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Dietary Patterns in Childhood

Relation to Growth, Obesity & Parental Characteristics

LOUISE BELTOFT BORUP ANDERSEN · PHD THESIS 2014

Dietary Patterns in Childhood

Relation to Growth, Obesity & Parental Characteristics

PhD thesis by

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Preface and Acknowledgements

This PhD thesis was based on the CHANCE consortium initiative which aims to increase knowledge and use of multivariate statistical and chemometric methods in a wide range of research fields at the University of Copenhagen. This was matched with the objective of obtaining a better understanding of the complex relations between growth, obesity and diet of children in the research group of Paediatric and International Nutrition. To fulfil this purpose I was supported by four competent supervisors: Christian Mølgaard and Kim F. Michaelsen, as experts within child nutrition, Rasmus Bro, as the chemometric expert, and Christian B. Pipper, representing the expertise of classic statistics. It has been challenging and interesting to be part of this interdisciplinary group. Thanks to all of you for the pedagogical explanations, the appropriate scepticism and fruitful discussions.

I will also like to thank all my colleagues for their praiseworthy cooperation. I appreciated all the hours together examining the children and the supporting comments after many hours in front of this document. Thanks to colleagues and co-authors of the papers for reading my thesis and paper-drafts respectively and supply me with useful feedback. I am grateful for the opportunity I got to work with data from three comprehensive human studies in this PhD. It should be emphasised that a long list of colleges has done a huge amount of work to collect all these data. Finally, I would like to thank all the children and parents participating in these studies.

List of papers

This thesis is based on the following four papers, referred to in the text as Paper I-IV.
Paper I: Maternal obesity and offspring dietary patterns at 9 months of age.
(*Current status Oct. 2014: Accepted for publication in The European Journal of Clinical Nutrition*)
Paper II: Indicators of dietary patterns in Danish infants at 9 month of age.
(*Current status Oct. 2014: Under revision before submission to a scientific journal*)
Paper III: Development of dietary patterns spanning infancy and toddlerhood: relation to body size, composition and metabolic risk markers at three years.
(*Current status Oct. 2014: Resubmitted to Maternal & Child Nutrition*)
Paper IV: The effects of water and dairy drinks on dietary patterns in overweight adolescents.
(*Current status Oct. 2014: Submitted to Public Health Nutrition*)

Data sources

Data from three human studies were used in this thesis:

● Two observational cohort studies, **SKOT I** and **SKOT II** (Danish abbreviation of: Småbørns kost og trivsel) following children from they were 9 to 36 mo of age with diverse inclusion criteria as the only main difference between the cohorts. SKOT I had no restriction on maternal BMI, while maternal pre-pregnancy BMI should be above 30kg/m^2 in SKOT II. In previous publications SKOT I was mentioned as SKOT, but to distinguish it from SKOT II it is from now named SKOT I.

● One intervention study **MoMS** (Milk components and Metabolic Syndrome; the effect of milk, whey and casein on biomarkers of metabolic syndrome in overweight children), where moderately obese adolescents (12-15 years) were randomised to either a dairy or water test drink for 12 weeks.

List of Abbreviations

ALSPAC	Avon Longitudinal Study of Parents and Children
BMI	Body Mass Index
CYCF	Complementary and Young Child Feeding
DXA	Dural-energy X-ray Absorptiometry
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology, and Nutrition
FFMI	Fat Free Mass Index
FFQ	Food Frequency Questionnaire
FMI	Fat Mass Index
HDL	High Density Lipoprotein
IGF-I	Insulin-like Growth Factor 1
IGFBP3	Insulin-like Growth Factor Binding Protein 3
IQR	Interquartile Range
LDL	Low Density Lipoprotein
mo	Month(s)
MOMS	Milk components and Metabolic Syndrome
MUFA	Monounsaturated Fatty Acids
PC	Principal Component
PCA	Principal Component Analysis
PLS	Partial Least Squares Regression
PUFA	Polyunsaturated Fatty Acids
RRR	Reduced Rank Regression
SFA	Saturated Fatty Acids
SKOT	Småbørns Kost og Trivsel (Danish abbreviation of small children's diet and wellbeing)
SWS	Southampton Women's Survey
TOP	Treatment of Obese Pregnant Women
WHO	World Health Organisation
wk	week

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English summary

Background

A healthy diet is essential for healthy growth and development during childhood and may prevent obesity, diabetes, and cardiovascular diseases throughout life. Traditionally diet has been investigated as single nutrients. However, people do not eat one single nutrient and they do not even eat one single food. People consume meals and these meals vary during a day, over a year and during a life course. Hereby it could be advantageous to investigate dietary patterns representing the whole diet as patterns might be better markers of growth and health compared to single nutrients. However, little is known about the development of dietary patterns in childhood both in relation to possible indicators and obesity related outcomes.

Aim

The overall aim of this PhD thesis was to make exploratory analyses of dietary patterns in childhood and to investigate associations to possible indicators and outcomes related to growth and obesity.

Subjects and methods

Data from three human studies were used; two observational cohort studies SKOT I (no restriction on maternal BMI) and SKOT II (maternal pre-pregnancy BMI>30 kg/m^2) following children at 9, 18 and 36 mo of age with similar design of data collection, and one intervention study, MoMS, where moderately obese adolescents (age-and sex-adjusted BMI corresponding to an adult BMI>25kg/m^2) were randomised to either a dairy or water test drink for 12 weeks. Dietary patterns were identified by principal component analysis (PCA) based on food diaries with portion size estimation in all three studies.

Results

The diet at 9 mo of age in SKOT I and SKOT II were compared in ***Paper I***. This showed that the dietary pattern FAMILY FOOD did not differ between cohorts, while infants in SKOT I had higher scores in a HEALTH-CONSCIOUS FOOD pattern than infants in SKOT II. This difference in dietary pattern was reflected at the food level e.g. as lower median intake of the food group *Wheat-BreadNoWholegrain*, plus higher intake of the food groups *Fruit*, *Vegetable*, and *BreastMilk* in SKOT I. Moreover, SKOT II had a higher percentage of energy intake from protein than SKOT I. These results indicated that infants in SKOT II, characterised by obese mothers, had a lower quality of complementary feeding than infants in SKOT I, mainly characterised by non-obese mothers of a higher social class. Maternal obesity and other possible indicators of dietary patterns at 9 mo of age, in a pooled sample of SKOT I and SKOT II, were further investigated in ***Paper II***. This showed e.g. that infants, who were younger at diet registration, who had higher BMI z-scores at 9 mo and infants with immigrant/descendant parents had lower scores in the FAMILY FOOD pattern. A lower score indicated a higher intake of baby food because this FAMILY FOOD pattern shows the transi-

tion from baby food towards the family's food. Moreover, a higher maternal BMI and a greater number of children in the household were associated with a lower score at the HEALTH-CONSCIOUS FOOD pattern, which suggests a less healthy diet. In ***Paper III*** the development in dietary patterns between 9, 18 and 36 mo was investigated based on the SKOT I cohort. This analysis indicated that the dietary patterns were tracking over time for a relatively large group of children. Furthermore, the development in the dietary patterns TRANSITION FOOD and HEALTHY FOOD were associated with obesity related outcomes at 36 mo of age. The TRANSITION FOOD pattern represents a transition from baby foods to family foods and HEALTHY FOOD is a gradient form less healthy to more healthy foods. Especially groups of children with lower adherence to the TRANSITION FOOD or HEALTHY FOOD pattern at two or all three ages had higher BMI z-scores, higher fat mass indices, and higher levels of metabolic risk markers and hence, could represent undesirable development of dietary patterns for toddlers. Finally, ***Paper IV***, exploring the effect of water or dairy test drinks on the dietary intake, showed that consumption of extra fluid seems to have a favourable influence on the rest of the diet, as a decrease in the dietary pattern CONVENIENCE FOOD was observed during both the water and dairy intervention. This included a decreased intake of the food group *SugaryDrink*. Moreover, low energy content in the extra fluid seemed favourable when considering the total energy intake in these overweight adolescents.

Conclusion

PCA seems to be suitable to grasp some of the complexity in child nutrition both in observational and intervention designs as well as for investigating development of dietary patterns over time. Findings based on dietary patterns are supported by including multiple dietary levels containing foods, nutrients, and energy intake. The explorative analysis of indicators of dietary patterns suggests that especially families with maternal obesity and multiple children, and possibly immigrant/descendant families had infants with less favourable dietary patterns which might indicate that they should be supported by the health care system to establish healthy dietary patterns for their infants. The relevance of early and sustained health promotion is indicated by the finding of tracking for some infants and changes in the adherence to dietary patterns for others during early childhood, and by the association of dietary patterns with obesity related outcomes already in toddlerhood. In relation to the specific content of dietary health promotions the findings in overweight adolescents support the recommendation stating that plain water should be promoted as the main source of fluid for children *instead* of sugar-sweetened beverages.

Danish summary (Dansk resumé)

Baggrund

Den rette kost er essentiel for sund vækst og udvikling igennem barndommen og kan formodentlig medvirke til at forebygge fedme, diabetes og hjerte-kar sygdomme igennem livet. Traditionelt set er kosten blevet undersøgt i forhold til ét næringsstof af gangen, men folk spiser ikke ét næringsstof af gange, de spiser ikke engang én fødevare af gangen. Folk spiser i stedet måltider og disse måltider varierer over en dag og gennem et livsforløb. Det kan derfor være en fordel at undersøge kostmønstre, der bredt dækker hele kosten, da disse kostmønstre måske er stærkere markører for vækst og sundhed sammenlignet med enkelte næringsstoffer. På nuværende tidspunkt har vi dog begrænset viden om udviklingen i kostmønstre igennem barndommen, om hvilke indikatorer der er for kostmønstrene, og om kostmønstrenes association med sundhedsmål relateret til vækst og fedme.

Formål

Det overordnede formål med denne ph.d. var at udføre eksplorative analyser af kostmønstre i barndommen og undersøge associationer med mulige indikatorer og sundhedsmål relateret til vækst og fedme.

Deltagere og metoder

Der er anvendt data fra tre humane studier: de to observationelle kohorte studier SKOT I (ingen krav til mors BMI) og SKOT II (krav: mors før-graviditets BMI>30kg/m^2), der har ens design og begge følger børn ved 9, 18 og 36 mdrs. alderen samt interventions studiet MoMS, hvor overvægtige unge (alders og køns justeret BMI ~voksent BMI>25kg/m^2) blev randomiseret til en mejeridrik eller vand i 12 uger. I de tre studier blev kostmønstrene identificeret vha. principal komponent analyse (PCA) baseret på kostdagbøger med estimeret portionsstørrelser.

Resultater

Kosten ved 9-mdrs. alderen i SKOT I og II blev sammenlignet i ***Artikel I***. Denne sammenligning viste at kostmønstret FAMILIE MAD ikke var forskelligt mellem kohorterne, mens børn i SKOT I havde en højere score på mønstret SUNDHEDSBEVIDST MAD end børn i SKOT II. Denne forskel i kostmønsteret blev afspejlet på fødevare niveau, da fx det mediane indtag af fødevaregruppen *HvedeBrødEjFuldkorn* var lavere og indtag af *Frugt*, *Grøntsager*, og *ModerMælk* var højere for børnene i SKOT I. Endvidere indtog SKOT II børnene en højere procentdel af deres energi fra protein end SKOT I børnene. Disse resultater indikerer at kvaliteten af overgangskosten i SKOT II, der havde svært overvægtige mødre, var lavere end i SKOT I, der hovedsageligt havde ikke-overvægtige mødre. At have en svært overvægtig mor og andre potentielle indikatorer for kostmønstrene ved 9 mdrs. alderen blev undersøgt nærmere i ***Artikel II*** i en samlet gruppe af børn fra SKOT I og SKOT II. Denne analyse viste bl.a. at børn med lavere alder på kostregistreringstidspunktet, højere BMI z-scores ved 9 mdr. og børn af immigranter/efterkommere, havde en lavere score på mønstret FAMILIE MAD. Denne lavere score indikerede et højere indtag af baby mad, da FAMI-

LIE MAD mønsteret dækker overgangen fra baby mad til mad, der begynder at ligne resten af familiens mad. Endvidere var et højere BMI hos mor og flere børn i husstanden associeret med en lavere score på mønstret SUNDHEDSBEVIDST MAD, som antyder en mindre sund kost. I ***Artikel III*** blev udviklingen i kostmønstrene mellem 9, 18 og 36 mdr. undersøgt baseret på SKOT I kohorten. Denne analyse antydede, at kostmønstrene trackede over tid for en relativt stor gruppe af børn. Desuden var udviklingen over tid i kostmønstrene OVERGANGS KOST og SUND MAD associeret med sundhedsmål relateret til fedme ved 36 mdrs. alderen. Kostmønstret OVERGANGS KOST repræsenterer overgangen fra baby mad til familiens mad og SUND MAD er en gradient fra mindre sund mad til sund mad. Især havde børn, med lavere score på OVERGANGS KOST eller SUND MAD mønstret, ved to eller alle tre aldre, højere BMI z-scores, højere fedt masse indekser og højere niveauer af metaboliske risikomarkører og kan derfor eventuelt repræsentere uønskede udviklingsforløb i kostmønstre for småbørn. Slutteligt udforskede ***Artikel IV*** effekten af vand- og mejeritestdrikke på unges øvrige kostindtag, hvilket viste, at et indtag af ekstra væske lader til at have en favorabel indflydelse på resten af kosten. Der blev, både i vandgruppen og i mejerigruppen, observeret en nedgang i mønstret NEMT OG BEKVEMT MAD inklusiv et lavere indtag fra fødevaregruppen *SukkersødeDrikke* under interventionen. I denne gruppe af overvægtige unge lod det desuden til at være favorabelt i forhold til det totale energiindtag, at den ekstra væske havde et lavt energiindhold.

Konklusion

PCA lader til at være en anvendelig metode til at favne noget af kompleksiteten i kosten hos børn både i observationelle studier, interventions studier såvel som til at undersøge udviklingen i kostmønstre over tid. Resultaterne på kostmønsterniveau kan med fordel underbygges ved at inkludere kostniveauerne energi, næringsstoffer og fødevaregrupper. De eksplorative analyser af indikatorer for kostmønstre antydede at familier med en svært overvægtig mor og flere børn samt måske immigrantfamilier havde småbørn med mindre favorable kostmønstre, hvilket eventuelt kan indikere at disse familier bør støttes i at introducere sunde kostmønstre for deres småbørn. Relevansen af tidlig og vedvarende sundhedsfremme indikeres af den viste tracking i kostmønstre for nogle børn og ændring i kostmønstre for andre gennem den tidlige barndom samt associationen mellem kostmønstre og markører for sundhedsmål allerede i småbørnsalderen. I forhold til det specifikke indhold af sundhedsfremmende tiltag indenfor kostområdet støtter undersøgelsen af kosten hos overvægtige unge den officielle anbefaling om at promovere vandindtag som den vigtigste kilde til væske *i stedet for* sukker-sødet drikke.

1 Introduction

Infancy, toddlerhood, and adolescence, are particularly interesting periods to study both because of the large changes in dietary intake and because these periods are characterised by rapid growth and development which is particularly demanding in relation to diet (1).

Many terms are applied for the first 18 years of life. In this thesis the flowering division is used:

Infancy:	Birth to 1 year of life
Toddlerhood:	1 to 3 years of life
Pre-schoolers:	3 to 5 years of life
Adolescence:	10 to 18 years of life
Childhood:	Birth to 18 years of life, but occasionally only 4 to 10 years of life

1.1 General trends of diet in childhood

The first food for a healthy new-born infant is breast milk or infant formula. The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) recommend exclusive breastfeeding for about 6 mo and partly breastfeeding as long as mutually desired by mother and child alongside complementary feeding (2). The official recommendation in Denmark is exclusive breastfeeding until about 6 mo and in Denmark nearly all mothers try to breastfeed their new-born infant and 50 % of the infants are still exclusively breastfed at 4 mo of age (3). If breastfeeding is not possible infant formula should be the alternative. Formulas are produced to mimic the qualities of breast milk by imitating the content. However, breast milk is recommended rather than formula because the content of formula currently does not perfectly match the content of breast milk e.g. in relation to protein content which may influence the growth of the child (2). The kidney, gut and motor skills of the new-born infant are only able to handle a milk based diet. At 4 to 6 mo of age the body functions of the infant are more mature and now able to handle other types of food than milk and the complementary feeding period starts. At that time the nutrient contents of energy, protein, iron, zinc, vitamin A and D in breast milk are also no longer sufficient to support the requirements of the child (4). Complementary feeding is recommended to be introduced in Denmark at about 6 mo of age and not before 4 mo of age (3). Complementary food is defined as all solids and liquid foods other than breast milk and formula introduced in early life (4) and should contain a mix of gruel, porridge, and mashed fruit, vegetable, fish and meat. Gradually the texture of the food should be lumpier and at around 12 mo of age the child has changed from complementary feeding to eat the family´s diet and only minor adaptions of the family's diet during toddlerhood is necessary to meet the special requirements of the child.

The intake of the family´s diet will continue throughout childhood and adulthood with minor or major changes e.g. when an adolescent begins to have more influence on own meals. Food is used to show identity and breakaway from parents especially for adolescents where dietary habits might differ both from childhood and later periods of life (5). Results from a national Danish survey showed that the diets of adolescents were less in agreement with the official recommendations than the diet of younger children because of fewer vegetables, less whole grain and a higher fast food intake. Moreover, intake of sugar-sweetened beverages is higher during childhood than adulthood (6). Specific official nutrition recommendations in Denmark exist for infants and toddlers and a few special recommendations apply to children up to about 10 years, while the recommendations for adolescents are the same as for adults (7-9).

1.2 Different approaches for investigating diet in childhood

Dietary intake can be evaluated at different levels and the traditional way to investigate diet is one nutrient or food at a time. The nutrient research holds valuable opportunities to dig into possible mechanistic relations between diet, bodily functions and health and is progressed by technology and advanced research methods. However, nutrition is often more complex and multifactorial than one nutrient causing or preventing a specific disease. This has given rise to a more holistic approach within nutrition research during the last approximately 30 years looking at whole dietary patterns (10). This holistic view is based on the fact that people do not eat nutrients, they eat foods and people do not eat one food at the time, they consume meals several times during a day and throughout life. Foods and nutrients are consumed in combinations and are therefore highly correlated and the effect is difficult to separate. Moreover, there may be a joint effect of the combination of nutrients where the synergetic effect is bigger than the sum of each single contribution (11).

Hereby the main reason for the focus on the whole dietary pattern approach is the hypothesis that the whole dietary pattern is more predictive of disease risk than a single nutrient or food and therefore provides a more comprehensive approach to prevention and treatment of diseases (11). It has been shown that dietary patterns in adults are associated with health outcomes like type 2 diabetes mellitus, hypertension, coronary heart disease, and metabolic syndrome, though with varying consistency (12-16). Nevertheless, few studies have attempted to verify the hypothesis that dietary patterns are stronger predictors than single nutrients or foods by direct comparison. In two reviews by Kant it is concluded that overall dietary patterns might be stronger (17) or equally strong (18) predictors of health outcomes compared to single nutrients or foods. However, this was concluded by comparing results from different papers and thereby on a rather limited basis with few data sources and fairly narrow patterns.

The new edition of the “Nordic Nutrition Recommendations 2012” for health professionals also acknowledge the holistic view by a stronger focus now, than in earlier editions, on dietary patterns and food based recommendations in addition to the nutrient recommendations. This is to support public guidelines because food based rather than nutrient based guidelines are easier to convert to practice in the public (19). Despite the presumable advantages of investigating dietary patterns it might not be optimal to solely focus on dietary patterns. Synergetic findings might appear when including multiple dietary levels such as energy, nutrients, foods, and dietary patterns to characterise dietary intake and this will ease the comparison with different nutrition recommendations and previous research at the nutrient and foods level.

1.3 Dietary patterns in childhood

1.3.1 Methods used in dietary pattern research

Dietary patterns are investigated by two different approaches: either by an theoretically-driven method defining a dietary index before the study from predefined criteria and previous empirical knowledge (the *a priori* approach) or by a data-driven method after data collection (the *a posteriori* approach) (11). The index approach is a scoring system based on current nutrition evidence in relation to a certain health outcome or reflects specific dietary guidelines (17;20) and it seeks to determine persons' adherence to a predefined presumed ideal diet, whereas the data-driven approach is used to define persons' actual diet (21). The data-driven approach is favourable when aiming at an explorative investigation of dietary patterns to generate new hypotheses, while the index approach is used for hypothesis-testing and is usable when having a thorough knowledge of associations between specific diets and health outcomes (11;12). The data-driven approach is applied in all four papers included in this PhD thesis and most of the referred studies because the purpose was to make exploratory analysis of dietary patterns that are practiced in Danish children. The data-driven approach will be elaborated in more details below.

The term "dietary patterns" has been used with diverse meanings in the literature. Sometimes dietary patterns refer to different meal structures or intake of a list of different nutrients or foods (18). Dietary patterns can also specifically refer to the index and data-driven approaches and in other papers it strictly refers to dietary patterns only identified by a data-driven approach. In this thesis, the term "dietary patterns" will primarily be used when the analysis more or less covers the whole diet with focus on the data-driven approach, and will not include intake of single nutrients, foods and structures of meals. Dietary patterns identified in this thesis are presented in capital letters and the specific food groups contributing to these patterns are presented in italic with first letter in capital.

1.3.1.1 Data –driven approach

Using a data-driven approach for identifying dietary patterns means that the dietary patterns appear from the data by multivariate techniques based on pattern recognition after data collection. These multivariate techniques could either be principal component analysis (PCA), factor analysis, cluster analysis, reduced rank regression (RRR) or partial least squares regression (PLS). PCA is the method most often used and is also the one used in this thesis.

Principal Component Analysis

PCA is the cornerstone method in the field called chemometrics. Chemometrics can be defined as "application and development of mathematical and statistical methods to extract maximum information from chemical data"(22). In the last couple of decades the discipline has been used in many other fields outside chemistry e.g. within nutrition research. The target of PCA is to simplify and reduce the sources of information in the sense of number of variables of the original dataset, while preserving the maximum amount of information. A few new summary variables named principal components are defined with the PCA method based on all the original variables. These principal components represent latent patterns in the data, which might not be possible to measure directly since these patterns only occur as a consequence of correlations between all the original variables.

Hereby the PCA makes a model of one or more hidden phenomena and when the original variables are food intake the hidden phenomena are named dietary patterns. One of the advantages of the PCA is that these dietary patterns can be visualised graphically (23).

