnancy increase fetal growth? A study of the size of the newborn, placental weight and gestational age in relation to fish consumption during pregnancy." Int J Epidemiol **19**(4): 971-977.

Olsen, S. F., M. L. Osterdal, et al. (2006). "Duration of pregnancy in relation to seafood intake during early and mid pregnancy: prospective cohort." Eur J Epidemiol **21**(10): 749-758.

Olsen, S. F. and N. J. Secher (2002). "Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study." BMJ **324**(7335): 447.

Picciano, M. F. (2001). "Nutrient composition of human milk." Pediatr Clin North Am **48**(1): 53-67.

Prado, E. L. and K. G. Dewey (2014). "Nutrition and brain development in early life." Nutr Rev **72**(4): 267-284.

Ptacek, R., H. Kuzelova, et al. (2014). "Disruptive patterns of eating behaviors and associated lifestyles in males with ADHD." Med Sci Monit **20**: 608-613.

Qawasmi, A., A. Landeros-Weisenberger, et al. (2013). "Meta-analysis of LCPUFA supplementation of infant formula and visual acuity." Pediatrics **131**(1): e262-272.

Qawasmi, A., A. Landeros-Weisenberger, et al. (2012). "Meta-analysis of long-chain polyunsaturated fatty acid supplementation of formula and infant cognition." Pediatrics **129**(6): 1141-1149.

Reinblatt, S. P., J. M. Leoutsakos, et al. (2014). "Association between binge eating and attention-deficit/hyperactivity disorder in two pediatric community mental health clinics." Int J Eat Disord.

Robinson, S., L. Marriott, et al. (2007). "Dietary patterns in infancy: the importance of maternal and family influences on feeding practice." Br J Nutr **98**(5): 1029-1037.

Roth, C., P. Magnus, et al. (2011). "Folic acid supplements in pregnancy and severe language delay in children." JAMA **306**(14): 1566-1573.

Rylander, L., U. Stromberg, et al. (2000). "Lowered birth weight among infants born to women with a high intake of fish contaminated with persistent organochlorine compounds." Chemosphere **40**(9-11): 1255-1262.

Sacker, A., M. A. Quigley, et al. (2006). "Breastfeeding and developmental delay: findings from the millennium cohort study." Pediatrics **118**(3): e682-689.

Salvig, J. D. and R. F. Lamont (2011). "Evidence regarding an effect of marine n-3 fatty acids on preterm birth: a systematic review and meta-analysis." Acta Obstet Gynecol Scand **90**(8): 825-838.

Schulze, M. B., K. Hoffmann, et al. (2003). "An approach to construct simplified measures of dietary patterns from exploratory factor analysis." Br J Nutr **89**(3): 409-419.

Silva, D., L. Colvin, et al. (2014). "Environmental risk factors by gender associated with attention-deficit/hyperactivity disorder." Pediatrics **133**(1): e14-22.

Simmer, K., S. K. Patole, et al. (2011). "Long-chain polyunsaturated fatty acid supplementation in infants born at term." Cochrane Database Syst Rev(12): CD000376.

Smith, A. D., P. M. Emmett, et al. (2013). "Dietary patterns obtained through principal components analysis: the effect of input variable quantification." Br J Nutr **109**(10): 1881-1891.

Smithers, L. G., L. Brazionis, et al. (2012). "Associations between dietary patterns at 6 and 15 months of age and sociodemographic factors." Eur J Clin Nutr **66**(6): 658-666.

Smithers, L. G., R. K. Golley, et al. (2011). "Characterizing whole diets of young children from developed countries and the association between diet and health: a systematic review." Nutr Rev **69**(8): 449-467.

Smithers, L. G., R. K. Golley, et al. (2012). "Dietary patterns at 6, 15 and 24 months of age are associated with IQ at 8 years of age." Eur J Epidemiol **27**(7): 525-535.

Sonneville, K. R., J. P. Calzo, et al. (2015). "Childhood hyperactivity/inattention and eating disturbances predict binge eating in adolescence." Psychol Med: 1-10.

Starling, P., K. Charlton, et al. (2015). "Fish intake during pregnancy and foetal neurodevelopment--a systematic review of the evidence." Nutrients **7**(3): 2001-2014.

Steenweg-de Graaff, J., H. Tiemeier, et al. (2014). "Maternal dietary patterns during pregnancy and child internalising and externalising problems. The Generation R Study." Clin Nutr **33**(1): 115-121.