To exemplify, we assume that we have collected information on the intake of three different foods (*Soft drinks*, *Cheese* and *Vegetables*) from 24 children and we would like to identify dietary patterns in this sample. First, we show data in a three dimensional coordinate system (**Figure 1**) with the original axis *Soft drinks*, *Cheese* and *Vegetables*. Information in a three dimension coordinate system can be difficult to handle, and with e.g. 20 different food axes it becomes impossible to visualise and interpret. Therefore the PCA method is used to identify patterns in data based on combinations of the original food axes and thereby the number of dimensions in data is decreased. The first new dimension or dietary pattern is placed in the direction with most variation between children in the intake of the three original foods and is named principal component (PC) 1. The second dietary pattern is placed in the direction with second most variation between children and orthogonal to PC1 and is named PC2. By this we now have a two dimensional, instead of a three dimensional coordinate system. Each child is given a new value for each new dietary pattern based on the orthogonal projection on to each new PC plane (broken line at **Figure 1**). This new value is named the score value. Each of the original food axes is also given a new value indicating their location in the new coordinate system. This value is named the loading value (23).

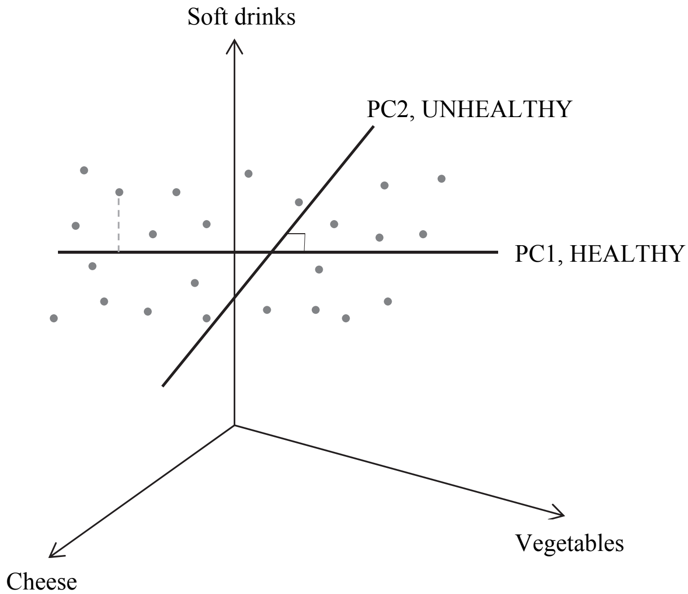

Figure 1 Concept of identifying latent dietary patterns by PCA
Coordinate system showing intake of three foods (original variables). Black bold lines indicate new dietary patterns (Principal components, PC). ● A child. ------- Projection of a child at the HEALTHY dietary pattern to define the score value at this pattern for this child (Figure inspired by Esbensen *et al.* (23)).

Scores can be visually mapped in a score plot (**Figure 2**) while loadings can be visualised in a loading plot (**Figure 3**). The score plot shows *how* the participants are distributed in relation to the dietary patterns. Participants close to each other have similar patterns. The loading plot shows how much each original food variable contributes to each dietary pattern and thereby the loading plot explains *why* the participants are placed where they are in the score plot. Foods placed far from the centre are the most important foods in defining the dietary pattern. Foods placed close to each other are positively correlated and foods placed at each end of a dietary pattern are inversely correlated. For interpretation it can be an advantage to show both scores and loadings in the same plot, a so-called biplot (**Figure 4**). Children placed close to a certain food indicate that they have a high intake of this food and a lower intake of foods far away (23).

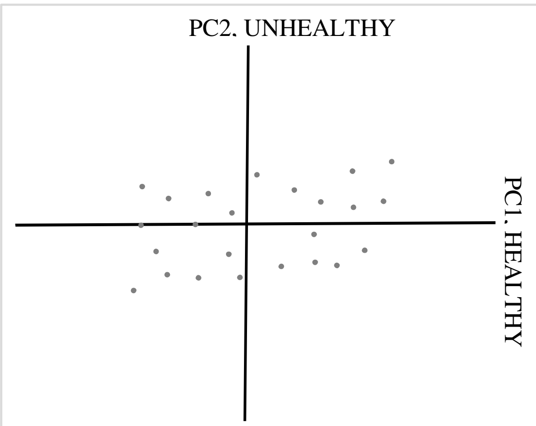

Figure 2 PCA score plot: "Map of children"
● Child (Figure inspired by Esbensen *et al.*(23)).

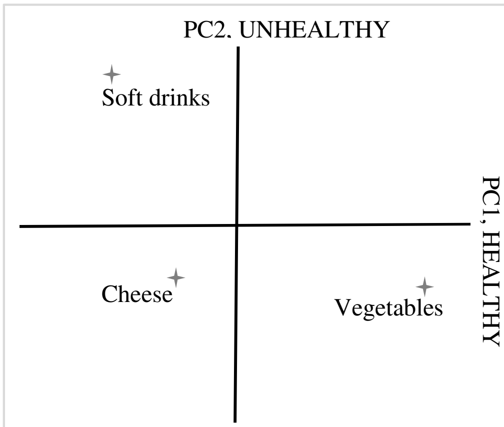

Figure 3 PCA loading plot: "Map of foods"
✦Food (Figure inspired by Esbensen *et al.*(23)).

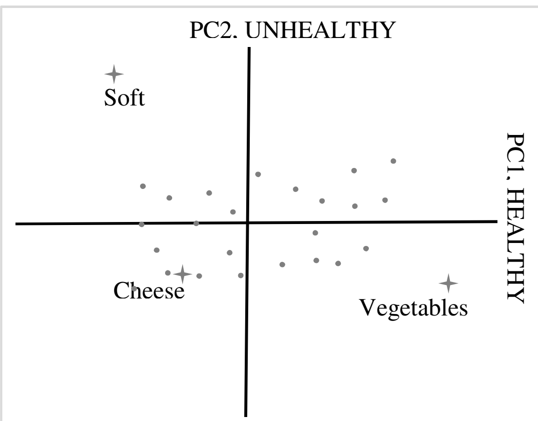

Figure 4 PCA Bi plot: "Map of foods and children"
✦Food, ● Child (Figure inspired by Esbensen *et al.* (23)).

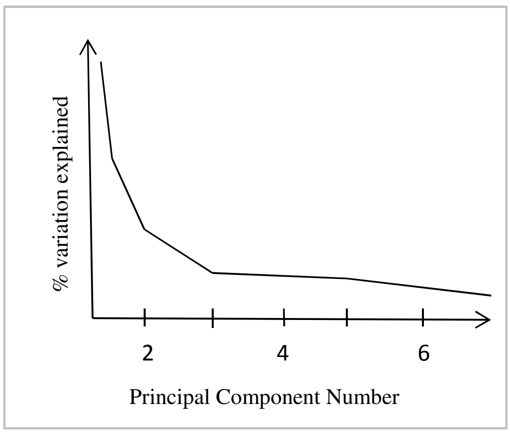

Figure 5 Scree plot: "How many patterns?"
Propose two to three relevant dietary patterns

Naming of the new dietary patterns may ease the interpretation of data. In the literature dietary patterns are named based on a subjective assessment of the food groups with the highest loadings within each pattern (10). In our example *Vegetables* are considered healthy and therefore PC1 could be named "HEALTHY". However, it should be emphasised that each dietary pattern is a gradient e.g. going from less healthy to very healthy but only named by highest loadings. Also worth noting is the fact that each child will have a value for each dietary pattern i.e. a child may have a high score on the HEALTHY pattern (consuming many *Vegetables*) but may at the same time have a high score on the UNHEALTHY pattern (consuming a high amount of *Soft drinks*) whereby the patterns does not exclude each other.

In this very simplified example we stop finding new dietary patterns after two; otherwise it would not be a simplification of the original three food axes (*Soft drinks, Cheese, Vegetables*). When analysing the whole diet we will have more than three food axes and need a technique to know when we can expect to have identified the most important dietary patterns in the data. For this propose the scree plot (**Figure 5**) is often used together with an evaluation of the interpretability of the identified dietary patterns. A clear change in the scree plot propose the number of dietary patterns to select (24).

The most common way in the dietary pattern literature to report the results from a PCA is by showing a table with loading values for the food groups, with the numerically highest loadings for each dietary pattern. The cut-off defining "highest loadings" is chosen arbitrary e.g. $>|0.15|$ or $>|0.35|$ (25;26). Nevertheless, the ability to visually inspect the dietary patterns may be useful to increase the understanding and interpretation. Therefor in Paper I, II, III and IV providing cut-off values was refrained and instead the results from the PCA were presented as either a loading plot or a bi-plot showing all included foods. After a dietary pattern has been identified by the PCA the individual score values are most commonly used as either a dependent or an independent variable in a multiple regression model to investigate associations with possible indicators or outcomes of this dietary pattern as shown in section 1.3.3 and 1.3.4.2 respectively.

<u>*Other data-driven methods*</u>

Apart from PCA, cluster analysis is the second most used data-driven method to generate dietary patterns. While PCA reduces data into patterns based upon inter-correlations between dietary variables, cluster analysis reduces data into patterns based upon differences between individuals. Cluster analysis places individuals into subgroups with most similar diets, maximally separated in a multi-dimensional space from other subgroups. Moreover, in cluster analysis each individual only belongs to one pattern and clusters can therefore be used in a categorical variable whereas each individual is represented on each pattern with an individual score value in the PCA and can be used in an continuous variable (10;27;28). In the literature PCA is often described as an example of factor analysis (10;18;29). However, the term factor analysis is also the name of an independent method sometimes used to identify dietary patterns. Factor analysis resembles PCA in many means but includes different assumptions (30) which is beyond the scope of this thesis.

Other methods occasionally used to identify dietary patterns are RRR and PLS. They are related to PCA, but the major difference is that the dietary patterns are not only identified with the goal of showing the maximum variation in dietary data. RRR and PLS both include a set of explanatory variables e.g. food groups and a set of outcome variables e.g. disease risk. In RRR the dietary patterns are derived with the goal to maximise the explained variance in the outcome variables. In PLS the goal is to find a balance both to maximise the explained variance in the dietary data as well as the outcome variables (31;32). While PCA is highly exploratory RRR and PLS incorporates current knowledge into the dietary pattern analyses so that specific hypotheses about a relation between diet and health outcomes can be explored (33). RRR has been used to identify dietary patterns in childhood a few times (33-36), while PLS to the best of my knowledge has not yet been used in a study population of children.

I am aware of two studies comparing PCA with other data-driven methods for dietary patterns in childhood. One compared PCA with cluster analysis and found the same main dietary patterns (37) and the other compares PCA with RRR and identified more or less revers patterns; a healthy and an unhealthy pattern respectively (35). The pattern identified by RRR was associated with increased risk of obesity, while PCA followed by multiple linear regression was not. Therefore it was concluded that RRR was better suited for that specific purpose (35). More, but still few studies have been carried out in adults and also show high resemblance in dietary patterns between especially PCA, factor analysis and cluster analysis (38-41). Similarities in associations between dietary patterns and health outcomes have also been observed across different methods (10;18;29). Hereby findings of dietary patterns based on PCA might with caution be compared with findings based on other data-driven methods (10;18;29). However, methodological differences need to be considered especially when evaluating dietary patterns in relation to health outcomes as RRR and PLS are designed to be associated with health outcomes. Comparability studies mention that no “golden standard” for identifying dietary patterns exist and the choice should depend on the skills available and the purpose of the analysis (37;38).

1.3.2 Characterisation of dietary patterns

In this section a short overview of the literature about dietary patterns in childhood based on the data-driven approach is given. The overview is subdivided into infants and toddlers, and adolescents encompassing the ages of the children in the SKOT and MOMS studies, respectively. Finally a subsection including literature about longitudinal tracking of childhood dietary patterns is included.

1.3.2.1 Infants and toddlers

Sixteen papers representing nine studies have been published about dietary patterns in infancy and toddlerhood (**Table 1**). Each of these studies identified from one to six different dietary patterns and most of them are based on FFQ followed by PCA. In the first about 4 to 6 mo of life most children in western countries are fed entirely by either breast milk or formula. Therefore no studies investigate the pattern of different foods before that age. Only two studies: the Southampton Women’s Survey (SWS) and the Avon Longitudinal Study of Parents and Children (ALSPAC) (42;43) have been found to include dietary patterns in the second half of the first year of life, where the complementary feeding takes place.

The studies in **Table 1** phrase the dietary patterns differently, but most of them define a presumably healthy (healthy/health-conscious/core foods/prudent/infant guideline) and a presumably unhealthy (less healthy/discretionary foods/western-like/junk/non-core foods/adult food) pattern (42-56). The healthy patterns were characterised by high intake of raw/cooked vegetables and fruit, legumes, fish, wholegrain bread, nuts, barriers, herbs, and water. The unhealthy patterns were characterised by high intake of soft drinks, ice-cream, sweets, chips, fried foods, burgers, biscuits, sweet, and salty snacks. Two studies with 3 year old children (49;57) also report a snack pattern which includes foods both from the healthy and unhealthy patterns like cakes, biscuits or fruit. Five papers also mention a traditional pattern (43;48;49;51) or a family food pattern, which is described as traditional family food (50). This pattern includes home-prepared meat, potato and vegetable dishes, stew, sauce, and butter/margarine and refers to the traditional food culture in the study setting. Thereby, the contribution from this pattern to a healthy lifestyle might vary from setting to setting. Moreover, three studies of the first 2 years of life identify a specific baby food pattern (infant guideline/breastfeeding/ready-prepared baby food/baby food) (43;48;53). This pattern shows the variation in intake of especially breast milk, formula, commercial baby food, porridge, fruit, and vegetables. The pattern is very age specific and indicates the rapid change in diet and the unique requirements in the first years of life. Paper I, II and III presented in this thesis contribute with more knowledge about dietary patterns in infancy and toddlerhood in a Danish context, where dietary patterns to my knowledge have not been reported. Moreover, these papers are based on dietary intake reported in 7-day pre-coded food diaries, not FFQ, as commonly seen.

1.3.2.2 Adolescents

Fourteen relevant papers have investigated dietary patterns in adolescence (**Table 2**). Several similarities are seen between the dietary pattern studies carried out in infancy or toddlerhood, (section 1.3.2.1) and adolescence. Most studies of adolescence are based on FFQ followed by PCA and more of the studies also report some kind of healthy (healthy/health-conscious) and unhealthy (convenient) pattern characterised more or less by the same foods as in infancy and toddlerhood (37;58-62). Equally to the infant and toddler studies some of the adolescent studies also identify a traditional pattern (37;59;63-66). However, the studies in **Table 2** are very diverse in relation to the countries where the study population lives also including non-Western countries like Brazil, Korea and China which are in the middle of a nutritional transition from a traditional towards a more westernised lifestyle. This is reflected in the dietary patterns named transitive, modified or in-transition patterns (62;63;65). Such patterns are not expected in a Danish setting. Two studies of adolescents identified a snack pattern as in infants and toddlers (59;67). Furthermore, in adolescence another pattern seems to arise called a fast food or junk pattern which is reported in five studies (59;63;67-69). The fast food/junk pattern includes high fat bakery products, hamburger, mayonnaise, French fries, fried food, soft drinks, cookies, crackers and, chocolate and seems to be a variety of the unhealthy pattern. This pattern might be a specific pattern for the adolescence age period (67) or at least be most pronounced at this age as it might appear earlier because it resembles a junk pattern observed at 36 mo of age (49). One study mention a dieting pattern (59) which also deviate from the infant and toddlers patterns and is probably related to the high focus on body appearance beginning in the years of adolescence.

Table 1 Dietary patterns in infancy and toddlerhood

Reference	Cohort, country	Age, n	Diet registration method	Dietary data processing	Dietary patterns
Robinson 2007 (53) /Baird 2008 (42) / Robinson 2009 (54)	SWS, England	6, 12mo, n=1434/ 1740/ 536	FFQ Incl. portion size	2xPCA	1)Infant guidelines, 2)Adult foods
Smithers 2012 (43)	ALSPAC, England	6,15 mo, n=7052/5610	FFQ Excl. portion size	2xPCA	1)Traditional style, 2)Ready-prepared baby food, 3)Discretionary foods, 4)Breastfeeding (6 mo)/Contemporary style (15mo)
Kiefte-de Jong 2013 (47)	Generation R, Netherland	14 mo, n=2420	FFQ Incl. portion size	PCA	1)Health-conscious, 2)Western like
Bell 2013 (44)	SAIDI/NOURISH, Australia	14, 24 mo, n=552/493	3x24h recall/ food diary Incl. portion size	2xPCA	1)14-mo-core-foods/24-mo-core-foods , 2) Basic combina-tion((14mo)/non-core foods (24mo)
Ystrom 2009 (55) Ystrom 2012 (56)	MoBa, Norway	18, 36mo, n=27763/14122	FFQ Excl. portion size	FA	1)Unhealthy diet , 2) Wholesome diet
Brazionis 2012 (45)	ALSPAC, England	6,15,24 mo, n=2169	FFQ Excl. portion size	PCA	1)Healthy (home prepared/ raw food), 2)Less healthy (ready prepared)
Northstone 2013 (50)	ALSPAC, England	24 mo n=9599	FFQ Excl. portion size	PCA	1)Family foods, 2)Sweet and easy, 3)Health-conscious
Kristiansen 2013 (48)	Norway	24 mo, n=1373(in 1999)/ 1472 (in 2007)	FFQ Incl. portion size	2xPCA	1)Unhealthy, 2)Healthy, 3)Bread and spread based (only in year 1999), 4)Low-fat milk, pancakes, fruits and berries (only in year 1999), 5)Traditional (only in year 2007), 6)Baby food (only in year 2007)
Fisk 2011(46)	SWS, England	36mo, n=1640	FFQ Partial incl. portion size	PCA	1)Prudent diet
North 2000 (49)/ Reilly 2005 (52)	ALSPAC, England	36 mo, n=10139/8234	FFQ Excl. portion size	PCA	1)Junk, 2)Healthy, 3)Traditional, 4)Snacks
Friedman 2009 (57)	Ukraine ELSPAC, Ukraine	36 mo, n=883	FFQ Excl. portion size	PCA	1)Snacks, 2)Fruit and vegetables, 3)Meats, 4)Noodles and pasta, 5)Breakfast foods
Pryer 2009 (51)	NDNS, England	1½-4½y n=1675	4 day food diary Incl. portion size	CA	1)Healthy diet, 2)Convenience diet, 3)Traditional diet
Larowe 2007 (70)	NHANES, USA	2-5y, n=541	24h recall Incl. portion size	CA	1)Mix/light drinker, 2)High-fat milk, 3)Water, 4)Fruit juices

ALSPAC: Avon Longitudinal Study of Parents and Children, CA: Cluster Analysis, ELSPAC: European Longitudinal Study of Parents & Children, FA: Factor Analysis, FFQ: Food Frequency Questionnaire, MoBa: Norwegian Mother and Child Cohort Study, NDNS: The National Diet and Nutrition Survey, NHANES: National Health & Nutrition Examination Survey, PCA: Principal Component Analysis, SAIDI/NOURISH: South Australian Infant Dietary Intake/ Nourishing Our Understanding of Role Modelling to Improve Support and Health, SWS: Southampton Women Survey

Table 2 Dietary patterns mainly in adolescence

Reference	Cohort, , country	Age, n	Diet registration method	Dietary data processing	Dietary patterns
Rothausen 2012 (61)	Danish National Survey of Dietary Habits and Physical Activity, Denmark	11-14y n=290	7 day diary records Incl. portion size	PCA	1)Processed, 2)Health-conscious
Shang 2012 (62)	China	6-13y n=5267	3x24h recall Incl. portion size	FA (+CA)	1)Healthy, 2)Transitive, 3)Western
Oellingrath 2010 (59) Oellingrath 2011 (60)	Norway	9-10y, follow up 12-13y n=924/427	FFQ Excl. portion size	2xPCA	1)Junk/convenient, 2)Varied Norwegian, 3)Snacking, 4)Dieting
Yannakoulia 2010 (68)	GENDAI Greece	9-13y n=1138	2x24h recall Incl. portion size + other lifestyle be-haviours	PCA	1)Unstructured eating, fast food/sugary foods and seden-tary lifestyle, 2)Dinner, cooked meals, and vegetables, 3)Breakfast
Ambrosini 2010 (58)	Raine Study Australia	14y n=1139	FFQ Incl. portion size	FA	1)Western, 2)Healthy
Cutler 2009 (67)	EAT USA	13-16y n=2516	FFQ Incl. portion size	PCA	1)Vegetable, 2)Fruit, 3)Sweet/salty snack, 4)Starchy foods, 5) Fast food (only identified at 5y follow up)
Richter 2012 (64)	KiGGS Germany	12-17y n=1272	Dietary history int. Incl. portion size	PCA	1)Western (and traditional in girls), 2)Healthy, 3)Traditional (only separate in boys)
Hearty 2011(37)	NTFS Ireland	13-17y n=441	7 day diary records Incl. portion size	PCA (+CA)	1)Healthy , 2)Traditional, 3)Sandwich, 4)Unhealthy
McNaughton 2008 (71)	NNS Australia	12-18y n=764	FFQ Excl. portion size	PCA	1)Fruit, salad, cereals, and fish, 2)High fat and sugar, 3)Vegetables
Song 2010 (65)	KNHANES Korea	10-19 y n=4337	24h recall Incl. portion size	PCA (+CA)	1)Traditional, 2)Modified, 3)Western
De Moraes 2012 (69)	Brazil	14-18y, n=991	FFQ Excl. portion size	PCA	1)Junk, 2)Healthy, 3)Protein rich
Dishchekenian 2011 (63)	Brazil	14-19y n=76	4 day diary records	FA	1)Traditional , 2)In-transition, 3)Fast Food
Rodrigues 2012 (66)	Brazil	14-19y n=1139	FFQ Incl. portion size	PCA	1)Western, 2)Traditional, 3)Mixed

CA: Cluster Analysis, FA: Factor Analysis, FFQ: Food Frequency Questionnaire, GENDAI: Gene-Diet Attica Investigation, KiGGS: The German Health Interview and Examination Survey for Children and Adolescents, KNHANES: Korean Nutrition Health and Nutrition Examination Survey, NNS: Australian National Nutrition Survey, NTFS: The National Teens Food Survey, PCA: Principal Component Analysis

From **Table 2** it is seen that more studies are needed to characterise the dietary patterns in adolescence in Western settings like Denmark. Paper IV is a contribution to this and will cover dietary aspects of the increasing group of overweight adolescents.

1.3.2.3 Tracking

Tracking defines the tendency for a member of a group to retain the same order in the rank of the group over time in relation to a specific characteristic (72). For dietary patterns this means maintaining the same adherence to a dietary pattern when measured more than once e.g. during childhood. Tracking of dietary patterns are of interest as this probably affects the impact on outcome measures of the dietary patterns. For example the impact of an unhealthy dietary pattern in childhood on obesity and related diseases later in life is probably smaller if the unhealthy lifestyle is only present for a very short period of time as opposed to being observed at several time points. However, few studies have been carried out investigating tracking of dietary patterns during childhood. Some similarities and thereby stability in dietary patterns from multiple time points have been reported at the group level both comparing infancy-toddlerhood (43), toddlerhood- later toddlerhood (44), childhood- later childhood (73), childhood-adolescence (74), and adolescence-later adolescence (67). However, some find specific dietary patterns related to a particular time point e.g. the baby food/breastfeeding pattern (43).

Individual tracking of dietary patterns is sparingly investigated in childhood. Only one study was found to investigate tracking of dietary patterns before 3 years of age (53). In this study the dietary patterns: “infant guidelines” and “adult foods” were both identified at 6 mo and again at 12 mo. Each of the dietary patterns was correlated between the two ages and this thereby indicated tracking. Moderate tracking was found during childhood from 3 to 9 years (75), 7 to 13 years (34) and 9 to 13 years (60). One study included children 3-18 years of age at baseline with a very long 21 years follow up. They found that 41% of the participants persisted in the uppermost quintile of scores in a traditional Finnish dietary pattern after 21 years and 38% persisted in the uppermost quintile of scores in a health-conscious pattern after 21 years (74). This shows very long term tracking but at the same time a considerable group of participants, 59% and 62% respectively, changed their dietary patterns over time.

In general, tracking of dietary patterns in childhood is highest, for the shortest time span and increase along with increasing age of the children (75). The strongest tracking seems to occur for people with the most extreme degree of a dietary pattern (highest/ lowest quintiles of scores from the PCA) (74). Additionally, tracking of dietary patterns might be stronger than tracking of individual food groups (34). Since tracking of dietary patterns during childhood is sparingly investigated so is the effect of this tracking. However, one study found that tracking of a dieting pattern (with high loadings for food products with artificial sweeteners) from middle childhood to early adolescence was associated with increased risk of being overweight and remaining overweight (60). Paper III in this thesis contributes with analysis of tracking across infancy and toddlerhood, and possible related outcomes, while Paper IV deals with short term stability of dietary patterns of adolescents during an intervention period. In both papers diet registrations from different time points are included in the same PCA, which is in contrast to most other papers (53;60;74;75). This was done as an attempt to compare the different time points exactly on the same scale for a particular dietary pattern instead of comparing presumably similar dietary patterns from separate PCAs of each time point which might be a limitation in previous studies.

1.3.3 Indicators of dietary patterns

Several possible indicators of dietary patterns in children have been investigated. The attention here will be on indicators for dietary patterns in infancy and toddlerhood because this age group has been the focus of Paper I and II. Possible indicators of dietary patterns in this age group have been investigated in seven observational studies published in twelve papers to the best of my knowledge (**Table 3**). These indicators can be divided into maternal, paternal, household and child characteristics. The association between maternal characteristics and offspring dietary patterns are the most widely examined; especially the age, socioeconomic factors, family structure, and health markers. All except one study, which investigated the association between maternal age and dietary patterns of the offspring found, that lower age of the mother was associated with higher scores in a unhealthy dietary pattern (adult foods/western like/biscuits, sweets and crisps/basic combination/less healthy/unhealthy/junk patterns, sweet & easy) (43-47;49;50;53;55). It is also relatively well established that children of less educated mothers (43-50;53;55;56) and mothers with higher BMI (43;45-47;49;53;55;56) have a less healthy dietary pattern. Moreover, children without siblings seem to have more healthy dietary patterns than children with siblings (43;45-50;53;55). However, for maternal education, BMI and number of siblings associations are only found between the indicator and dietary patterns for one of several ages of the child (44;53) or one of several different dietary patterns investigated in a study. This might suggest different indicators for different dietary patterns and ages of the children. The paternal impact on offspring dietary patterns is far less reported than maternal characteristics. To the best of my knowledge, only two studies examine paternal indicators (47;48). They found that lower age and educational level of the father were associated with less healthy dietary patterns (western like/unhealthy pattern) (47;48). The BMI of the father was not associated with offspring dietary patterns, opposite the finding of maternal BMI (47). Income is like parental education and work situation a measure of the socioeconomic status of the child's family. Lower household income has been associated with higher intake of a less healthy dietary pattern (western like/ unhealthy patterns) (47;55).

Nine papers investigated the gender of the child in relation to dietary patterns and show mixed results. Two studies showed lower scores for all identified dietary patterns for girls at 14 and 24 mo (47;48). Contrary one study found lower scores in both a healthy and unhealthy pattern at 18 mo for boys (55) but at 36 mo this was only confirmed for the healthy pattern in the same group of children (56). Another study found higher scores in a healthy pattern for boys at 24 mo (50) but an opposite association for a similar pattern at 36 mo (49). Three found no association (43;44;46). Associations between dietary patterns and the body size of the child at birth and at the time of diet recording has been investigated in two studies (46;47). This is presumably with the hypothesis that parents feed their children differently depending on whether the child is small or big for its age but this was not explicit stated. They did not find any association between size of the child and dietary patterns. However, more studies are needed before any definite conclusions can be made and an important notion is that such analysis bares the risk of reverse causality; meaning that the child is big because it is fed differently and not fed differently because it is big.

A relation between sedentary behaviour (time spent watching TV) and dietary patterns in toddlerhood has been investigated in two studies (46;47) finding that less healthy dietary patterns are associated with more time spent watching TV. No studies have investigated the opposite of sedentary behaviour, i.e. physical activity, in relation to dietary patterns in infants and toddlers. Moreover the relation between early feeding like breastfeeding duration and age at introduction of solids and toddlers dietary patterns is not well documented. Four studies investigated early feeding as possible indicators of toddlers' dietary patterns (44;47;48;53). They found that less breastfeeding (duration or yes/no) and early age at introduction of solids were associated with a less healthy dietary pattern. Only one of these four studies investigated the association in infants below 12 mo (53). People preparing food for the child, i.e. the person who does most of the cooking in the home, and the place, where meals are eaten (home or in day care), have also been investigated as possible indicators of dietary patterns in toddlerhood, but only sporadically. Being in day care at 18 mo was associated with lower adherence to a healthy dietary pattern in a Norwegian study population (55), while no association between day care and dietary patterns was seen in 14 mo old toddlers in the Netherlands (47). These associations might be very culturally dependent as both the percentages of children in day care, the age normally sent to day care and the food served in day care varies from place to place. In relation to ethnicity, toddlers in an Australian cohort with a mother born in Australia had higher adherence to a dietary pattern in accordance with dietary recommendations compared to toddlers with a mother not born in Australia at 24 mo of age but no association was found at 14 mo (44). Contrary non-white toddlers in an English cohort had higher scores in a healthy pattern than white toddlers at 24 and 36 mo (49;50). The association is presumably highly dependent on the definition of ethnical differences.

Additional possible indicators of dietary patterns investigated in older children and adolescents are physical activity, differences between week or weekend days, lunch at school, cooking skills, does the family have joint family meals, smoking habits of the adolescent, does the adolescent still live together with parents, education and employment of the adolescent (59;61;64;69;71;73), while the number of parental indicators investigated in these studies are fewer than in younger children. Studies of gender differences in older children found more healthy dietary patterns in girls down to 4 years of age in two studies (59;73). Moreover, in older children a higher level of physical activity was associated with a healthier dietary pattern (59;64;69;71) but not consistent in both genders (64;69).

More studies investigating indicators for dietary patterns such as maternal BMI before 12 mo are needed and will be addressed in Paper I. More studies are also required to investigate paternal characteristics, ethnicity, child gender, body size, early feeding practice, activity level, and the impact of who takes care of the feeding as possible indicators of dietary patterns in infancy, which is addressed in Paper II.

Table 3 Possible indicators of dietary patterns in infancy and toddlerhood

Reference	Robinson 2007 (53)	Kiefte-de-Jong 2013(47)	Smithers 2012 (43)	Bell 2013 (44)	Ystrom 2009 (55)	Brazionis 2012 (45)	Kristiansen 2013 (48)	Northstone 2013 (50)	Fisk 2011 (46)	North 2000 (49)	Pryer 2009 (51)	Ystrom 2012 (56)	Paper II
Cohort	SWS	Generation R	ALSPAC	SAIDI/ NOURISH	MoBa	ALSPAC	-	ALSPAC	SWS	ALSPAC	NDNS	MoBa	SKOT I+II
Age of children	6,12mo	14 mo	6,15 mo	14,24 mo	18 mo	6,15,24 mo	24 mo	24 mo	36 mo	36 mo	18-54mo	36mo	9 mo
Maternal													
Age	X*	X*	X*	X*	X*	X*	X*	X*	X	X*		X*	X
Education	X*	X	X*	X*	X*	X*	X*	X*	X*	X*	X	X*	X
Social class			X	X		X*			X		X*		
Work situation/has a job/ homemaker							X*	X*		X*		X*	X
Marital status/has a partner/number of adults in household		X*	X*	X	X		X	X	X	X		X*	X
Parity/no. siblings/birth order/no. of children in household	X*	X*	X*	X	X*	X*	X*	X*	X*	X*			X
Diet (Dietary pattern/ Vegetarian/Diet pregnancy)	X*	X*						X*	X*	X*			
Smoking/Smoking in home	X*	X*	X	X*	X*	X			X*	X*	X	X*	X
Alcohol consumption										X*			
Time spent watching TV	X*												
Current/pre-pregnancy BMI	X*	X*	X*	X	X*	X*			X*			X*	X
Diabetes/hypertension/ hypercholesterolemia		X*											
Born in cohort country				X*									
Negative affectivity					X*							X*	
Paternal													
Age		X*											X
Education		X*					X*						X
Work situation/has a job													X
Smoking/smoking in home		X*											X
BMI		X											X
Diabetes/hypertension/ hypercholesterolemia		X*											

Table continued next page

Table 3 continued

Reference	Robinson 2007 (53)	Kiefte-de-Jong 2013(47)	Smithers 2012 (43)	Bell 2013 (44)	Ystrom 2009 (55)	Brazionis 2012 (45)	Kristiansen 2013 (48)	Northstone 2013 (50)	Fisk 2011 (46)	North 2000 (49)	Pryer 2009 (51)	Ystrom 2012 (56)	Paper II
Cohort	SWS	Genera-tion R	ALSPAC	SAIDI/ NOURISH	MoBa	ALSPAC	-	ALSPAC	SWS	ALSPAC	NDNS	MoBa	SKOT I+II
Age of children	6,12mo	14 mo	6,15 mo	14,24 mo	18 mo	6,15,24 mo	24 mo	24 mo	36 mo	36 mo	18-54mo	36mo	9 mo
Household													
Income/Deprivation score/Financial difficulties		X*			X*			X*	X*	X*	X*		X
Housing tenure								X*		X*	X*		
Person who cooks mostly										X*			X
Child													
Age		X*		X*									X
Gender		X*	X	X	X*		X*	X*	X	X*		X*	X
Ethnicity/immigrant/ de-scendant status			X*					X*	X	X*			X
Multiple birth			X										
Body size (Birth weight/ weight-for-age/ height-for-age/BMI-for-age)		X							X				X
Eating habits (vegetarian, based on snacking or meals, difficult eater, nibbling behaviour)								X*	X*				
Age at introduction of solids	X*	X*		X*									X
Breastfeeding duration		X*		X*			X*						X
Food allergy		X											
Time spent watching TV		X*							X*				
Physical activity level													X
Age when crawling													X
Day care		X			X*								X
Feeling unwell during diet survey											X*		

ALSPAC: Avon Longitudinal Study of Parents and Children, MoBa: Norwegian Mother and Child Cohort Study, NDNS: The National Diet and Nutrition Survey, SAIDI/NOURISH: South Australian Infant Dietary Intake/ Nourishing Our Understanding of Role Modelling to Improve Support and Health, SKOT: Småbørns Kost og Trivsel (Danish abbreviation), SWS: Southampton Women Survey, *Significant associations, $p<0.05$(not disclosed for Paper II, instead see section 4.2)

1.3.4 Dietary patterns and health outcomes

1.3.4.1 Growth, obesity and metabolic risk markers

Growth is the natural increase in body size during childhood. In the past, a chubby infant was perceived as a healthy infant and it was assumed that the child would lose the extra fat during growth. Now it is well established that childhood obesity tracks into late childhood and further into adulthood. The predictive power of childhood BMI for adult BMI increase with increasing age of the child (76). About 26-41% of obese pre-schoolers are still obese as adults, while 42-63% of school age children are still obese as adults (77). In 2010 the prevalence of childhood overweight and obesity was 7% worldwide (78) and 12% separately for developed countries (78). Among Danish pre-schoolers born in 1995 10–13% were overweight and 1.5–2% were obese (79). A steep increase has been reported globally (78;80) as well as in Denmark (81). The obesity epidemic is hereby critical, but a levelling off in the increasing prevalence in countries such as Denmark appears to be emerging in present time (82-84).

Overweight and obesity are generally defined as excessive body fat affecting health (85). Different measures of body fat and different definitions of cut-offs for overweight and obesity in childhood exists. For example weight is a very rough proxy of body fat, while BMI also take height into account and is therefore a frequently used proxy. BMI is closely associated with fat mass in children and adults (86). The BMI cut-offs for overweight (25 kg/m^2) and obesity (30 kg/m^2) in adults are based on risk of morbidity and mortality (87). In 2000, Cole et al. defined age and sex specific BMI cut-offs for children aged 2-18 years extrapolated from the adult BMI cut-offs (88). Cut-offs for overweight and obesity in children were defined as the BMI percentile which passed through BMI of 25 and 30kg/m^2 respectively at 18 years (88). Contrary, the World Health Organisation (WHO) does not define overweight and obesity based on health risks. Instead their cut-offs are based on the statistical distribution with overweight defined as BMI >+2 z-scores at 0-5 years and >+1 z-score at <5-19 years, and obesity as >+3 z-scores at 0-5 years and >+2 z-scores at 5-19 years on their age and sex specific growth curves (85). The anthropometric measures such as weight and BMI are indirect measures of body fat that do not take individual differences in the proportion of body fat mass and fat free mass into account. Fat mass is more directly measured e.g. with bioelectrical impedance (89).

Short term consequences of childhood obesity are psychological and social problems as well as increased prevalence of respiratory, skeletal, neurological, gastroenterological, endocrine and cardiovascular difficulties (90). However, long-term consequences of childhood obesity manifested in adults have often been the main reason for weight reducing interventions in children (91;92). The evidence is relatively robust and fairly consistent that childhood obesity is related both to morbidity and early mortality in adulthood (92). An extra-long term consequence of childhood obesity is the possible adverse effects on the next generation, when an obese child grow up to be an obese pregnant woman and mother (93). These consequences seem to include delivery complications and increased risk of obesity and diabetes in the offspring contributing to a vicious cycle (93;94). Nevertheless, most diseases related to obesity develop over long time of persistent obesity and will not appear in early life. Therefor most studies in children use risk markers and subclinical levels of e.g. type 2 diabetes and cardiovascular diseases rather than actual morbidity symptoms, as outcome measures to predict risk-groups and enable an early prevention (91). Risk markers could be insulin, insulin-like growth factor 1 (IGF-I), glucose, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triacylglycerol levels in the blood. Tracking of metabolic risk markers during childhood from the first year of life (95) and into adulthood (96) has been found.

The causes of obesity are multifactorial and imply different risks from individual to individual. Examples of factors associated with childhood obesity are intrauterine exposure to gestational diabetes and maternal adiposity, high and low birth weight, early adiposity rebound, epigenetics, low physical activity level and high sedentary behaviour, limited sleep, low socioeconomic status and an unfavourable diet (97). Examples of unfavourable diets are lack of breastfeeding and high intake of sugar-sweetened beverages (98). Moreover, the quality of the food in the complementary feeding period and toddlerhood are potentially important in relation to obesity risk but this is not widely investigated (4). Sugar-sweetened beverages have been extensively discussed as a possible important factor in the increasing prevalence of obesity in childhood because the intake has increased simultaneously with the increasing prevalence of obesity (99;100). Despite contrasting findings a recent review concludes that consumption of sugar-sweetened beverage promotes weight gain in children and adults (101). It has been discussed what carries the effect and evidence e.g. shows that liquid energy seems to result in lower satiety compared to energy consumed in solid form. This poses a risk of incomplete compensatory reduction in energy intake at subsequent meals (101). The question then arise; what to drink instead of sugar-sweetened beverages? ESPGHAN recommends that plain water should be promoted as the main source of fluid for children *instead* of sugar-sweetened beverages (102). This is also the official recommendation in Denmark, which adds a limit of maximum ½ L sugar-sweetened beverages per week (9). However, only few studies have investigated if and how the rest of a meal, or the whole diet, changes when drinking more water (103-105) and as far as I know, this has only been investigated in adults. One of these studies investigated the effect of water intake on body weight and thereby obesity risks (106). Moreover, only a couple of studies have investigated if children in practice drink water *instead* of sugar-sweetened beverages when both are available (107-109). Milk is another beverage which has been investigated in relation to obesity based on the hypothesis that it poses a protective effect on obesity and risk markers of the metabolic syndrome in children. However, the evidence is still inconclusive (110). For example, studies have investigated the effect of different whey and casein fractions of the milk on appetite regulation (111-115).

1.3.4.2 Associations between dietary patterns, growth, obesity and metabolic risk markers

Only few studies have investigated the relations between dietary patterns and growth, obesity, and markers for diabetes and cardiovascular diseases in infancy and toddlerhood (**Table 4**) and findings are ambiguous. Two studies investigated the relation between dietary patterns and current BMI in infancy and toddlerhood. One found that higher intake at a meat pattern was associated with higher BMI (57), while the other study did not found an association (44).

Table 4 Growth, obesity and metabolic outcomes possibly related to dietary patterns in infancy and toddlerhood

Reference	Baird, 2008 (42)	Robinson, 2009 (54)	Bell, 2013 (44)	Friedman, 2009 (57)	Reilly, 2005 (52)	Paper III
Cohort	SWS	SWS	SAIDI/ NOURISH	Ukraine ELSPAC	ALSPAC	SKOT
Age of children at diet registration	6 mo	12mo	14, 24 mo	3y	3y	9, 18,36mo
Age of children when measuring outcome	12 mo	4y	14, 24 mo	3y	7y	36mo
Body size						
Weight	X					
Weight gain (6-12mo)	X*					
Length/Length-for-age z-scores	X					X
Length gain (6-12mo)	X					
BMI/BMI-for age z-scores		X	X			X
Overweight(BMI≥85th perc.) /obesity (BMI≥95th perc.)				X*	X*	
Skinfold thickness (triceps and subscapular)	X*					
Skinfold thickness (triceps and subscapular) gain (6-12mo)	X*					
Body composition						
Fat mass / Fat mass index		X				X
Lean or fat free mass / lean or fat free mass index		X*				X
Blood parameters						
Total cholesterol						X
LDL						X
HDL						X
Triglycerides						X
Glucose						X
Insulin						X
IGF-I						X
IGFBP3						X
IGF-I/IGFBP3						X

ALSPAC: Avon Longitudinal Study of Parents and Children, ELSPAC: European Longitudinal Study of Parents & Children, SAIDI/NOURISH: South Australian Infant Dietary Intake/ Nourishing Our Understanding of Role Modelling to Improve Support and Health, SKOT: Småbørns Kost og Trivsel (Danish abbreviation), SWS: Southampton Women Survey, *Significant associations, p<0.05 (not disclosed for Paper III, instead see section 4.3).

In addition, two studies investigated the association between dietary patterns in infancy or toddlerhood and BMI later in life. Again, one study found an association between higher intake of a junk food pattern at 3 years and higher BMI at 7 years (52), while the other did not find an association between dietary patterns at 12 mo and BMI, nor fat mass, at 4 years (54). Instead this study found an association between a dietary intake most similar to official guidelines at 12 mo and a higher lean mass at 4 years (54). In the last study dietary patterns was also investigated as early as 6 mo of age and adherence to an infant guideline pattern at 6 mo was associated with higher gain in weight and skinfold thickness from 6 to 12 mo (42). When looking at the relation between dietary patterns and BMI later in childhood or adulthood (116-118) and including other lifestyle factors such as activity level in a broader lifestyle pattern (119;120) findings indicate that higher scores on an unhealthy dietary pattern or lower scores on a healthy pattern are associated with higher BMI. Contrary, length has only been investigated in one study and was not significantly associated with dietary patterns (42). A possible relation between dietary patterns and blood parameters in infancy and toddlerhood has not yet been investigated but is called for in a large review about dietary patterns (26). Because of the relative few and ambiguous studies within this area, dietary patterns in infancy and toddlerhood are investigated in relation to anthropometry, body composition, and blood parameter outcomes in Paper III.

1.3.5 Dietary patterns in intervention studies

All studies describing dietary patterns displayed by PCA referred to until now have been observational studies. To my knowledge, only one study has taken advantages of PCA to describe dietary patterns as part of the evaluation of an intervention study in children (121). In this study the intervention was a number of group sessions for parents with children at 18 mo facilitated by a dietician with the focus of improving dietary intake and food-related behaviour of the child. In general, it could intuitively be an advantage to evaluate diet interventions by a data-driven approach such as PCA taking the whole diet into account to be sure not to miss a possible effect, oppose to investigating the effect on single nutrients or foods. However, in this intervention study they also discuss the risk of blurring the intervention effect directed at specific foods (here fruit, vegetables, and noncore foods) by including the whole diet in a PCA (121). To validate the usefulness of PCA in this intervention they also assessed effectiveness of the intervention by an index specially designed to capture changes in fruit, vegetables, and noncore foods (the Obesity Protective Dietary Index). An intervention effect was found based on the index but not on the PCA. However, other types of nutrition interventions might show different results. Paper IV will contribute to illuminate the possible advantages of dietary patterns identified by PCA in the evaluation of nutrition interventions.

2 Hypotheses and research questions

The overall aim of this PhD thesis was: *To make exploratory analysis of dietary patterns in childhood, with the purpose of characterising the diet and investigating associations to possible indicators and outcomes related to growth and obesity*.

A conceptual frame work of this thesis is visualised in **Figure 6** and research questions and hypotheses considered in each of the four included papers are listed below.

Paper I (based on SKOT I + SKOT II)
<u>*Hypothesis:*</u>
● Children born to obese mothers, compared to non-obese mothers, already at 9 mo of age have a less healthy diet, when evaluated both at energy, macronutrients, foods, and dietary pattern levels.
<u>*Research questions:*</u>
● How does the dietary patterns at 9 mo differ between children born to obese mothers compared to infants born to mainly non-obese mothers?
● How does the intake of single food groups, nutrients, and energy at 9 mo differentiate between children born to obese mothers compared to infants mainly born to non-obese mothers?
● What are the main differences in background characteristics of children in SKOT I and SKOT II?

Paper II (based on SKOT I+ SKOT II)
<u>*Hypothesis:*</u>
● Dietary patterns in infancy are associated with factors such as child and parental characteristics.
<u>*Research questions:*</u>
● How are dietary patterns at 9 mo of age associated with child characteristics, maternal characteristics, paternal characteristics and household characteristics?
● Which are the strongest indicators of dietary patterns at 9 mo of age?

Paper III (based on SKOT I)
<u>*Hypotheses:*</u>
● Development of dietary patterns can be identified by PCA for infants and toddlers.
● Development of dietary patterns across infancy and toddlerhood are associated with body size, body composition, and metabolic risk markers in toddlerhood.
<u>*Research questions:*</u>
● What characterises the development of dietary patterns in SKOT I from 9 to 36 mo of age?
● Is it possible to make an informative classification of toddlers based on their development of dietary patterns from 9 to 36 mo of age?
● How is the development of dietary patterns related to body size, body composition, and metabolic risk markers at 36 mo of age?