Steer, C. D., G. Davey Smith, et al. (2010). "FADS2 polymorphisms modify the effect of breastfeeding on child IQ." PLoS One **5**(7): e11570.

Suren, P., C. Roth, et al. (2013). "Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children." JAMA **309**(6): 570-577.

Swanson, S. A., S. J. Crow, et al. (2011). "Prevalence and correlates of eating disorders in adolescents. Results from the national comorbidity survey replication adolescent supplement." Arch Gen Psychiatry **68**(7): 714-723.

Symonds, M. E., S. P. Sebert, et al. (2009). "Nutritional programming of the metabolic syndrome." Nat Rev Endocrinol **5**(11): 604-610.

Szajewska, H., A. Horvath, et al. (2006). "Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials." Am J Clin Nutr **83**(6): 1337-1344.

Szajewska, H., M. Ruszczynski, et al. (2010). "Effects of iron supplementation in nonanemic pregnant women, infants, and young children on the mental performance and psychomotor development of children: a systematic review of randomized controlled trials." Am J Clin Nutr **91**(6): 1684-1690.

Theodore, R. J., J. Thompson, et al. (2009). "Dietary patterns and intelligence in early and middle childhood." Intelligence **37**: 506-513.

Theodore, R. J., J. Thompson, et al. (2006). "Dietary patterns of New Zealand European preschool children." N Z Med J **119**(1235): U1998.

Thomas, R., S. Sanders, et al. (2015). "Prevalence of Attention-Deficit/Hyperactivity Disorder: A Systematic Review and Meta-analysis." Pediatrics.

Thorsdottir, I., B. E. Birgisdottir, et al. (2004). "Association of fish and fish liver oil intake in pregnancy with infant size at birth among women of normal weight before pregnancy in a fishing community." Am J Epidemiol **160**(5): 460-465.

Uauy, R., D. R. Hoffman, et al. (2003). "Term infant studies of DHA and ARA supplementation on neurodevelopment: results of randomized controlled trials." J Pediatr **143**(4 Suppl): S17-25.

Vassilaki, M., L. Chatzi, et al. (2014). "Smoking and caesarean deliveries: major negative predictors for breastfeeding in the mother-child cohort in Crete, Greece (Rhea study)." Matern Child Nutr **10**(3): 335-346.

Ventura, A. K. and J. Worobey (2013). "Early influences on the development of food preferences." Curr Biol **23**(9): R401-408.

Viana, V., S. Sinde, et al. (2008). "Children's Eating Behaviour Questionnaire: associations with BMI in Portuguese children." Br J Nutr **100**(2): 445-450.

Walfisch, A., C. Sermer, et al. (2013). "Breast milk and cognitive development--the role of confounders: a systematic review." BMJ Open **3**(8): e003259.

Wall, C. R., J. M. Thompson, et al. (2013). "Dietary patterns of children at 3.5 and 7 years of age: a New Zealand birth cohort study." Acta Paediatr **102**(2): 137-142.

Wardle, J., C. A. Guthrie, et al. (2001). "Development of the Children's Eating Behaviour Questionnaire." J Child Psychol Psychiatry **42**(7): 963-970.

Wild, C. P. (2005). "Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology." Cancer Epidemiol Biomarkers Prev **14**(8): 1847-1850.

Xiong, T., F. Gonzalez, et al. (2012). "An overview of risk factors for poor neurodevelopmental outcome associated with prematurity." World J Pediatr **8**(4): 293-300.

Xu, M. Q., W. S. Sun, et al. (2009). "Prenatal malnutrition and adult schizophrenia: further evidence from the 1959-1961 Chinese famine." Schizophr Bull **35**(3): 568-576.

Ystrom, E., S. Niegel, et al. (2009). "The impact of maternal negative affectivity on dietary patterns of 18-month-old children in the Norwegian Mother and Child Cohort Study." Matern Child Nutr **5**(3): 234-242.

Zhou, S. J., R. A. Gibson, et al. (2006). "Effect of iron supplementation during pregnancy on the intelligence quotient and behavior of children at 4 y of age: long-term follow-up of a randomized controlled trial." Am J Clin Nutr **83**(5): 1112-1117.

Zimmermann, M. B. (2007). "The adverse effects of mild-to-moderate iodine deficiency during pregnancy and childhood: a review." Thyroid **17**(9): 829-835.

Zimmermann, M. B. (2009). "Iodine deficiency in pregnancy and the effects of maternal iodine supplementation on the offspring: a review." Am J Clin Nutr **89**(2): 668S-672S.