Paper IV (based on MoMS)

Hypotheses:

● Adolescents will change their diet to compensate for extra energy intake during an intervention with increased intake of dairy products.

● Adolescents will change their diet to compensate for extra volume intake during an intervention with increased water intake despite no extra energy intake.

● The dietary changes during the intervention will reflect food habits in the study population whereby the test drink will substitute appropriate meal elements.

Research questions:

● What characterises the diet of moderately overweight Danish adolescents participating in the MoMS study, when evaluated both at energy, macronutrients, foods, and dietary pattern levels?

● How does the dietary intake change during the intervention period?

● Are the changes in dietary intake during the intervention different according to intervention groups?

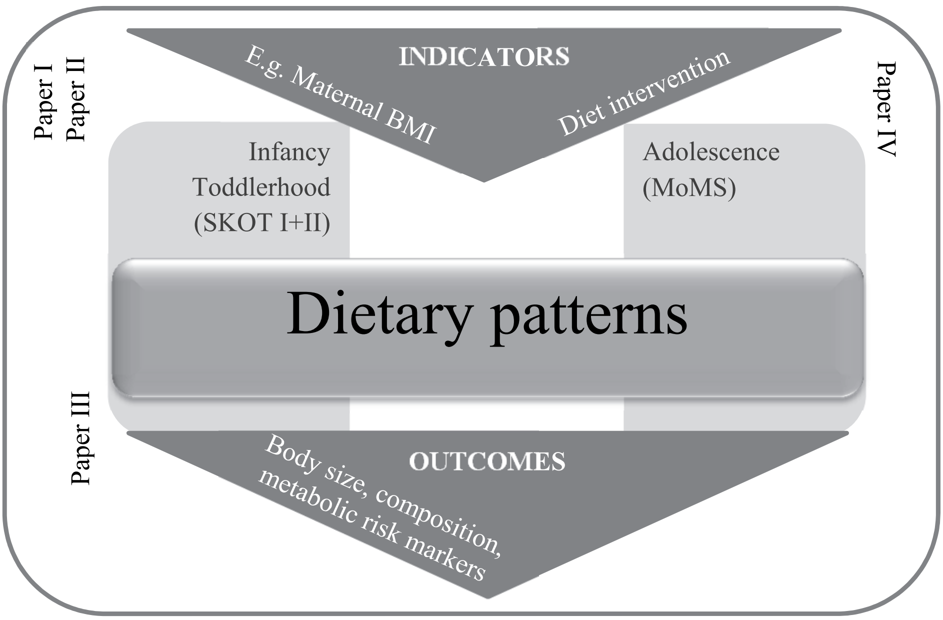

Figure 6 Conceptual framework of this PhD thesis

3 Subjects and Methods

This section provides a methodological overview of the three studies; SKOT I, SKOT II and MoMS and is supplementary to the subjects and material sections in Paper I, II, III and IV.

3.1 SKOT I & SKOT II

3.1.1 Study design

The SKOT studies are two similar prospective cohorts monitoring healthy children at 9 mo (±2 weeks), 18 mo (± 4 weeks) and 36 mo (± 12 weeks) of age. Anja L. Madsen, Katrine T. Ejlerskov, Laurine BS. Harsløf, Line B. Christensen, Christian Mølgaard, and Kim F. Michaelsen were the main responsible for design and data collection at SKOT I. Emma LM. Carlsen, Katrine T. Ejlerskov, Birgitte Hermansen, Christian Mølgaard, Kim F. Michaelsen, and I were the main responsible for design and data collection at SKOT II. I participated in data collection at 9, 18, and 36-mo examinations in SKOT II.

The overall aim of both studies was to investigate the impact of complementary and young child feeding on short and long term development and health. This, to be able to contribute to the scientific basis for dietary and life style strategies, policies, and dietary guidelines for infants and young children in Denmark. The focus was prevention of obesity and specific nutrition related diseases such as type 2 diabetes, cardiovascular disease, and osteoporosis that develops later in life, either during childhood or adulthood. Furthermore, for SKOT II, the aim was to investigate the health consequences for a child being born to an obese mother, when comparing data with SKOT I. Therefore, the structure of the two studies, and the whole battery of data collection, was kept as similar as possible. The only main difference in design between the two cohorts was the recruitment and inclusion criteria. Data collection at SKOT I was carried out year 2007-2010 and data collection at SKOT II began in year 2011 and the 36-mo examinations are expected to finish spring 2015.

3.1.2 Recruitment and drop-out

3.1.2.1 SKOT I

The SKOT I cohort was one of six work packages of the major project “Complementary and Young Child Feeding (CYCF)-impact on short and long term development and health” which cover different scientific approaches in relation to complementary feeding such as sensory, sociologic, and natural science. The participants for SKOT I were recruited by postal invitations to parents of infants living in the Copenhagen area based on extractions from the National Civil Registration System. Inclusion criteria for participation were healthy singletons born at term (37-43 weeks of gestation) with an age of 9 mo ±2 weeks at the first examination and having Danish-speaking parents. Children were excluded if diseases influencing food intake and growth were diagnosed. A total of 329 children were included in SKOT I (**Figure 7**) and 95% completed 9-mo examination, 88% completed 18-mo examination and 80% completed 36-mo examination. The percentage of children with complete diet registration both at 9, 18 and 36 mo was 95% of the diet registers.

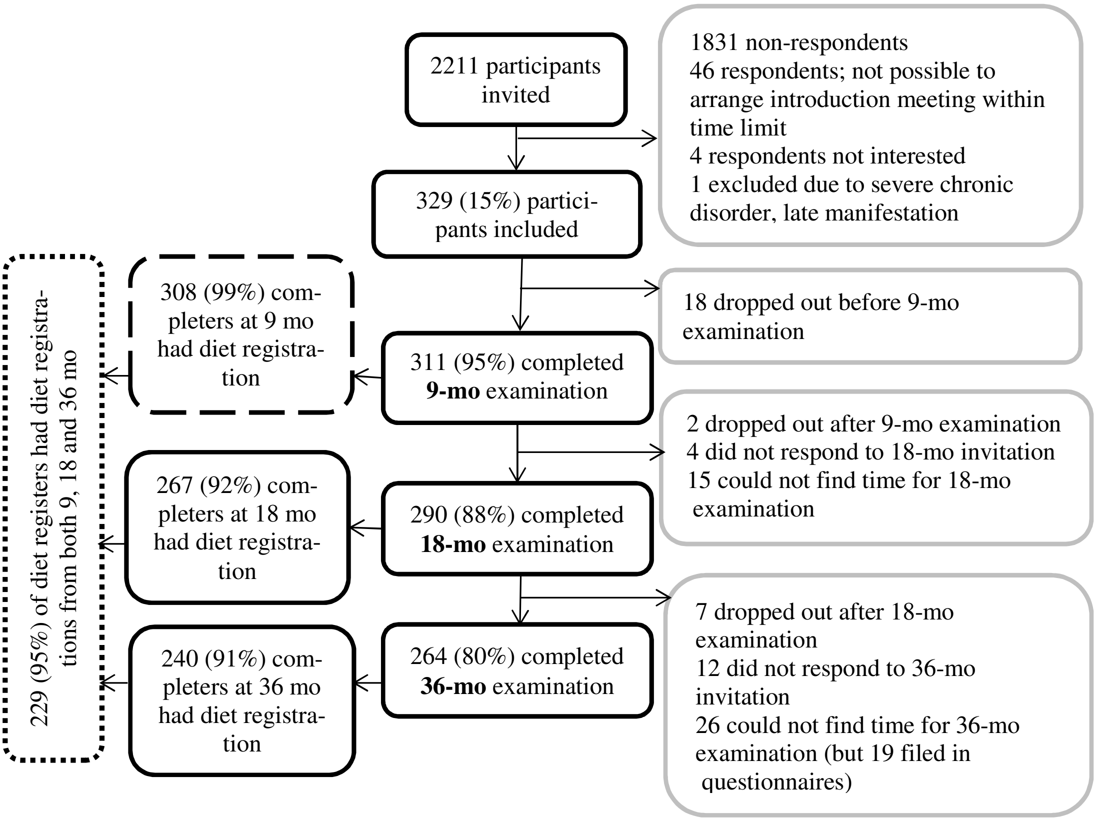

Figure 7 Participants and drop outs in SKOT I
---- Included in Paper I and II, ... Included in Paper III

3.1.2.2 SKOT II

Participants in SKOT II were recruited among offspring of pregnant women participating in the intervention study; "Treatment of Obese Pregnant Women" (TOP) at Hvidovre Hospital, Denmark. All participants (n=420) in the TOP study had a pre-pregnancy BMI above 30 kg/m^2. At week 11-14 of gestation the pregnant women were randomised into four intervention groups; exercise (11,000 steps daily monitored by pedometer), exercise and diet (11,000 steps daily monitored by pedometer + counselling by a dietician every second week) or control (standard procedure). The aim of the TOP-intervention was to limit weight-gain during pregnancy to 5 kg or less (122). After birth a subsample of 200 mother-infant pairs from TOP was randomised into two additional intervention groups, either intensive breastfeeding counselling or standard procedure with the aim of prolonging breastfeeding (123). At Hvidovre Hospital the offspring of TOP were DXA scanned and anthropometric measurements were carried out at birth and 6 mo of age. At the 6-mo visit they were invited to participate in SKOT II, which was carried out at the Department of Nutrition, Exercise and Sports, Frederiksberg, Denmark. Hereby the inclusion criteria for SKOT II were the same as

SKOT I plus all participants should be offspring of women with pre-pregnancy BMI above 30 kg/m^2. Because of late approval of protocols 299 infants were invited and 208 infants participated at the 6-mo visit and were further invited to SKOT II whereby 184 infants were included into SKOT II and 90% completed the 9-mo examination (**Figure 8**). Comparing the drop-out in SKOT I and SKOT II is difficult because of the different recruitment methods. However, 95% versus 90% of included infants completed 9-mo examination in SKOT I and SKOT II respectively and 99% versus 86% of infants completing the 9-mo examination provided diet registration at 9 mo in SKOT I and SKOT II respectively.

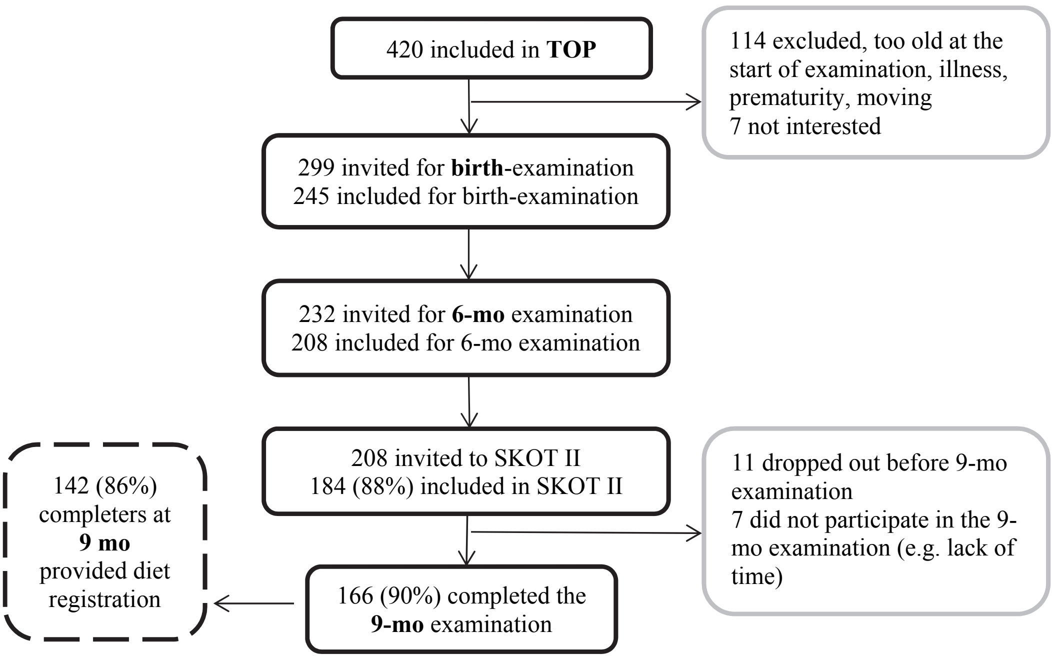

Figure 8 Participants and drop outs in SKOT II, until 9 mo examination
---- Included in Paper I and II (Only 9 mo data are included in this PhD)

3.1.3 Measurements and questionnaires

Table 5 shows an overview of data collected in SKOT I and SKOT II with indication of the data which are used in this PhD thesis.

Table 5 Overview of data collection in SKOT I and SKOT II

Age	9 mo	18 mo	36 mo
Anthropometry	I / II	I	I
Blood pressure			
Faeces sample			
Urine sample		†	
Blood sample			I
Bioelectrical impedance analysis			I
DXA scan			†
Diet registration	I / II	I	I
Background interview	I / II	I	I
Questionnaire-general	I / II	I	I
Questionnaire-psychomotor skills			
7-day physical activity monitoring			
Parental blood pressure, height, weight	I / II	I	I

Grey cells: data collected in SKOT I and SKOT II, †: data only collected in SKOT I, I: data applied in this PhD thesis from SKOT I, II: data applied in this PhD thesis from SKOT II.

3.1.3.1 Diet

Both in SKOT I and SKOT II diet was recorded prospectively by 7-day food diaries at 9, 18 and 36 mo (4 days at 36 mo in SKOT II) with the same method as used in the national survey (124). Each diary was divided into four sections based on a usual Danish meal pattern (breakfast, lunch, dinner and in-between meals) and pre-coded with usual foods within each section and open fields for foods not pre-coded. Portion sizes were estimated based on a photo booklet with 12 dishes shown in four to six different portion sizes each. Household measures (e.g. cup, spoon) were used for portion size estimation if no suitable photo was available. Two versions of food diaries were used; one for infants (9 mo) and one for toddlers (18 and 36 mo). They were similar in layout but the infant diary contained extra pre-codes with baby food such as infant formula, porridge, and mash. This diet recording method has been validated against a weighed diet registration and double labelled water in 9 and 36 mo old children (125).

The diaries were handed out at the examination visit and should be sent by post back to the department, except at the first visit in SKOT I where the diaries were handed out at the information meeting and filled out before the 9-mo examination where they could be handed in personally. If the food diaries were not returned within a couple of weeks the parents were contacted by phone repeatedly to support the registration and return of diaries together with other data materials. Before the diet registration parents had a careful oral and written instruction which was refreshed before each registration period. The aim was diet registration for 7 consecutive days but a minimum of 4 days were accepted to obtain an estimate of usual intake. At 9 mo of age many of the children were still fulltime taking care of at home and the parents did the entire diet registration. For children in day care a separate sheet for day care registration was handed out. The parents were requested to ask the staff in the day care to write down as detailed as possible what the child ate during the day and portion sizes should be estimated in household measures. The parents were then to transfer the information from the day care sheet into the food diary.

Following data collection dietary data were digitalised, exposed to quality control and nutritional content was calculated. The National Food Institute, Denmark was responsible for all these three parts of the data pre-processing in SKOT I whereas the digitalisation in SKOT II was carried out at the Department of Nutrition, Exercise and Sports and the quality control and assessment of nutritional content was executed at the National Food Institute. There were differences in the procedure for digitalisation of data between SKOT I and SKOT II but a test sample of food diaries from SKOT I was included in the pre-processing of SKOT II data to verify comparability. The National Food Institute used the GIES software and the Danish food composition databank (www.foodcomp.dk) in the pre-processing of diet data in both studies. After data pre-processing, The National Food Institute provided the dietary data as the mean intake of the 7 days registration for each child and the intake was presented in three separate ways; 1) intake based directly on the recorded foods represented in very detailed groups (such as Banana) or 2) total intake calculated from standard recipes and combined in few broad categories (such as Fruit and fruit products) or lastly 3) energy and nutrients calculated from the food composition tables.

The PCA analyses presented in this thesis are based on a selection of food group variables which is a mixture of type 1 and 2 dietary data (**Table 6**) and the variables are mainly the same across all four papers. Variables based on type 1 data are the sum of a number of more specific subgroups. The main principle for selection of variables was to cover the whole diet. Furthermore, the degree of details and thereby the number of food variables were selected based on nutritional knowledge trying to cover most aspects of the official recommendations, nutrition evidence and typical childhood diet in Denmark. By mixing type 1 and 2 dietary data these principles were attempted to be balanced. Type 1 data were especially chosen when more detailed variables were thought to be favourable such as the *BreakfastCereals, WheatBread, RyeBread,* and *PastaRice* variables which are based on type 1 data instead of the overall type 2 food group "Bread and other cereal products". Type 2 diet data were often chosen when the food in focus frequently is part of a mixed dish such as *Vegetable*. Here a considerable amount of intake would be overlooked if using type 1 dietary data. Even though being careful in forming the food variables by mixing of data types this pose a small risk of overlap between some variables. Food variables in gram per day were divided by total body weight to increase the comparability across different requirements and ages.

The intake of breast milk is the only food variable not based on the food diaries because portion size per feed is difficult to estimate by mothers. The intake of breast milk was estimated based on the question posed during the examination visit: *how many times during 24 hours does the child consume breast milk as a meal?* In the PCA analyses number of breastfeeding sessions was used directly to minimise sources of uncertainties. However, the portion size was estimated for descriptive purpose and comparison with other foods using the estimate of 99 g/feeding (126).

3.1.3.2 Anthropometry, body composition, blood samples, background questions

The procedures about measuring anthropometry, body composition, blood samples and background questions are described in each paper and will not be reproduced here.

Table 6 Description and data origin of food variables

Food group	Description (g/kg body weight/day)	Data layer
Porridge	Cereal gruel, porridge; homemade or ready-prepared	1
BreakfastCereals AddSugar†	Sugar puffs and sugary cereals	1
BreakfastCerealsNoAddSugar†	Oatmeal, muesli, cornflakes	1
WheatBreadWholegrain	Grainy bread, crisp bread	1
WheatBreadNoWholegrain	White bread, biscuits	1
RyeBread	Rye bread with and without seeds	1
PastaRice	Pasta, rice	1
Potato	Potatoes boiled, baked, mashed or prepared in potato salad	1
Fruit	Fresh fruit and berries, fruit porridge/soup/compote; homemade or ready-prepared	1
Vegetable	All vegetables eaten raw/cooked/mashed alone or in a dish	2
Fish	All fish and fish products eaten as sandwich spread or in a dish	2
Meat	All meat and meat products eaten as sandwich spread or in a dish, except poultry and fish	2
Poultry	All poultry and poultry products eaten as sandwich spread or in a dish	2
Egg	All egg and egg products eaten as sandwich spread or in a dish	2
FatsAnimal	Butter, spreadable butter, sauce made from butter	1
FatsVegetable	Oil, margarine, mayonnaise, remoulade, ketchup, low-fat sauce	1
Cheese	All cheese and cheese products eaten as sandwich spread or in a dish	2
Milk	All milk and milk products eaten alone or in a dish except human milk or infant formula	2
Formula	Infant formula, follow-up formula	2
BreastMilk††	Human milk from the mother	-
FruitNutSnack	Cereal bar, nuts, almonds, dried fruit and fruit spread, jam, honey, peanut butter, seeds, peanuts	1
Chips †††	Potato chips, popcorn	1
SweetsCake	Ice cream, chocolate, liquorice, soufflé, croissant, Danish pastry, cookies, cream cake, pancake, cream puff, light/not light versions	1
SugaryDrink	Soda, juice, lemonade, chocolate milk, milk shake and yogurt drink, mix of light/not light versions	1
FastFood †††	Fried potatoes, French fries, hotdogs, pizza, burgers, spring rolls	1

1: intake based directly on the recorded foods, 2: total intake calculated from standard recipes. †In Paper I and II *BreakfastCerealsAddSugar* and *BreakfastCerealsNoAddSugar* were combined and named *BreakfastCereals*. †† *BreastMilk* is not categorised according to data layer because the estimation is not based on the diet diaries. †††In Paper I and II *Chips* and *FastFood* were combined and named *FastFood*

3.1.4 Statistics

In Paper I dietary patterns were displayed by PCA and the data structure for this PCA is shown in **Table 7**. Here all score values in each principal component in the PCA represents different children all at the age of 9 mo and some of the children belongs to SKOT I and some belongs to SKOT II. The PCA displayed in Paper I was also the basis for Paper II. In Paper III dietary patterns were also displayed by PCA with food variables from 9, 18 and 36 mo diet registrations in one PCA based on a data set in long format (**Table 8**) meaning that each participant constitutes three rows in the data matrix, one for each age. Herby each participant will appear with three score values in each principal component in the PCA.

Table 7 Data structure of the variables used in the PCA in Paper I and II

ID	Cohort	Registration age	Intake Food1	Intake Food2	…	Intake Food23
1	SKOT I	9 mo				
2	SKOT I	9 mo				
…	SKOT I	9 mo				
307†	SKOT I	9 mo				
1	SKOT II	9 mo				
2	SKOT II	9 mo				
…	SKOT II	9 mo				
142	SKOT II	9 mo				

†One participant in SKOT I had a missing weight recording and amount of food per kg body weight could not be calculated and the participant could not be included in the PCA, giving a total of n=307.

Table 8 Data structure of the variables used in the PCA in Paper III

ID	Cohort	Registration age	Intake Food1	Intake Food2	…	Intake Food25
1	SKOT I	9 mo				
2	SKOT I	9 mo				
….	SKOT I	9 mo				
229	SKOT I	9 mo				
1	SKOT I	18 mo				
2	SKOT I	18 mo				
….	SKOT I	18 mo				
229	SKOT I	18 mo				
1	SKOT I	36 mo				
2	SKOT I	36 mo				
….	SKOT I	36 mo				
229	SKOT I	36 mo				

3.2 MoMS

3.2.1 Study design

MoMS is a randomised parallel intervention study. The participants in MoMS were asked to drink 1 L/day of skim milk, whey, casein, or mineral water for 12 weeks (**Figure 9**). Karina Arnberg, Julie Brønholm, Anni Larnkjær, Christian Mølgaard, and Kim F. Michaelsen were the main responsible for design and data collection in MoMS. I participated in the last part of data collection.

The overall aim of MoMS was to investigate the effect of a milk, whey or casein test drink on biomarkers of metabolic syndrome in overweight adolescents, compared to water. Data collected in MoMS at week 0, just before the intervention period and at week 12, the last week during intervention, were used in Paper IV. However, MoMS included two additional points of data collection (**Figure 9**). A subgroup of 33 participants had a physical examination and diet registration 12 weeks before the randomisation to the intervention (-12 wk) and served as a pre-test control group. This pre-test control group was randomised into the four intervention arms together with the rest of the participants after the examination at week 0. To insure an equal gender distribution between intervention groups the participants were randomised in blocks of 12 (3 skim milk, 3 whey, 3 casein, 3 water) stratified by gender.

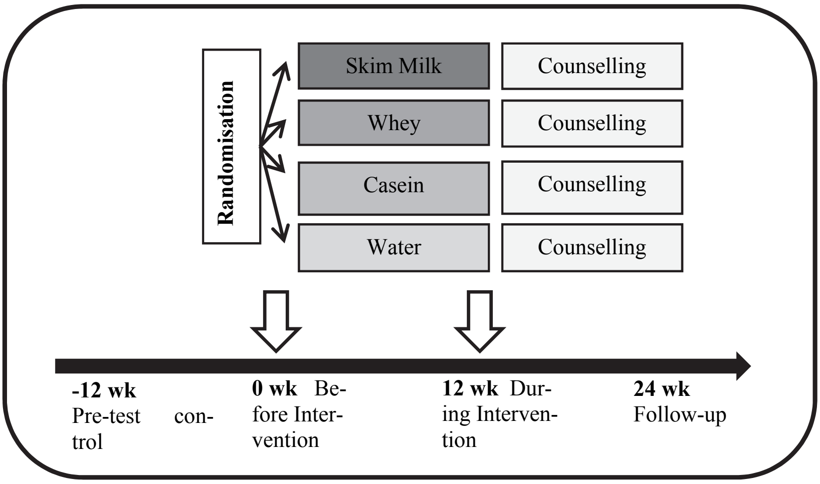

Figure 9 The study design in MoMS
wk: week

After the intervention all participants were offered lifestyle group counselling facilitated by a psychologist with the aim of providing tools to obtain a healthier lifestyle and to meet overweight adolescent fellows. Twelve weeks after ending the intervention a physical follow-up examination was carried out. The duration of the intervention was based on the consideration that it is long enough to observe body weight changes.

3.2.2 Recruitment and drop-out

The participants were recruited by postal invitations to adolescents born in year 1995 to 1998 living in the Copenhagen area based on extractions from the National Civil Registration System and flyers. Participants were recruited over a 2 year period leaving out the summer holiday (July-August) to avoid bringing test drinks on vacation. Data collection ran from autumn year 2008 to spring 2011. Inclusion criteria were healthy, overweight (age-and sex-adjusted BMI corresponding to an adult BMI>25kg/m^2(88)) adolescents at 12-15 years of age and with a habitual milk and yogurt intake below 250 ml/dag. Low habitual milk and yogurt intake was demanded in order to be able to increase the milk intake considerably and thereby observe potential effects. About 20-25% of Danish adolescents drink less milk and milk products than 250 ml/day according to a national survey (127). The exclusion criteria were adolescents who took antibiotic less than one month before intervention start or who smoked regularly or had chronic disease or serious long-term illness after the person was included in the study. Based on sample size calculations the recruitment goal was 200 participants with 50 in each intervention arm whereby the study should be able to detect a significant weight difference during intervention of about 1 kg and an expected drop-out of 10%. The inclusion rate from the invitation letters was approximately 0.7 % and a total of 203 were included and of these, 173 participants had complete diet registrations both before and during intervention (**Figure 10**).

3.2.3 Test drinks and compliance

The two milk fractions whey and casein were tested in addition to skim milk because former studies have shown that these two fractions seem to have different qualities e.g. in relation to satiety, which might affect weight control (112;113;115). Moreover, other studies investigating the effect of dairy supplementation on body weight and factors of metabolic syndrome use an isocaloric glucose solution as control drink (128;129). However, such a control drink may in itself have a negative effect on weight and risk factors. Therefore water was used for comparison in the MoMS study. However, the water group should not be assumed to be an inactive control because studies in adults have shown increased weight loss when water intervention was combined with a hypocaloric diet compared to only a hypocaloric diet (130). Therefor the water group was perceived as an intervention group equally to the dairy groups and is the focus of Paper IV.

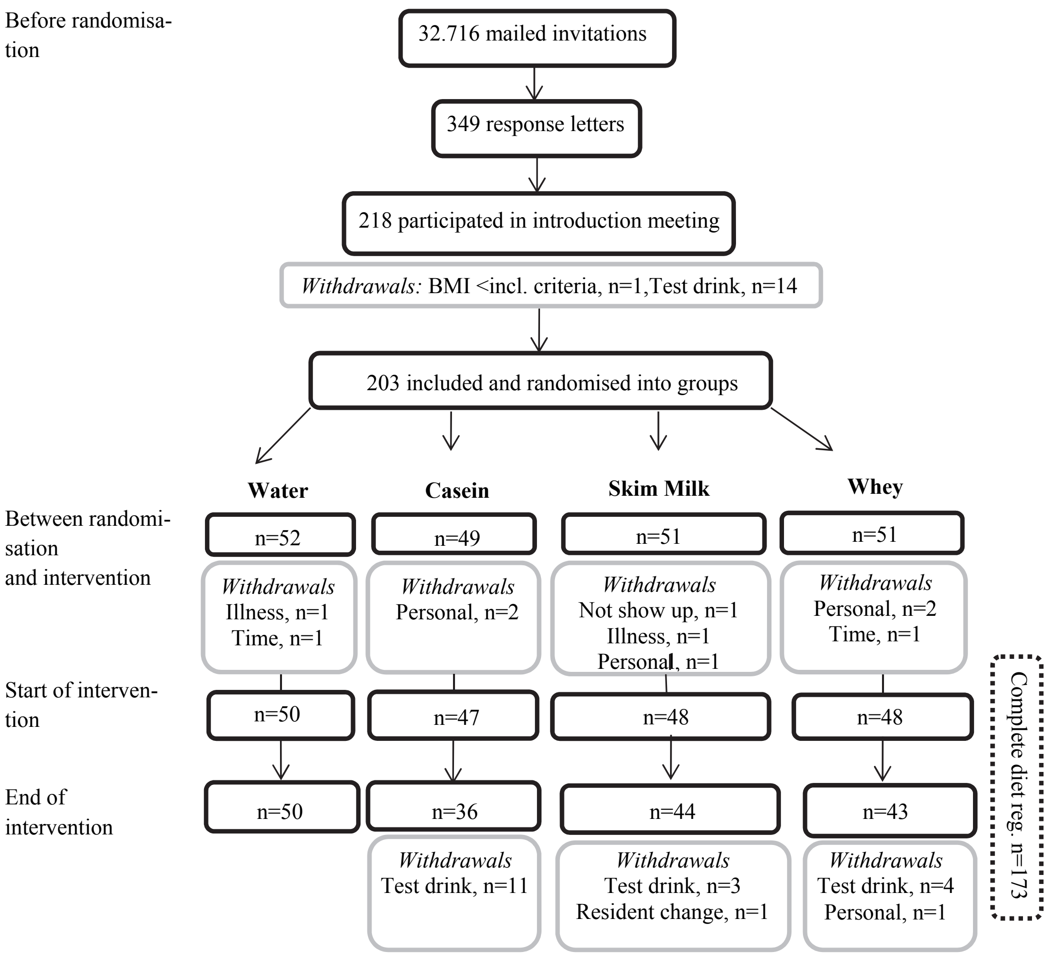

Figure 10 Participants and drop outs in MoMS from recruitment to 12-wk examination

·········· Included in Paper IV

The dairy test drinks were provided by Arla Food Ingredients and the water was provided by Jørgensen Engros A/S. The water was packed in 0.5 L transparent plastic bottles, while the dairy test drinks were long life products and packed in identical 200 ml white cartons and coded by a technical assistant who took care of randomisation but was not involved in data collection. Hereby the dairy drinks were blinded for participants and investigators, while the water group was not blinded. The nutrient content of the test drinks is shown in **Table 9**. Arla Food Ingredients aimed at similar amounts of calcium, phosphor, and lactose in the three dairy test drinks, but this was not possible due to sensory difficulties. Especially the whey test drink would have been too sour if this should be fulfilled. Compliance was monitored by three measures. The participants were asked to record consumption of test drinks in a booklet and to count leftover cartons or bottles. Moreover, serum urea nitrogen (SUN) was analysed as a measure of resent protein intake (131). Agreement across compliance measures was observed (132) and in Paper IV data from the booklet and counts of leftovers were used.

Table 9 Nutritional composition of the test drinks

Nutrients	Skim Milk	Whey	Casein	Water
Energy, kJ/100g	156	137	136	0
Fat, g/100g	0.47	0.004	0.05	0
Lactose, g/100g	4.68	4.45	4.44	0
Protein, g/100g	3.47	3.48	3.46	0
Casein/Whey ratio	80:20	0:100	100:0	0/0
Na, mg/100g	30	10	140	0.8
P, mg/100g	100	60	40	-
Ca, mg/100g	120	10	60	3.1
Mg, mg/100g	10	0	0	0.2
K, mg/100g	160	10	90	0.1

3.2.4 Measurements and questionnaires

3.2.4.1 Diet

The participants were requested to eat their normal diet *ad libitum* (defined as "background diet") in addition to the test drink during the study. Diet was recorded prospectively using 4-day food diaries (3 weekdays, 1 weekend day) with the same pre-coded concept and potion size estimation as used in the SKOT cohorts (section 3.1.3.1) but adapted for older children as in national surveys (127). The National Food Institute, Denmark did the digitalisation, quality control and assessment of nutritional content after data collection. The selected food variables were identical to foods investigated in the SKOT cohorts (section 3.1.3.1) except omission of baby food (*Porridge, Formula* and *BreastMilk*). Moreover, the Milk variable in MoMS was subdivided into two variables; *HighFatMilk* and *LowFatMilk* because MoMS had a special focus on milk and because detection of possible changes of subgroups of dairy products in the background diet was of special interest.

3.2.4.2 Anthropometry
Measurements of anthropometry are described in Paper IV and will not be reproduced here.

3.2.5 Statistics
Dietary patterns in Paper IV were displayed by PCA with food variables from before and during intervention together in long format **(Table 10)** meaning that each participant constitutes two rows in the data matrix; one representing intake *before intervention* and one representing intake *during intervention*. Herby each participant will appear with two score values in each principal component in the PCA – one for each time point.

Table 10 Data structure of the variables used in the PCA in Paper IV

ID	Study	Registration	Intake Food1	Intake Food2	…	Intake Food23
1	MoMS	Before				
2	MoMS	Before				
….	MoMS	Before				
173	MoMS	Before				
1	MoMS	During				
2	MoMS	During				
….	MoMS	During				
173	MoMS	During				

4 Results

The results are divided into four scientific manuscripts (section 4.1 to 4.4). Each of these should be perceived as an independent peace of work including its own section of introduction, subjects & methods, results, discussion, reference list, and numbering of illustrations.

4.1 Paper I: Maternal obesity and offspring dietary patterns at 9 months of age

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Tables and figures can be found at the end of the manuscript

Maternal obesity and offspring dietary patterns at 9 months of age

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Abstract

Background/ Objectives: Differences in the quality of complementary feeding between infants of obese and non-obese mothers have not been examined sufficiently. The aim of this paper was to compare dietary patterns, foods, nutrients, and energy intakes of 9-mo-old Danish infants in a cohort comprising obese mothers (SKOT II, n=184) with a cohort consisting mainly of non-obese mothers (SKOT I, n=329). **Subjects/Methods:** Dietary intake was assessed by 7-day records, and dietary patterns were identified by principal component analysis. **Results:** SKOT I was characterized by a lower maternal BMI and a higher social class than SKOT II in relation to parental education and occupation. Infants in SKOT II had lower scores on a HEALTH-CONSCIOUS FOOD pattern reflected at the food group level, e.g. with lower intake of the food groups *Fruit* and *Vegetable* but higher intake of *WheatBreadNoWholegrain* in SKOT II compared to SKOT I. Moreover, SKOT II had shorter durations of breastfeeding, earlier introductions of complementary feeding, higher energy intake from protein but lower energy intakes from monounsaturated fatty acids and polyunsaturated fatty acids at 9 mo. SKOT II had higher weight-for-age and length-for-age z-scores, but no differences in BMI z-scores, than SKOT I at 9 mo. **Conclusions:** Infants of obese mothers from a lower social class seem to have a less healthy diet and higher weight and length z-scores at 9 mo. Therefore, the promotion of healthy complementary feeding might be beneficial for the prevention of health implications, such as obesity, later in life for these infants.

Keywords: obese, mother, dietary patterns, infants

Introduction

It is well established that maternal obesity is associated with early childhood obesity and it seems that environmental factors like the intrauterine nutrient environment[1] and breastfeeding practice[2], which have been widely investigated, are likely to play a role in the causal link[3,4]. For example, obese mothers have lower rates of initiation and duration of breastfeeding[5,6]. Therefore, children of obese mothers are more frequently formula-fed, which increases the risk of obesity in later life. The higher protein content in infant formula compared to breast milk is likely to be part of the explanation[7]. The relation between maternal obesity and complementary feeding practice is much less investigated but is potentially important for the intergenerational transfer of obesity risk because the infants are still nutritionally vulnerable, and this period seems to contribute to the establishment of life-long eating habits[8]. However, the focus has mainly been on the timing of complementary feeding and less on the quality of food[8].

The quality of complementary food can be investigated at different levels. When the infant changes from a milk-based diet to the family's diet, the complexity increases and so also the need for a diversified characterization of dietary patterns comprising the whole diet, supported by information about intake of energy, nutrients, and foods. To our knowledge, the relation between maternal BMI and offspring dietary patterns within the first 2 years of life has been investigated in 4 studies[9-12], and only 2 of these have investigated the relation within the first year of life[10,11].

The aim of this paper is to compare the dietary patterns, foods, nutrients, and energy intake of Danish infants at 9 mo in a cohort consisting of obese mothers with a cohort consisting mainly of non-obese mothers.

Subjects and Methods

Study design and participants

This study is based on two comparable observational cohorts, SKOT I and SKOT II (SKOT; Danish abbreviation of small children's diet and wellbeing), when infants were 9 mo old. Infants in SKOT I were recruited by postal invitations to randomly selected parents of infants based on extractions from the National Civil Registration System. SKOT I required participants to be healthy singletons, born at term, with an age of 9mo±2 weeks at the first examination and having Danish-speaking parents[13]. Participants for SKOT II were recruited among offspring of obese pregnant women participating in the intervention study, "Treatment of Obese Pregnant Women" (TOP) at Hvidovre Hospital with dietetic and physical activity counseling[14] followed by breastfeeding counseling for a subgroup of the participants[15]. Hereby, the inclusion criteria for SKOT II was equal to SKOT I, except that all participants were required to be offspring of women with pre-pregnancy BMI>30kg/m^2. Of 2 211 random selected infants from the National Civil Registration System invited to participate 329 (15%) participants were included in SKOT I, of which 95% completed the 9-mo examination. In SKOT II, 208 infants whose mothers had participated in the TOP intervention were invited and 184 (88%) were included, of which 90% completed the 9-mo examination. In SKOT I, 99% of the infants participating in the 9-mo examination completed diet registration, while this was 86% in

SKOT II. Collection of 9-mo data was carried out in the year 2007-2008 and year 2011-2012 for SKOT I and SKOT II respectively because the TOP study started later. Official Danish guidelines for infant feeding did not change during this period. SKOT I (H-KF-2007-0003) and SKOT II (H-3-2010-122) were approved by The Committees on Biomedical Research Ethics for the Capital Region of Denmark.

Dietary data
The diet was recorded by parents using validated 7-day food records[16]. Portion sizes were estimated with household measures and food photograph series and noted in a pre-coded food diary. The calculation of nutrient intake has previously been described[16]. Possible over- and under-reporters were identified based on the estimated daily energy requirement of 338kJ/kg for both genders, as an average between 6- and 12-mo estimates[17] and cut-off values of +/-46%[18]. The food groups were selected based on nutritional knowledge in an attempt to cover most aspects of the official recommendations, nutrition evidence, and typical infant diet in Denmark. The intake (g/day) of all food groups, except breast milk, was divided by total body weight (kg) for each participant. Intake of breast milk was estimated as number of breast feedings per day (<1, 1–2, 3–5, 6–8, >9) and recoded as the mean of each interval. These breastfeeding categories were used in the PCA, while an estimate of 99g/feeding[19] was used in the box plot of food groups and in the calculation of total energy intake.

Background characteristics
Information about parental and household factors was collected via interviews and questionnaires when the infants were aged 9 mo. Total household income was divided into more or less than 650 000DKK(~120 000US$) per year (country average for families is 684 000DKK(~127 000US$) (20)). The weights and heights of the mothers in SKOT I were self-reported, while, for the mothers in SKOT II, they were measured during the 9-mo examination (weight: Tanita WB-100MA, Tanita Corporation, Tokyo, Japan; height: 235 Heightronic Digital Stadiometer). Duration of breastfeeding and age for introduction of complementary feeding were recalled at 9 mo. Exclusive breastfeeding was defined as receiving only breast milk, water, and vitamins. Birth weight and length measurements were carried out by midwives and obtained from health records. Weight and length measurements at 9 mo were carried out at the Department of Nutrition, Exercise and Sports, University of Copenhagen, by trained research staff. Using a digital scale (Sartorius IP65; Sartorius AG, Göttingen, Germany), weight was measured, without clothes, to the nearest 0.1kg. Recumbent length was a mean of 3 measurements carried out with a digital measuring board (Force Technology, Brøndby, Denmark) and recorded to the nearest 0.01cm. Weight, length, and BMI were converted to z-scores, using the WHO growth standards as a reference and the software program WHO Anthro 2005(ref. 21). Parents were requested to start the dietary assessment just before and just after the examination in SKOT I and SKOT II respectively for logistic reasons, but the examination visit did not include any dietary advises.

Statistical analyses

For categorical variables, cohorts were compared via Pearson's chi-squared test or Fisher's exact test. For continuous variables cohorts were compared using the 2-sample t-test, Welsh's t-test or Mann-Whitney test. All statistical tests were conducted in the statistical programming environment R, version 3.0.2(www.r-project.org). Significant dietary differences between SKOT I and SKOT II are reported as P<0.001***, <0.01**, <0.05*. For the identification of dietary patterns, PCA was carried out in MATLAB R2010b using PLS_Toolbox, version 7.3.1.on a pooled sample of SKOT I and SKOT II data, centered and scaled to unit variance. The influence plot (sample sum squared residuals versus Hotellings T^2 within the model), which provides a quantitative measure of how well the data of individual persons fit the PCA model in terms of the underlying patterns, was used to verify the pooling of dietary data from SKOT I and SKOT II. Naming of dietary patterns was based on food groups with highest loadings. The number of principal components was selected based on a clear change in the scree plot[22].

Results

Background characteristics of SKOT I and SKOT II

As expected, the maternal BMI in SKOT II was distinctively higher than in SKOT I (**Table 1**). At 9 mo, 4% and 92% of the mothers were obese and 19% and 7% were overweight in SKOT I and SKOT II, respectively. A smaller difference was seen for paternal BMI. The social class was higher in SKOT I compared to SKOT II, both in relation to maternal and paternal job situations and educational levels.

According to the child characteristics, the breastfeeding duration was shorter in SKOT II, and the introduction of complementary feeding began earlier. The median duration of exclusive breastfeeding was 129 days in SKOT I versus 75 days in SKOT II. In SKOT I, 59%, compared to 80% in SKOT II, introduced complementary feeding at 4 mo or earlier. At birth, there was a trend of higher BMI z-scores in SKOT II, caused by higher weight-for-age z-scores. At 9 mo, SKOT II had higher weight-for-age and length-for-age z-scores, but no differences in BMI-for-age z-scores.

Comparing intake of energy in SKOT I and SKOT II

Dietary registrations at 9 mo were available for 308 and 142 infants in SKOT I and SKOT II respectively. The mean energy intake recorded at 9 mo in SKOT I was 3 531±885 kJ/day, which was higher (p=0.002) than 3 250±842 kJ/day in SKOT II. Both in SKOT I and II, 1% were possible under-reporters; in SKOT I, 15% were possible over-reporters, compared to 9% in SKOT II, but none of these were excluded.

Comparing intake of nutrients in SKOT I and SKOT II

Protein intake, expressed as a percentage of energy, was significantly lower in SKOT I, while intake of energy from monounsaturated fatty acids (MUFA), energy from polyunsaturated fatty acids (PUFA), and fiber (mg/kJ) was significantly higher in SKOT I compared to SKOT II (**Table 2**). In SKOT I 9% of infants and in SKOT II 18% of infants had percentages of energy intake from protein that were higher than the Nordic Nutrition Recommendations.

Comparing intake from food groups in SKOT I and SKOT II

The diet was divided into 23 food groups representing the whole diet (**Table 3**). *BreastMilk, BreakfastCereals, SweetsCake* and *SugaryDrink* were eaten by fewer than 50% of the participants in both cohorts at 9 mo. Infants in SKOT I had a significantly lower median intake of *Milk, PastaRice, WheatBreadWholegrain, WheatBreadNoWholegrain* but a higher intake of *Fruit, Vegetable, Potato, Porridge, BreastMilk, FruitNutSnack, FatsVegetable,* and *FastFood* compared to SKOT II (**Figures 1A and B**).

Comparing dietary patterns in SKOT I and SKOT II

A visual inspection of the influence plot showing an equal distribution of residuals in SKOT I and SKOT II indicated similar underlying dietary patterns in both cohorts, verifying that the diets in the cohorts were suitable for pooling in one PCA. Two dietary patterns were identified in this pooled sample of the cohorts at 9 mo of age (**Figure 2**). The first pattern was named FAMILY FOOD pattern and explained 13% of the variation in the intake of food groups. This pattern was characterized by low loadings for the baby foods *Formula* and *BreastMilk* and high loadings for family foods like *Meat, Milk, RyeBread* and *FatsAnimal*. The second pattern was named HEALTH-CONSCIOUS FOOD because it had low loadings for foods like *SweetsCake, WheatBreadNoWholegrain,* and *SugaryDrink* and high loadings for foods like *Potato*, *Vegetable, Fruit,* and *Fish*, and it explained 9% of the variation. No significant difference was seen between cohorts in relation to the FAMILY FOOD pattern, while infants in SKOT I had higher score values in the HEALTH-CONSCIOUS FOOD pattern than infants in SKOT II (**Figure 3**).

Discussion

Significant differences in the infant diet between SKOT I and SKOT II were found. This included lower scores on the HEALTH-CONSCIOUS FOOD pattern and higher intake of energy from protein for infants in SKOT II. Both of these are likely to be associated with an increased risk of obesity in later life. The findings are of public health relevance, since infants in SKOT II, from birth, are already at a higher risk of developing obesity and metabolic complications because they are all born to obese mothers with a lower social class than those in SKOT I.

Comparing dietary patterns in SKOT I and SKOT II

Lower scores on a healthy dietary pattern for young children of mothers with higher BMI are in agreement with previous findings[9,10,12,23,24]. If maternal diet was included in the analysis, this was the strongest indicator for the dietary pattern of the infant[10], whereby a considerable part of the association between maternal BMI and infant dietary patterns is probably mediated through the mothers' own food choices. The health implications, including the risk of obesity, related to the difference in the HEALTH-CONSCIOUS FOOD pattern at this age, are uncertain. Studies investigating the association between dietary patterns and the BMIs of young children have been inconclusive[25-28]. Moreover, the differences between the cohorts in the HEALTH-CONSCIOUS FOOD pattern might increase with age because the complementary period is a period with special baby food products and parents are often very conscious about food choices in this period but this might be difficult to maintain after the complementary feeding period.

Comparing intake from food groups in SKOT I and SKOT II

The difference in the HEALTH-CONSCIOUS FOOD pattern between cohorts is reflected in differences in individual food groups. The higher intake of *Fruit*, *Vegetable,* and *FatsVegetable* indicates a healthier diet in SKOT I. Unexpectedly, SKOT I had a higher intake of *FastFood,* which mainly represented intake of sausages and fried potatoes. However, the intake of *FastFood* at 9 mo was low and most likely does not have health implications at this age. The slightly older age of infants in SKOT II could imply a more advanced stage in complementary feeding transition and thereby a lower intake of *Porridge* and a higher intake of *Milk* than SKOT I. In Denmark, cow's milk is recommended to be gradually introduced as a drink at 9 mo of age[29]. However, the age difference of 9 days is probably irrelevant, and it might rather be an expression of the earlier age of introduction of complementary foods and different complementary feeding practices in the cohorts. Even though infants in SKOT I had shorter times between their first introduction of complementary foods and the diet registration, as complementary food was introduced later, the variation in the share of the whole diet coming from *BreastMilk* or *Formula* does not seem to pose differences between the cohorts. This is based on the finding of no differences in the FAMILY FOOD pattern, which is an overall expression of the transition from *BreastMilk* and *Formula* (left side of Figure 2) to other foods (right side of plot). Differences in feeding practices between the cohorts were more dependent on choices within the other foods, and the difference in *BreastMilk* versus *Formula* intake at this age as the adherence to the HEALTH-CONSCIOUS FOOD pattern differed. Health implications of the differences in intake of foods on the right side of Figure 2 is uncertain, but the sole difference in current breastfeeding practice, together with the finding of longer duration of exclusive breastfeeding, might contribute to different risks of future obesity in the cohorts. A shorter duration of breastfeeding and early introduction of complementary feeding are often highly correlated[30] and have been associated with increased risk of childhood obesity[31;32].

Comparing intake of nutrients in SKOT I and SKOT II

The higher percentage of energy intake from protein in SKOT II was expected because of the higher intake of *Milk* and *Formula* (although non-significant), which have higher protein content than *BreastMilk*. We are not aware of studies reporting a higher energy percentage from protein in the complementary feeding period in infants of obese mothers, but higher percentages of energy from carbohydrates in complementary foods and a higher total energy intake has been reported[33]. The 9% of infants with a percentage of energy from protein above the recommendation in SKOT I was comparable with the 10% found in a previous national survey[34]. Yet the higher percentages of energy from protein in SKOT II resulted in a considerably higher proportion (18%) of infants with an intake above the recommendations. The higher protein intake might contribute to the higher weight-for-age z-scores observed in SKOT II, as a high protein intake during infancy has been associated with higher weight-for-age z-scores[35]. However, no differences were observed in BMI z-scores between the SKOT cohorts; neither did the study that reported a higher energy intake from carbohydrates in infants of obese mothers find differences in current BMIs[33]. Still, higher BMIs might appear later in life, as it was concluded in a recent review that a high intake of protein, particularly dairy protein in infancy, could be associated with increased BMI later in childhood[8]. Nevertheless, it cannot be excluded that the percentage of energy from protein is influenced by a differentiated under-reporting of fat- and carbohydrate-rich products, especially in SKOT II.

Strengths and limitations

The main strengths of this study are the dietary assessment with thorough portion size estimation in infancy plus the analysis of different dietary levels, including dietary patterns, in two very diverse cohorts of infants that represent a wide range of the Danish spectrum in relation to maternal BMI and social class[36,37]. The main limitation of the study was a possible reduced generalizability of identified dietary differences between the cohorts to other Danish infants with parents of similar BMIs and social classes. This is due to a potential effect of the maternal intervention in SKOT II during pregnancy and postpartum, with a focus on prolonging breastfeeding and healthy diet for the pregnant woman, which also might affect her complementary feeding practice. The interventions were able to increase the duration of breastfeeding considerably[15] and to reduce the maternal gestational weight gain[14]. Despite this focus, the breastfeeding duration in SKOT II was considerably shorter than in SKOT I, and the diet quality at 9 mo was lower. Information on duration of breastfeeding was collected at 9 mo which could result in some recall bias, but we do not believe it is an important problem and the effect of recall bias is not expected to differ substantially between the cohorts. There was a large difference in the success rate of inclusion between the studies; in SKOT I (15%) compared to SKOT II (88%). SKOT I is a highly selected group from a random sample of families invited to participate, while SKOT II is recruited from a health intervention study during pregnancy, and thereby both cohorts are most likely families with some interest in healthy living which may also reduce the generalizability. The success rate in SKOT II shows the accomplishment recruiting infants born to obese mothers through the intervention study which otherwise is a difficult group to reach. Over-reporting might explain the higher energy intake in SKOT I, but it is unknown if the observed over-reporting was random or mostly related to healthy foods. A further limitation in this study is the relatively rough portion size estimation of breast milk intake, which differed from the rest of the dietary assessment. This might explain the location of *BreastMilk* at the lower end of the HEALTH-CONSCIOUS FOOD pattern.

Conclusion

Compared to infants born mainly to non-obese mothers from a higher social class (SKOT I), at 9 mo, infants of obese mothers (SKOT II) had earlier introductions of complementary feeding and less healthy diets, including less breastfeeding, a higher energy intake from protein but a lower energy intake from MUFA and PUFA, and a possibly less healthy overall dietary pattern, including a lower intake of *Fruit* and *Vegetable* and a higher intake of *WheatBreadNoWholegrain*. A promotion of healthy complementary feeding seems highly relevant for these infants because, from birth, they have a higher risk of future obesity, and the observed nutritional differences might increase the risk further. Already at 9 mo of age, they have higher weight and length z-scores than the infants born to non-obese mothers of a higher social class. However future studies should contribute to our knowledge about possible health implications of the dietary differences observed.

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Table 1 Characterization of SKOT I and SKOT II, including comparison of the two cohorts

		SKOT I[1]	SKOT II[1]	p
Maternal factors				
Work situation	Has a job, %	79	77	
	Student, %	12	7	
	Not at labor market or student, %	9	16	**0.02[2]**
Education level	Basic education, %	18	36	
	Short education, %	10	13	
	Medium education, %	33	33	
	Long education, %	39	18	**< 0.0001[2]**
Age at childbirth	Years, mean ± SD	31.8 ± 4.6	31.7 ± 4.5	0.82[3]
BMI when child is 9 mo[4]	kg/m^2, median (IQR)	22.4 (20.5;24.7)	35.2 (32.3;37.7)	**< 0.0001[5]**
Paternal factors				
Work situation	Has a job, %	89	82	
	Student, %	9	6	
	Not at labor market or student, %	2	7	**0.03[2]**
Education level	Basic education, %	29	46	
	Short education, %	8	12	
	Medium education, %	21	25	
	Long education, %	43	17	**< 0.0001[2]**
BMI when child is 9 mo	kg/m^2, mean ± SD	25.4 ± 3.3	27.9 ± 4.8	**< 0.0001[6]**
Household factors				
Number of adults in household	1, %	5	6	
	2, %	95	93	
	3 to 4%	0.3	1	0.74[2,7]
Number of children in household	1, %	57	57	
	2, %	31	34	
	3 to 7, %	11	9	0.66[2]
Household income[8]	Less than 650 000 DKK, %	41	49	
	650 000 DKK or more, %	59	51	0.13[2]
Parental immigration /descendant status	Danish mother and father, %	80	72	
	Mother and/or father not Danish, %	20	28	0.07[2]
Child factors				
Weight-for-age at birth	Z-score, median (IQR)	0.5(0.01;1.0)	0.7(0.09;1.5)	**0.013[5]**
Length-for-age at birth	Z-score, median (IQR)	1.5 (0.6;2.1)	1.5(0.6;2.2)	0.21[5]
BMI-for-age at birth	Z-score, mean ± SD	-0.3 ± 1.0	-0.1±1.2	**0.046[6]**
Age at diet registration	mo, median (IQR)	8.8(8.5;9.0)	9.1 (8.9;9.4)	**< 0.0001[5]**
Weight at 9 mo	kg, median (IQR)	9.0 (8.3;9.7)	9.3 (8.7;10.1)	**0.0002[5]**
Weight-for-age at 9 mo	Z-score, mean ± SD	0.4 ± 0.9	0.8 ± 1.0	**< 0.0001[3]**
Length-for-age at 9 mo	Z-score, mean ± SD	0.3 ± 1.0	0.8 ± 1.0	**< 0.0001[3]**
BMI-for-age at 9 mo	Z-score, median (IQR)	0.3 (-0.3;1.0)	0.4 (-0.2;1.2)	0.15[5]
Duration exclusive BF	Days, median (IQR)	129 (91;152)	75 (14;122)	**< 0.0001[5]**
Age at introduction of complementary feeding	0–3 mo, %	4	11	
	4 mo, %	55	69	
	5 mo, %	28	17	
	6 mo, %	12	3	
	7 mo, %	1	0	**< 0.0001[7,2]**
Day care or home care	Home, %	94	83	
	Day care, %	6	17	**0.0002[2]**
Sex	Girls, %	52	46	
	Boys, %	48	54	0.27[2]

[1]n varies between 303 and 329 in SKOT I and between 127 and 183 in SKOT II; [2]by Pearson's chi-squared test; [3]by unpaired t-test; [4]SKOT I, self-reported in questionnaire; SKOT II, measured at 9-mo examination; [5]by Mann-Whitney test; [6]by unpaired Welsh's t-test; [7]categories collapsed for the test (one or more parents, 6 mo or more); [8]country average for families, 684 000 DKK (~127 000 US$); BF: Breastfeeding

Table 2 Nutrient intake at 9 mo in SKOT I and SKOT II, comparing cohorts, and comparing with recommendations

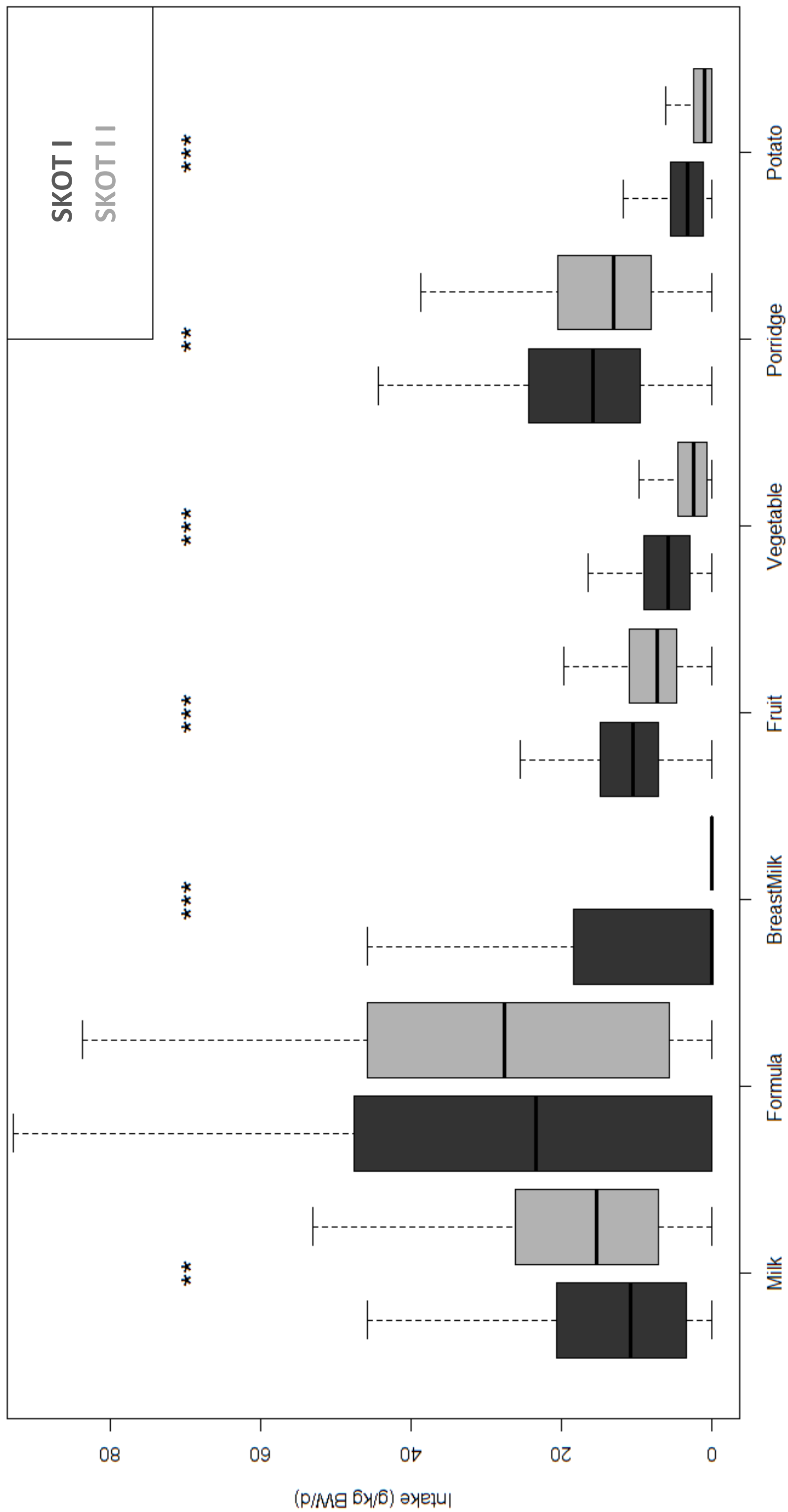
	SKOT I, n=308			**SKOT II,** n=142				**Recommendation**
	Mean ± SD or Median (IQR)	% with intake < rec.	% with intake > rec.	Mean ± SD or Median (IQR)	% with intake < rec.	% with intake > rec.	p[1]	NNR 2012 (17) 6-11mo
Protein (E%)	12 ± 2	0	9	13 ± 2	0	18	***< 0.0001***[2]	7-15E%
Fat (E%)	38 ± 6	7	13	38 ± 5	6	6	0.36[2]	30-45E%
SFA (E%)	15 ± 4	-	90	16 ± 4	-	94	0.11[2]	<10E%
MUFA (E%)	13 ± 3	14	0	12 ± 3	20	0	***0.01***[2]	10-25E%
PUFA (E%)	6(5;7)	34	6	5(4;6)	56	1	***< 0.0001***[3]	5-10E%
Carbohydrate (E%)	52 ± 6	12	8	51 ± 6	13	6	0.29[2]	45-60E%
Added sugar (E%)	1(0;3)	-	1	1(0;3)	-	1	0.59[3]	<10E%
Fiber (mg/kJ)	2.4 ± 0.8	32	21	2.2 ± 0.7	42	13	***0.003***[2]	No rec. <1y[4]

[1]Comparisation of nutrient intake in SKOT I and SKOT II; [2]By unpaired t-test; [3] By Mann-Whitney test if not normally distributed; [4]2–3mg/kJ (> 1y); E%: percentages of total energy from each macronutrient; NNR: Nordic Nutrition Recommendations; rec: recommendation

Table 3 Description of food groups and the percentages of infants with intake > 0 g/day at 9 mo in SKOT I and SKOT II

Food group	Description	SKOT I, % n=307	SKOT II, % n=142	p
Porridge	Cereal gruel, porridge; homemade or ready-prepared	98	98	0.71[1]
BreakfastCereals	Oatmeal, muesli, cornflakes, sugar puffs, and sugary cereals	15	14	0.99[2]
WheatBreadWholegrain	Grainy bread, crisp bread	72	81	0.054[2]
WheatBreadNoWholegrain	White bread, biscuits	76	84	0.089[2]
RyeBread	Rye bread with and without seeds	75	73	0.67[2]
PastaRice	Pasta, rice	62	74	***0.020***[2]
Potato	Potatoes boiled, baked, mashed, or prepared in potato salad	94	83	***0.0006***[2]
Fruit	Fresh fruit and berries, fruit porridge/soup/compote; homemade or ready-prepared	100	98	***0.031***[1]
Vegetable	All vegetables eaten raw/cooked/mashed alone or in a dish	100	96	***0.0048***[1]
Fish	All fish and fish products eaten as sandwich spread or in a dish	85	75	***0.016***[2]
Meat	All meat and meat products eaten as sandwich spread or in a dish, except poultry and fish	98	96	0.53[1]
Poultry	All poultry and poultry products eaten as sandwich spread or in a dish	59	60	0.89[2]
Egg	All egg and egg products eaten as sandwich spread or in a dish	74	78	0.40[2]
FatsAnimal	Butter, spreadable butter, sauce made from butter	93	95	0.42[2]
FatsVegetable	Oil, margarine, mayonnaise, remoulade, ketchup, low-fat sauce	73	66	0.15[2]
Cheese	All cheese and cheese products eaten as sandwich spread or in a dish	79	89	***0.020***[2]
Milk	All milk and milk products eaten alone or in a dish, except human milk or infant formula	98	99	1.00[1]
Formula	Infant formula, follow-up formula	74	77	0.55[2]
BreastMilk	Human milk from the mother	42	23	***<0.0001***[2]
FruitNutSnack	Cereal bar, nuts, almonds, dried fruit and fruit spread, jam, honey, peanut butter, seeds	62	44	***0.00073***[2]
SweetsCake	Ice cream, chocolate, licorice, soufflé, croissant, Danish pastry, cookies, cream cake, pancake, cream puff, mix of light/not light versions	27	30	0.55[2]
SugaryDrink	Soda, juice, lemonade, chocolate milk, milkshake and yogurt drink, mix of light/not light versions	19	15	0.41[2]
FastFood	Fried potatoes, French fries, hot dogs, pizza, burgers, spring rolls, chips	52	47	0.42[2]

[1] Fisher's Exact Test, [2] Pearson's chi-squared test


SKOT I
SKOT I I
Intake (g/kg BW/d)
0
20
40
60
80
Milk
Formula
BreastMilk
Fruit
Vegetable
Porridge
Potato
**
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***
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**
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Figure 1A

Figure 1B

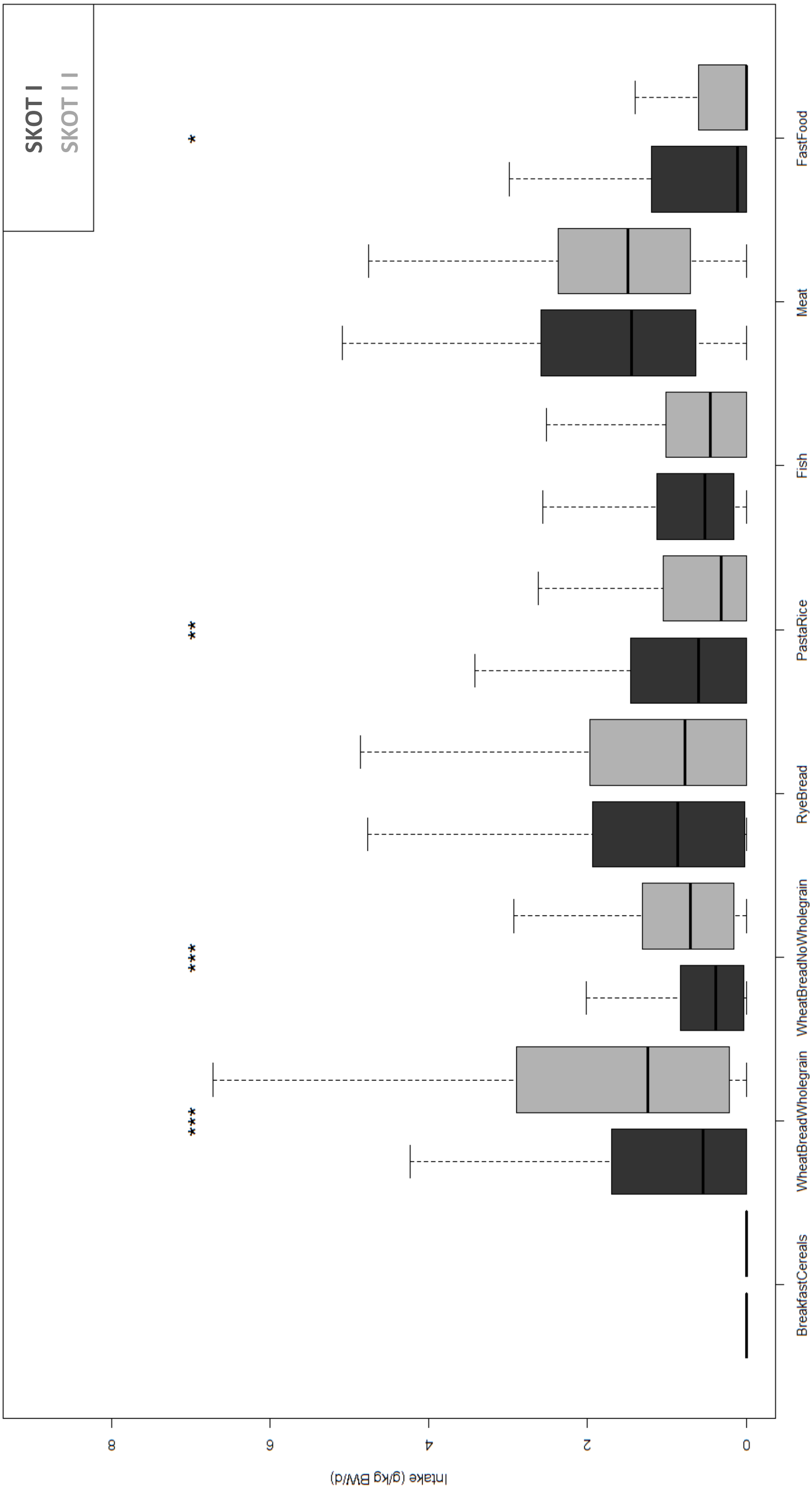
SKOT I
SKOT II
Intake (g/kg BW/d)
0
2
4
6
8
BreakfastCereals
WheatBreadWholegrain
WheatBreadNoWholegrain
RyeBread
PastaRice
Fish
Meat
FastFood
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Figure 1C

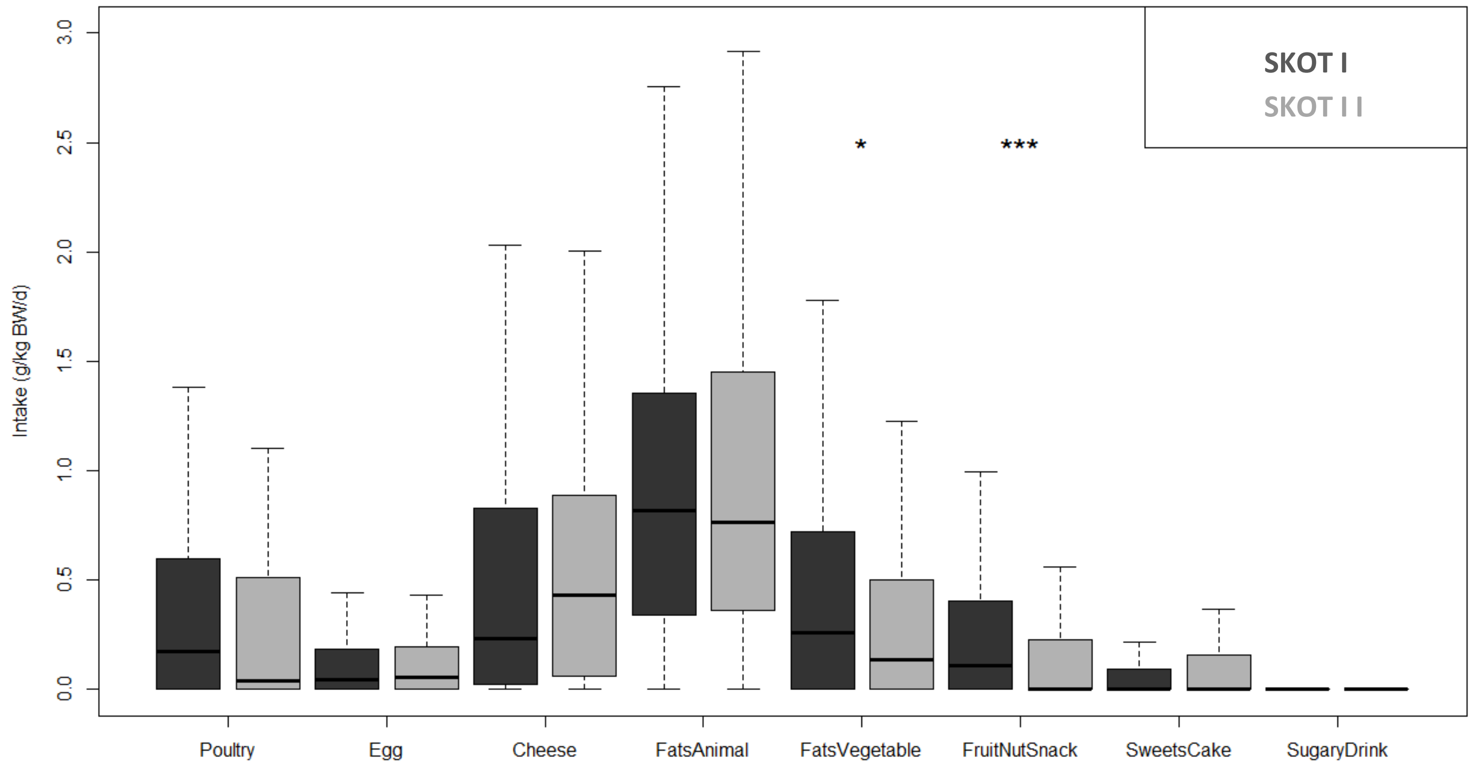

Figure 1 Foods intake at 9 mo in SKOT I and SKOT II, and comparison of cohorts
A: Median intake of the foods *Milk, Formula, BreastMilk, Fruit, Vegetable, Porridge, Potato.* **B:** Median intake of the foods *BreakfastCereals, WheatBreadWholegrain, WheatBreadNoWholegrain , RyeBread, PastaRice, Fish, Meat* and *FastFood.* **C:** Median intake of the foods *Poultry, Egg, Cheese, FatsAnimal, FatsVegetable, Fruit-NutSnack, SweetsCake* and *SugaryDrink.* Significant differences between SKOT I and SKOT II are indicated with asterisks (P < 0.001***, < 0.01**, < 0.05*). If no asterisks, the difference was not significant. Cohorts compared by Mann-Whitney test. Boxes indicate the interquartile range (IQR) around the median and are extended by lines of +/- 1.5 *IQR (or maximum/minimum, if these are within 1.5*IQR). BW: body weight

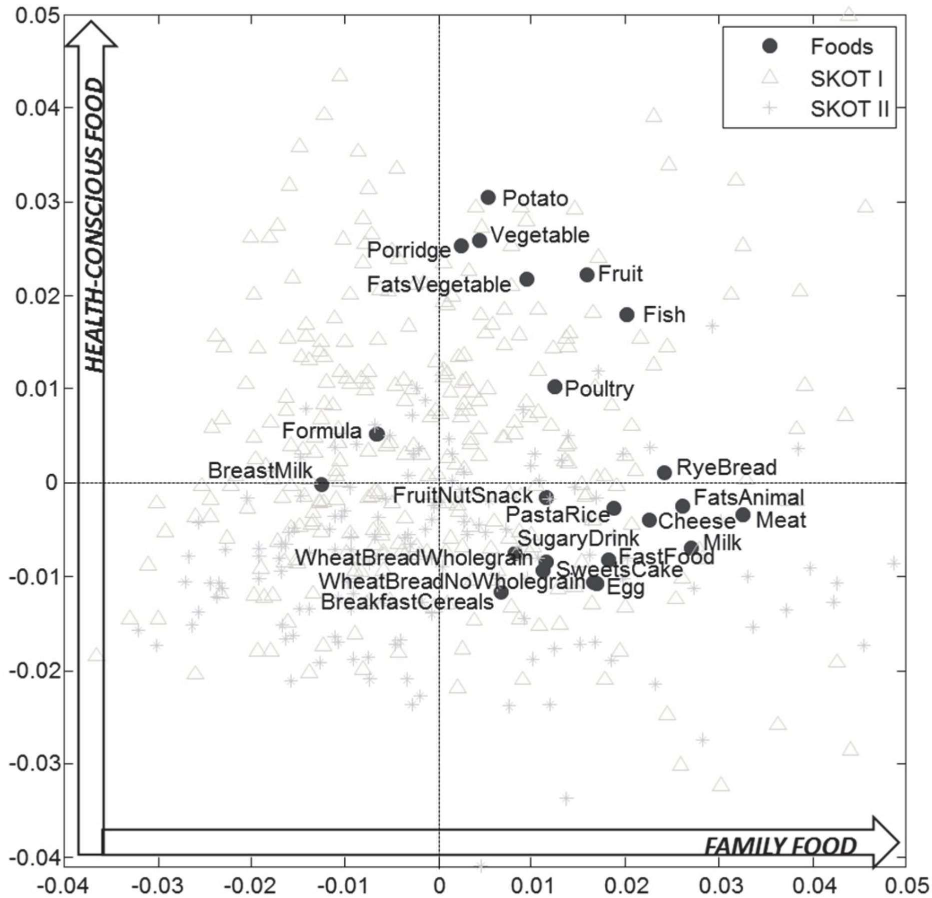

Figure 2 Dietary patterns at 9 mo in SKOT I and SKOT II

The 2 dietary patterns FAMILY FOOD and HEALTH-CONSCIOUS FOOD at this loading plot are based on a PCA with intake of foods in g/kg body weight/d (except BreastMilk which is in feedings /d). The foods causing the patterns are indicated with ●. Scores of participants are indicated with Δ SKOT I, * SKOT II. Foods close to each other are correlated while participants placed close to a certain food variable (loading) have a high intake of this food and a lower intake of foods far away, relative to the rest of the participants in the SKOT cohorts. n(SKOT I) = 307, n(SKOTII) = 142.

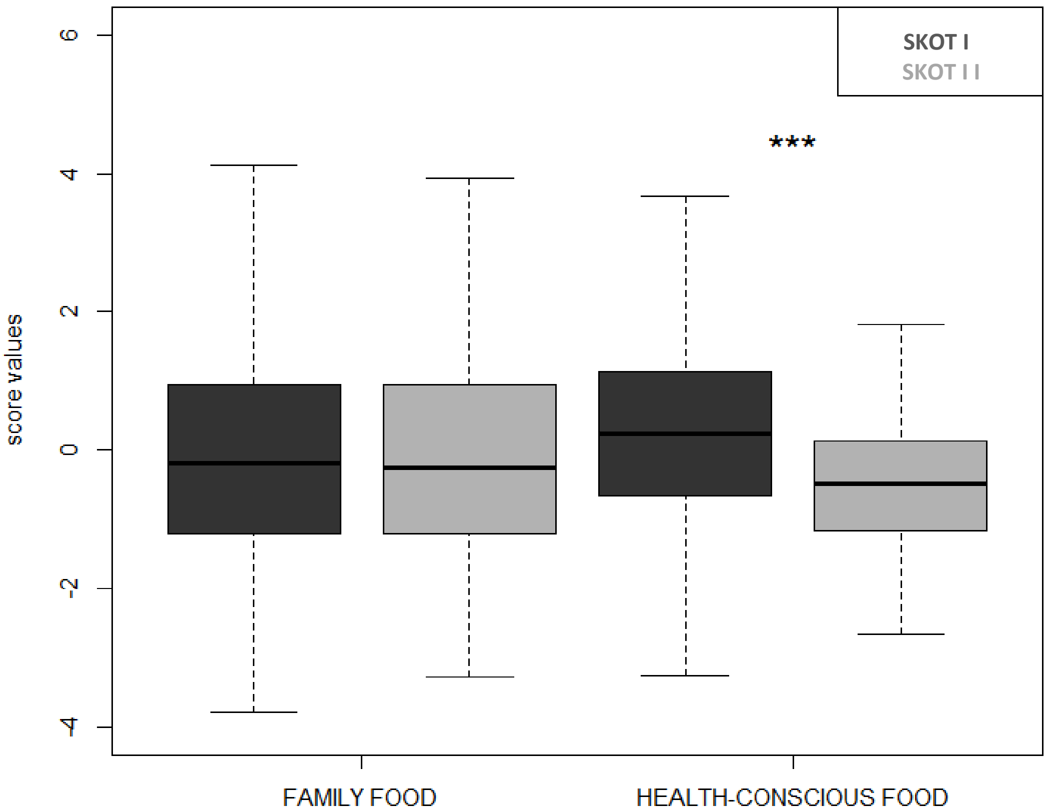

Figure 3 Median score values for the dietary patterns at 9 mo in SKOT I and SKOT II, and comparison of cohorts
Significant differences between SKOT I and SKOT II are indicated with asterisks (P < 0.001***). If no asterisks, the difference was not significant. Cohorts compared by Mann-Whitney test. Boxes indicate the interquartile range around (IQR) the median and are extended by lines of +/-1.5 *IQR (or maximum/minimum, if these are within 1.5*IQR).

4.2 Paper II: Indicators of dietary patterns in Danish infants at 9 month of age

Current status (Oct. 2014): Under revision before submission to a scientific journal

Tables and figures can be found at the end of the manuscript

Indicators of dietary patterns in Danish infants at 9 months of age

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Abstract

It is important to increase the awareness of indicators associated with infant dietary patterns to be able to prevent or to improve undesirable dietary patterns early on. The aim of this study was to investigate the association between a wide range of possible indicators and adherence to dietary patterns for infants aged 9 mo. Dietary patterns were displayed by principal component analysis, and associations with possible indicators were analysed by multiple linear regressions in a pooled sample of two comparable observational cohorts, SKOT I and SKOT II. These cohorts comprised infants with obese mothers versus infants with mainly non-obese mothers, respectively. Infants who were younger at diet registration and had higher BMI z-scores at 9 mo, and those with immigrant/descendant parents, parents who shared cooking responsibilities and fathers in the labour market, had lower FAMILY FOOD pattern scores. Lower FAMILY FOOD scores indicate a higher intake of baby food, as this pattern shows transition from baby food towards the family's food. A higher maternal BMI, a greater number of children in the household, a higher BMI z-score at 9 mo and a higher infant age at diet registration were associated with a lower score on the HEALTH-CONSCIOUS FOOD pattern, indicating a less healthy diet. To conclude, the identification of associations between infant dietary patterns and maternal, paternal, household and child characteristics may improve the possibility of identifying infants with an increased risk of developing unfavourable dietary patterns and potentially enable an early targeted preventive support in families with these characteristics.

Keywords: indicators, dietary patterns, infants

Introduction

Diet during infancy is important for health, both early and later in life, and tracking of eating habits into later childhood and adulthood has been observed[1-3]. When the infant's diet gradually change from breast milk or infant formula to the family's diet during the complementary feeding period, the complexity of the diet increases. In research, the dietary intake has traditionally been characterised by one or a few nutrients. However, infants do not eat nutrients they consume foods and meals; therefore, a characterisation based on a few nutrients might not be the most appropriate. The investigation of dietary patterns representing the whole diet rather than single nutrients might grasp more of the diet's complexity and thereby increase our understanding of the relation between infant diet and health[4;5]. To be able to prevent or to improve undesirable dietary patterns in infancy, it is important to increase our awareness of indicators that are associated with different dietary patterns.

As far as we are aware, indicators of dietary patterns in children aged 0 to 36 mo have been investigated in seven studies that have been published in twelve papers[6-17]. The indicators can roughly be divided into maternal, paternal, household and child characteristics. These studies indicate that children with siblings[7-11;13-15;17], with low-educated mothers[6-11;13-17], with young mothers[6;7;9-11;13-17], and with mothers who have high BMIs[7-9;13-16] have higher scores in unhealthy dietary patterns. However, little is known about these associations in infants who are younger than 12 mo of age. Other possible indicators, such as sex, body size, activity level of the child, person responsible for the cooking at home, and immigrant status are less investigated, and, to our knowledge, possible paternal indicators are nearly unexplored[9;10]. Therefore, in our study, we aimed to investigate the association between a wide range of possible indicators and adherence to dietary patterns in infants aged 9 mo.

Subjects and Methods

Study design and participants

Data were collected when infants were 9 mo of age in the two observational cohorts, SKOT I and SKOT II, which had similar data-collection designs. In this paper, the data from the two cohorts are pooled, which is possible because of the similar designs. In SKOT I, the participants were infants randomly selected from the Danish capital area based on extractions from the National Civil Registration System and with no restrictions on maternal BMI. Inclusion criteria for participation in SKOT I required participants to be healthy singletons, born at 37–43 weeks of gestation, aged 9 mo ±2 weeks at the first examination and having Danish-speaking parents. Inclusion criteria for SKOT II were equal to SKOT I; in addition, all participants were required to be offspring of women with pre-pregnancy BMIs above 30 kg/m^2 and who had participated in the intervention study, "Treatment of Obese Pregnant Women" (TOP) at Hvidovre Hospital in the capital of Denmark[18;19]. Data were collected for SKOT I when infants were aged 9 mo, in the year 2007–2008, and in the year 2011–2012 for SKOT II. The studies were conducted according to guidelines laid down in the declaration of Helsinki, and all procedures involving human subjects were approved by The Committees on Biomedical Research Ethics for the Capital Region of Denmark (SKOT I: H-KF-2007-0003; SKOT II: H-3-2010-122). Written informed consents were obtained from all parents.

Dietary data and dietary patterns

Using a validated seven-day food record method[20], the dietary intake of the infants was recorded by parents for seven consecutive days. Portion sizes were estimated with household measures and a photo booklet and noted in a pre-coded food diary. Intake of food items was calculated for each participant using the software General Intake Estimation System (GIES, version 1.000d, developed at National Food Institute, Technical University of Denmark). In this study, the dietary intake was divided into 23 food groups (*Porridge, BreakfastCereals, WheatBreadWholegrain, WheatBread-NoWholegrain, RyeBread, PastaRice, Potato, Fruit, Vegetable, Fish, Meat, Poultry, Egg, FatsAnimal, FatsVegetable, Cheese, Milk, Formula, BreastMilk, FruitNutSnack, SweetsCake, SugaryDrink, FastFood*) that encompassed the whole diet. These food groups represented intake in g/kg body weight/day, except *BreastMilk*, which represented feedings/day. The division of food items into groups, based on nutritional knowledge, was an attempt to cover most aspects of the official recommendations, the typical infant diet in Denmark and issues addressed in scientific studies on infant diet. A principal component analysis (PCA) was displayed with these food groups to identify latent dietary patterns, with the purpose of reducing the number of dietary variables, but reserving the information of the whole dietary intake representing underlying dietary concepts.

Possible indicators

The selection of possible indicators for dietary patterns in infants aged 9 mo was based on published literature within this area[6-11;13-15]. Data regarding parental and household factors were collected when the infants were aged 9 mo. Weights and heights of the fathers in both cohorts and of mothers in SKOT I were self-reported, while, for the mothers in SKOT II, they were measured during the 9-mo examination. BMI was calculated as weight (kg)/height $(m)^2$. Parental immigrant/descendant status was recorded beginning with the infants' grandparents and, in a few cases, with the great-grandparents of the infant. Total household income was divided into more or less than 650,000DKK (~120,000US$) per year. Average income for families in Denmark is 684,000DKK (~127,000US$).

Duration of breastfeeding, age at introduction of complementary feeding and age of crawling were recalled by an interview and questionnaire at 9 mo. Exclusive breastfeeding was defined as receiving only breast milk, water and vitamins. A question asking for the age at which the child first ate different kinds of typical complementary foods was used to estimate the age at introduction of complementary feeding (0–3, 4, 5, 6, 7, 8 mo). Relative physical activity level was estimated by the parents to be more or less active as that of the infant's peers. Birth weight and length were obtained from health records kept by parents, with measurements carried out by midwives. At 9 mo, weight and length were measured at the department of Nutrition, Exercise and Sports, University of Copenhagen, by trained research staff. Recumbent length was a mean of three measurements carried out with a digital measuring board (Force Technology, Brøndby, Denmark) and recorded to the nearest 0.01 cm. Using a digital scale (Sartorius IP 65; Sartorius AG, Göttingen, Germany), weight was measured, without clothes, to the nearest 0.1 kg. BMIs (kg/m^2) were converted to z-scores using the software program WHO Anthro 2005 and the WHO growth standards as a reference[21]. The parents were requested to start the dietary assessment as close as possible to the 9-mo visit.

Statistical analyses

Analyses were based on complete cases and infants with missing values are referred to as 'non-completers'. Categorical variables are represented as %, and completers were compared with non-completers via Pearson´s chi^2 test. Continuous variables are represented as mean and SD, and completers were compared with non-completers via the unpaired t-test (for equal variance) or Welsh's t-test (for unequal variance) if normally distributed, and represented as median, 25; 75 percentiles and compared via Mann-Whitney test if not. The numbers of children and adults in the households were descriptively presented as categorical variables but treated as continuous variables in the statistical tests. Dietary patterns were identified by PCA, which was carried out in MATLAB R2010b using PLS_Toolbox Version 7.3.1, and included both complete and non-complete cases. Data was auto-scaled before the PCA, and the number of principal components was selected based on a clear change in the scree plot[22]. Dietary patterns were named based on subjective assessments of food groups with the highest loadings within each principal component. Individual score values from each identified dietary pattern were used as continuous variables. A multivariate linear regression model was fitted for each of the dietary patterns identified as the outcome variable and simultaneously included all the possible indicators as explanatory variables. The initial model included maternal work situation, maternal education level, maternal age at child's birth, maternal BMI when the infant was 9 mo, paternal work situation, paternal education level, paternal age at child's birth, paternal BMI when the infant was aged 9 mo, number of adults in the household, number of children in the household, household income, smoking in the home, parental immigrant/descendant status, person responsible for cooking at home, infant BMI z-score at birth, infant age at diet registration, infant BMI z-score at 9 mo, duration of exclusive breastfeeding, infant age at the introduction of complementary feeding, whether the infant was crawling at 9 mo, infant physical activity level at 9 mo, infant in day care or home care at 9 mo and sex of the infant. Estimates from the reduced model after backward stepwise selection, with $p < 0.05$ as the cut-off, are reported. When reporting the reduced multivariate models, standardised regression coefficients were obtained by refitting the models after standardising both the outcomes and explanatory variables. For each variable, standardisation consists of subtraction of the sample mean and dividing by the sample standard deviation. Categorical explanatory variables were broken down into dummy variables before standardisation. Consequently, the standardised regression coefficient refers to how many standard deviations an outcome variable will change, per standard deviation increase in the explanatory variable. Univariate linear regressions with each of the dietary patterns and one indicator at a time were also conducted for comparison. All statistical tests were conducted in the statistical programming environment R version 3.0.2 (www.r-project.org). All p-values were evaluated at a 5% significance level.

Results

Characterisation of participants and dietary patterns

Two dietary patterns were identified, and they explain 13% and 9% of the variation in the intake of the food groups respectively (**Figure 1**). The first pattern was named FAMILY FOOD and is an overall expression of the transition from *BreastMilk* and *Formula* to other foods because *BreastMilk* and *Formula* had the lowest loadings for this pattern, while *Meat*, *FatsAnimal*, *RyeBread* and *Milk* had the highest loadings. The second pattern was named HEALTH-CONSCIOUS FOOD because foods such as *SweetsCake* and *SugaryDrink* had the lowest loadings and *Potato*, *FatsVegetable*, *Fruit* and *Vegetable* had some of the highest loadings in this pattern.

The group of participants is characterised by including infants from very versatile backgrounds in relation to parental socioeconomic status, parental obesity status, and breastfeeding history (**Table 1**). The infants included in the regression analyses in this paper all had complete datasets. These completers differed from non-completers in a number of characteristics, e.g. a higher proportion of completers came from SKOT I, had more highly educated parents, a lower maternal BMI, and less immigrant/descendant parents than non-completers.

Indicators of the dietary pattern FAMILY FOOD

The initial regression model included all the maternal, paternal, household and child characteristics shown in Table 1 as possible indicators for the FAMILY FOOD pattern. Both the full and the reduced model (**Table 2**) showed that a higher infant age at diet registration and lower BMI z-scores at 9 mo were associated with higher scores in the FAMILY FOOD pattern. Moreover, infants with immigrant/descendant parents, parents who share the cooking responsibilities and fathers with jobs had lower scores in the FAMILY FOOD pattern compared to infants of Danish parents, infants from families where the mother is the only home cook and infants with fathers who are students. Based on the confidence interval, including zero, no differences were seen between infants with a father without a job versus a father who has a job or between mothers versus fathers as the only home cook. Univariate regressions, including the FAMILY FOOD pattern as the outcome variable and one of the possible indicators, by turn, as the explanatory variable, showed comparable results (data not shown). Based on the standardised coefficients, it is seen that BMI z-scores at 9 mo are the strongest indicator for the FAMILY FOOD pattern followed by the infant's age at diet registration. There was a trend in the reduced model, in which a greater number of children in the household ($p=0.08$) were associated with higher scores in the FAMILY FOOD pattern, but this was excluded from the reduced model presented. The variability accounted for in the reduced model was 12%.

Indicators of the dietary pattern HEALTH-CONSCIOUS FOOD

The initial regression model included all the maternal, paternal, household and child characteristics shown in Table 1 as possible indicators for the HEALTH-CONSCIOUS FOOD pattern. Both the full and reduced regression models (**Table 3**) showed that higher maternal BMIs, greater numbers of children in the household, higher ages of the infant at diet registration and higher BMI z-scores of the infants at 9 mo were associated with lower scores in the HEALTH-CONSCIOUS FOOD pattern. Univariate regressions, including the HEALTH-CONSCIOUS FOOD pattern as the outcome variable and one of the possible indicators, by turn, as the explanatory variable, showed comparable results (data not shown). Based on the standardised coefficients, it is seen that maternal BMI was the strongest indicator for the HEALTH-CONSCIOUS FOOD pattern, followed by the age of the infant at diet registration. With one SD increase in maternal BMI, the score value of the HEALTH-

CONSCIOUS FOOD pattern decreased with 0.22 SD. The variability accounted for in the reduced model was 13%.

Discussion

Pooling of the two cohorts SKOT I and SKOT II increased the heterogeneity in the sample of infants, especially in relation to breastfeeding history, parental socioeconomic status and maternal obesity status, and thereby served as an interesting basis for investigating a wide range of possible indicators for the dietary patterns at 9 mo of age. The strongest indicator for a lower FAMILY FOOD score was a higher BMI z-score of the infant at 9 mo, while a higher maternal BMI was the strongest indicator for a lower HEALTH-CONSCIOUS FOOD score. This, together with the significant association between the dietary patterns and parental immigrant/descendant status plus a greater number of children in the household, suggests the relevance of investigating indicators for dietary patterns as early as 9 mo of age.

Indicators of the dietary pattern FAMILY FOOD

Only two studies that we know of, the Southampton Women's Survey (SWS)[13] and the Avon Longitudinal Study of Parents and Children (ALSPAC)[14], have investigated dietary patterns with PCA within the first year of life, both at 6 mo of age. These two studies report dietary patterns specifically related to the complementary feeding period as we do, but their patterns are not directly equal to our FAMILY FOOD pattern because of different methodologies and populations. However, our study does confirm findings from these two studies: we show that, at this early stage, dietary patterns are associated with characteristics of the infant as well as with characteristics of the infant's family. At 9 mo, contrary to 6 mo of age, the infant should be well under way in the complementary feeding transition, and it is thereby an important age on which to focus, though, at this age, the most desirable score value in the FAMILY FOOD pattern is less obvious. Nevertheless, the FAMILY FOOD pattern is useful for exploring indicators associated with the complementary feeding practice; however, confirmatory analyses, e.g. by investigating the relation to nutrient intake, are needed to endorse health implications. This should be kept in mind when interpreting the direction of associations with indicators.

A higher rate of lifestyle-related diseases in immigrants compared to non-immigrants has previously been observed in Denmark[23], but the knowledge about dietary intake in early life among immigrants is sporadic[24]. Hereby, it is interesting to observe that infants with immigrant/descendant parents had significantly lower scores in the FAMILY FOOD pattern than infants whose parents were both Danish. This, even though our classification of immigrant/descendant also includes, e.g. other Scandinavian countries with a relatively similar culture and only included infants with Danish speaking parents that may be a proxy of integration. Nevertheless, this linguistic integration might not necessarily be synonymous with adherence to Danish health recommendations to the same extent as parents with a longer Danish history. However, it should be stressed that it is unknown if our results indicate that the infants with immigrant/descendant parents are too far behind in the complementary feeding process towards family food at this age in a way that poses negative health consequences. Nevertheless, a previous questionnaire survey for Danish health visitors showed that infants of immigrant parents more often had an unbalanced diet and late introduction of complementary foods[24]. These findings might therefore be an enticement to continue research within this area and to pay special attention to this group of infants in the primary health care system. In the ALSPAC cohort, an association between the dietary patterns, specifically related to the complementary feeding period, and ethnicity was not found. However, it is interesting to note that, in the British ALSPAC cohort, infants and toddlers of non-white parents, compared to offspring of white parents, had higher scores in a healthy pattern[11;14;17]. This contradicts our finding of no association between immigrant/descendant status and the HEALTH-CONSCIOUS FOOD pattern. The authors at ALSPAC suggest that the association is influenced by the ethnicities included and the degree of adherence to the original food culture.

As a measure of socioeconomic status, paternal work situation was associated with the FAMILY FOOD pattern. It indicated that infants with a father in the labour market ate more baby food and were less advanced in the transition towards family food compared to families in which the father was a student. No association was seen for fathers without jobs, but only few infants with complete data had fathers without jobs, which made a significant association less likely. We do not know of other studies investigating dietary patterns in early life with respect to the paternal work situation. However, two-year-old Norwegian toddlers ate less baby food if the mother worked full-time compared to if the mother did not have a job[10]. The finding of a lower FAMILY FOOD score for infants in homes in which the cooking responsibility was shared amongst family members compared to families in which the mother alone cooked, is also interesting; however, a possible underlying mechanism can be only speculative. We are not aware of other studies investigating this in the context of infancy, but one study has previously reported a healthier dietary pattern in three-year-old children if the mother was the primary cook compared to another person[11], though we did not find any association between the HEALTH-CONSCIOUS FOOD pattern and the person who cooked at home. No difference was seen between mothers versus fathers as the only home cook. However, only few infants had fathers who did most of the cooking at home, which made a significant association less likely.

It could be hypothesised that the largest 9-mo-old infants would be ahead in the complementary feeding period, as reported previously[25;26], perhaps because they had an earlier need (or parents presumed they had an earlier need) for food other than breast milk or infant formula. Therefore, a positive association between BMI z-scores at 9 mo and the FAMILY FOOD pattern, instead of the inverse association we observed, would be expected. However, this could be a matter of reverse causality: the intake of high amounts of energy from family food is more demanding on the fine motor skills and the family food eaten may possibly have a lower energy density than baby food. It might therefore be easier for an infant with a relatively low FAMILY FOOD score to quickly consume a greater amount of energy (and to thereby be larger) during this transition period.

Indicators of the dietary pattern HEALTH-CONSCIOUS FOOD

The association between higher maternal BMI and lower scores in the HEALTH-CONSCIOUS FOOD pattern confirms previous findings[8;13-16]. This association appeared although a higher proportion of SKOT II infants compared to those in SKOT I were excluded because of non-complete data. Even though our analysis showed this association, it does not disclose whether maternal weight loss will result in healthier dietary patterns of the offspring. However, this might be possible because maternal and offspring dietary patterns are correlated[13], and maternal weight loss reduces offspring obesity risk[27]. In contrast, no association was observed between the HEALTH-CONSCIOUS FOOD pattern and paternal BMI, which is in agreement with a study of 14-mo-old toddlers[9]. Unfortunately, this is the only other study we know of reporting an investigation of the association between paternal BMI and dietary patterns in early life. However, it could be speculated that such an association will appear later in childhood, when maternity leave and breastfeeding is finished, making the parents more equal with respect to the child-feeding task. The association between the presence of siblings in the household and lower scores at a healthy dietary pattern has been repeatedly observed[8;11;13;15]. As suggested by North[11], older siblings might introduce less healthy food to the infant. The trend of higher scores in the FAMILY FOOD pattern with higher numbers of children in the household also suggests that the complementary feeding period might be more rapid for infants with older siblings.

The association between age at diet registration and the HEALTH-CONSCIOUS FOOD pattern probably indicates that this pattern, in addition to the FAMILY FOOD pattern, also holds some information about how far the infant is in the complementary feeding process. *Potato*, *Vegetable*, *Fruit,* and *FatsVegetable*, which are used in mash, plus *Porridge* and *Formula,* are all typical complementary foods that have high loadings in the HEALTH-CONSCIOUS FOOD pattern. An association between infant age at diet registration and healthy/unhealthy dietary patterns was also observed in two other studies with 14-mo-old toddlers[6;9], but not at a later examination at 24 mo[6], which indicates that this association is transient and might fade when children have finished the complementary feeding period.

As suggested for the FAMILY FOOD pattern, it could be hypothesised that parents feed their children differently depending on whether the child is small or big for its age. This might be supported by the association between a higher BMI z-score at 9 mo and a lower score in the HEALTH-CONSCIOUS FOOD pattern, indicating that infants with the largest body sizes eat less healthfully. However, the reverse causality that infant body size depends on the diet, is perhaps more intuitive for this pattern. One other study with which we are familiar investigated current body size (weight-for-age and height-for-age z-scores but not BMI z-scores) as a predictor of dietary patterns at 14 mo[9]. Contrary to our results that study did not find any associations in these toddlers, who were five mo older than the infants in our study.

Strengths and limitations

The main strengths of this study are as follows: *first*, the focus on dietary patterns rather than on single nutrients; *second*, the wide range of possible indicators investigated; *third,* the inclusion of infants with versatile backgrounds obtained by pooling two comparable cohorts and *last,* the contribution of findings obtained from infants in the first year of life, a period for which studies are needed.

The study also comprises some limitations. Including infants only with complete data ensures that one unique data set is used throughout the model reduction. However, results may be biased if missing data do not occur at random. A number of possible indicators differed between completers and non-completers; therefore, the findings from the regression analyses probably represent those families with the largest resources and high health motivation. In addition, the fact that infants in SKOT II are offspring from the TOP study with a focus on maternal diet, physical activity and breastfeeding-support, might influence the associations we report in the present paper. Moreover, it is uncertain if the strength of the association between indicators and dietary patterns are strong enough to pose a biologically relevant difference in health outcomes of the infant. For example, the decrease of 0.22 SD in the HEALTH-CONSCIOUS FOOD pattern per one SD increases in maternal BMI. In addition, the regression models leave rather large proportions of unexplained variation in the dietary patterns, suggesting that indicators other than those on the long list included here should be investigated, such as parental diet[13]. Finally, the methodological concern whether or not to adjust for a cohort effect could be posed. The full model of the multiple regression analyses have been carried out, both with and without a cohort variable. The cohort variable was significantly associated with the FAMILY FOOD and HEALTH-CONSCIOUS FOOD patterns. However, when the cohort variable was included, paternal work situation and maternal BMI did not reach significant levels because these two are main characteristic differences between the cohorts; therefore, including the cohort variable will blur their association with dietary patterns. To be able to investigate the association between these important indicators and infant dietary patterns, the regression analyses were not adjusted for cohort origin.

Conclusion

Both parental, household and child characteristics were associated with the dietary patterns FAMILY FOOD and HEALTH-CONSCIOUS FOOD already at 9 mo of age. Our explorative analysis suggests that families with maternal obesity and multiple children, and perhaps immigrant families, had infants with less favourable dietary patterns which might indicate that they should be supported by the health care system to establish healthy dietary patterns for the infant. This investigation of early indicators of dietary patterns might improve the possibility of identifying infants with an increased risk of developing unfavourable dietary patterns and potentially enable an early targeted preventive support in families with these characteristics.

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Table 1 Characteristics of infants at 9 mo, comparison of completers and non-completers

		Completers (n = 374)		Non-completers (n = 139)		p*
		% or Mean or Median	SD or 25;75 perc.	% or Mean or Median	SD or 25;75 perc.	
Cohort origin	SKOT I, %	74		37		
	SKOT II, %	26		63		***< 0.0001*†**
Dietary patterns						
FAMILY FOOD	Scores, mean, SD	0.032	1.699	-0.162	1.717	0.37‡
HEALTH-CONSCIOUS FOOD	Scores, mean, SD	0.12	1.43	-0.64	1.03	***< 0.0001*§**
Maternal characteristics						
Work situation	Has job, %	79		74		
	Student, %	11		11		
	No job, %	10		15		0.36†
Education level	Basic education, %	21		34		
	Short education, %	11		12		
	Medium education, %	33		32		
	Long education, %	35		22		***0.010*†**
Age at child's birth	Years, mean, SD	31.9	4.4	31.2	5.5	0.29§
BMI when child is 9 mo‖	Kg/m^2, median, 25;75 percentiles	23.7	21.1;30.1	34.5	27.8;37.2	***< 0.0001*¶**
Paternal characteristics						
Work situation	Has job, %	88		88		
	Student, %	9		6		
	No job, %	3		6		0.33**
Education level	Basic education, %	31		48		
	Short education, %	9		9		
	Medium education, %	22		23		
	Long education, %	39		20		***0.0032*†**
Age at child's birth	Years, mean, SD	33.9	5.3	32.8	4.9	0.098‡
BMI when child is 9 mo	Kg/m^2, median, 25;75 percentiles	25.3	23.6;28.3	25.8	23.3;29.9	0.68¶
Household characteristics						
No. of adults in household	1, %	2		15		
	2, %	98		83		
	> 2, %	1		2		***< 0.0001*¶**
No. of children in household	1, %	57		59		
	2, %	33		30		
	3, %	7		10		
	> 3, %	3		1		0.72¶

Table continued next page

Table 1 continued

		Completers (n = 374)		Non-completers (n = 139)		p*
		% or Mean or Median	SD or 25;75 perc.	% or Mean or Median	SD or 25;75 perc.	
Household characteristics						
Household income ††	< 650,000DKK, %	40		60		
	> 650,000DKK, %	60		40		***0.0011***†
Smoking in the home	Yes, %	98		90		
	No, %	2		10		***0.0045*****
Parental immigrant /descendant status	Danish mother and father, %	80		68		
	Mother and/or father not Danish‡‡, %	20		32		***0.0090***†
Person who cooks at home	Mother/woman, %	57		64		
	Father/man, %	17		12		
	By turn or jointly, %	26		24		0.25†
Child characteristics						
BMI at birth	z-score, mean, SD	-0.26	1.00	-0.30	1.19	0.72§
Age at diet registration	months, mean, SD	8.9	0.4	9.0	0.6	***0.013***§
BMI at 9 mo	z-score, mean, SD	0.42	1.0	0.35	0.88	0.50‡
Duration exclusive BF	days, median, 25;75 percentiles	122	57;152	91	14;141	***0.022***¶
Age at introduction of complementary feeding	0–3 mo, %	5		11		
	4 mo, %	60		58		
	5 mo, %	26		20		
	6–7 mo, %	9		12		0.069†
Crawling at 9 mo	Yes, %	49		63		
	No, %	51		37		***0.047***†
Physical activity level at 9 mo	More than others at same age, %	32		45		
	Same level as others at same age, %	62		53		
	Less than others at same age, %	6		3		0.083**
Day care or home care at 9 mo	Home care, %	94		79		
	Day care, %	6		21		**< *0.0001***†
Sex	Girls, %	49		53		
	Boys, %	51		47		0.49†

BF, Breastfeeding.* Pooled group of children from the SKOT I and SKOT II cohorts, in which completers (with complete data for diet and indicators used in regressions, Tables 2 and 3) are compared with non-completers (with at least one missing piece of data for diet or indicators). † By Pearson's chi^2 test. ‡By unpaired t-test. §By unpaired Welsh's t-test.|| SKOT I, self-reported in questionnaire; SKOT II, measured during the 9-mo examination.¶ By Mann-Whitney test. **By Fisher's exact test. ††Country average for families is 684,000DKK (~127,000 US$). ‡‡ 14% were from Scandinavia, 43% were from other European countries and 42% were from outside Europe.

Table 2 Indicators associated with the dietary pattern FAMILY FOOD at 9 mo of age (n = 374) *

		Reduced multivariate regression model after stepwise backward selection†			
Indicators	Comparison of subgroups	β	95% CI	std. β‡	p
Infant age at diet registration (months)		0.90	0.52, 1.30	0.22	***< 0.0001***
Infant BMI 9 mo (z-score)		-0.39	-0.56, -0.23	-0.23	***< 0.0001***
Paternal work situation					***0.0043***§
	Student vs. Has job *(ref)*	0.93	0.35, 1.5	0.15	
	No job vs. Has job *(ref)*	0.61	-0.32, 1.5	0.063	
Parental immigrant/descendant status					***0.017***
	Mother and/or father not Danish vs. Danish mother and father *(ref)*	-0.50	-0.91, -0.089	-0.12	
Person who cooks at home					***0.016***§
	Father/man vs. Mother/woman *(ref)*	0.23	-0.22, 0.68	0.051	
	By turn or jointly vs. Mother/woman *(ref)*	-0.45	-0.84, -0.071	-0.12	
					Adj. $R^2 = 0.12$

*Based on multiple linear regression with the initial full model, including maternal work situation, maternal education level, maternal age at child's birth, maternal BMI when the infant was 9 mo, paternal work situation, paternal education level, paternal age at child's birth, paternal BMI when the infant was 9 mo, number of adults in household, number of children in household, household income, smoking in the home, parental immigrant/descendant status, person who cooks at home, infant BMI z-score at birth, infant age at diet registration, infant BMI z-score at 9 mo, duration of exclusive breastfeeding, infant age at introduction of complementary feeding, if the infant was crawling at 9 mo, infant physical activity level at 9 mo, infant in day care or home care at 9 mo and sex of the infant. †Model reduction by backward stepwise selection until all $p < 0.05$ in the model. ‡ Standardised regression coefficient. § P-value based on the collected variables before broken down to dummy-variables.

Table 3 Indicators associated with the dietary pattern HEALTH-CONSCIOUS FOOD at 9 mo of age (n = 374)*

	Reduced multivariate regression model after stepwise backward selection†			
Indicators	β	95% CI	std. β‡	p
Infant age at diet registration (months)	-0.52	-0.88, -0.17	-0.15	***0.0040***
Infant BMI at 9 mo (z-score)	-0.16	-0.30, -0.023	-0.11	***0.022***
Maternal BMI when child is 9 mo (kg/m^2)	-0.051	-0.075, -0.027	-0.22	***< 0.0001***
Number of children in household	-0.20	-0.36, -0.037	-0.12	***0.017***
				Adj. $R^2 = 0.13$

*Based on multiple linear regression with the initial full model, including maternal work situation, maternal education level, maternal age at child's birth, maternal BMI when the infant was 9 mo, paternal work situation, paternal education level, paternal age at child's birth, paternal BMI when the infant was 9 mo, number of adults in household, number of children in household, household income, smoking in the home, parental immigrant/descendant status, person who cooks at home, infant BMI z-score at birth, infant age at diet registration, infant BMI z-score at 9 mo, duration of exclusive breastfeeding, infant age at introduction of complementary feeding, if the infant was crawling at 9 mo, infant physical activity level at 9 mo, infant in day care or home care at 9 mo and sex of the infant. †Model reduction by backward stepwise selection until all $p < 0.05$ in the model. ‡ Standardised regression coefficient.

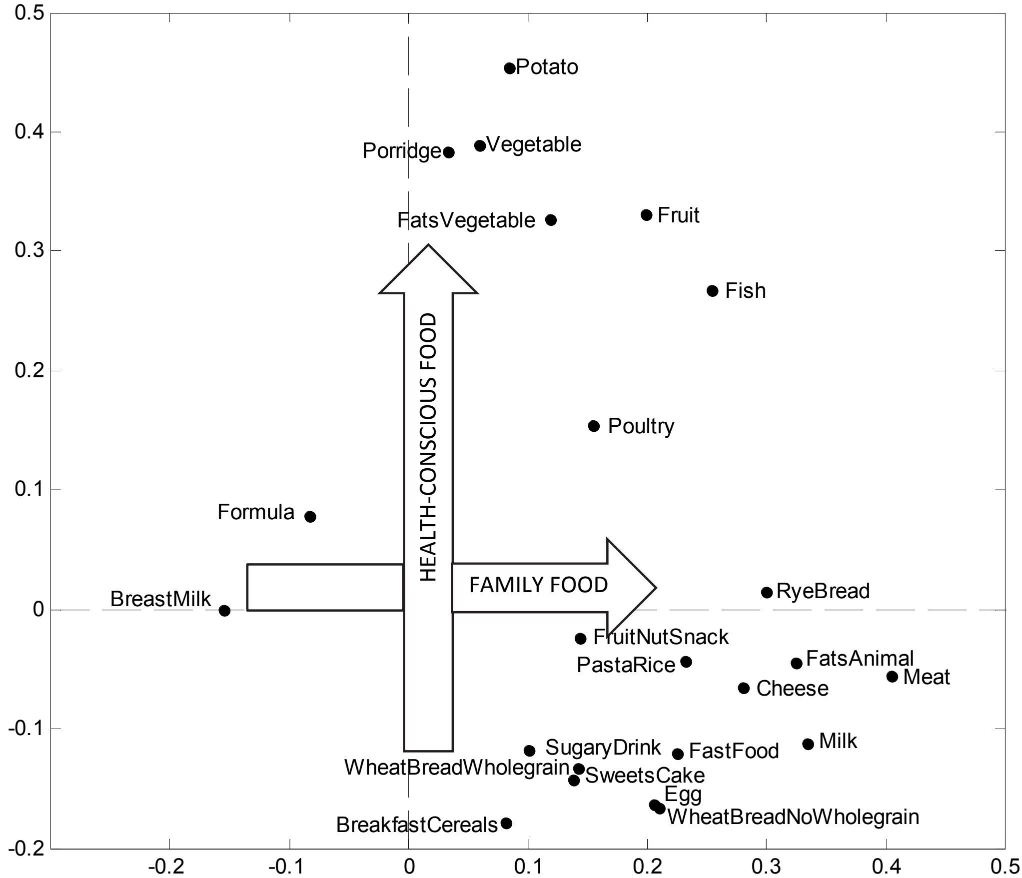

Figure 1 The two dietary patterns; FAMILY FOOD and HEALTH-CONSIOUS FOOD at 9 mo of age
Loading plot based on a PCA with intake of foods in g/kg Body Weight /day (except *BreastMilk*, which is in feedings /day) in a pooled sample of infants from the two cohorts SKOT I and SKOT II.

4.3 Paper III: Development of dietary patterns spanning infancy and toddlerhood: relation to body size, composition and metabolic risk markers at three years

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Tables and figures can be found at the end of the manuscript

Development of dietary patterns spanning infancy and toddlerhood:

relation to body size, composition and metabolic risk markers at three years

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Abstract

Little is known about the development of dietary patterns during toddlerhood and the relation to growth and health. The study objective was to characterise the development of dietary patterns from 9-36 mo of age and investigate the association to body size, body composition and metabolic risk markers at 36 mo. Food records were filled out at 9, 18 and 36 mo of age (n=229). Dietary patterns were identified by principal component analysis (PCA); the development of these patterns across the three ages was described; and association to body size, body composition and metabolic risk markers were analysed. Three dietary patterns were identified: TRANSITION FOOD, HEALTHY FOOD and TRADITIONAL FOOD. The course of development in dietary patterns from 9-36 mo indicated tracking for a relatively large group of participants in the three patterns. TRANSITION FOOD and HEALTHY FOOD were associated with some of the investigated outcomes. Children with lower adherence to the TRANSITION FOOD or HEALTHY FOOD pattern at two or all three ages had higher BMI z-scores, higher fat mass indices, and higher metabolic risk markers. E.g. BMI z-score was 0.7-0.9 higher than in other groups. Hence, this could represent undesirable development of dietary patterns in toddlers. In conclusion, development of dietary patterns can be exploratory characterised by PCA and related to potential cardiovascular risk markers in toddlers even within a homogeneous population with a high socioeconomic status. The tracking of dietary patterns from 9 mo of age indicates a need for early and sustained promotion of healthy diets.

Keywords: dietary patterns, risk markers, tracking, toddlerhood, principal component analysis, longitudinal

Introduction

Within the first years of life, infants and toddlers substantially change their sources of nutrients from a milk-based diet to the same diet as the rest of the family. This is the most extensive dietary change made throughout life, and furthermore this is the period of the most rapid growth. The first years of life are a window of opportunities because early nutrition and growth seem to have long-lasting consequences for the risk of obesity and cardiovascular diseases (Osmond and Barker, 2000). Furthermore, some evidence shows that early dietary patterns seem to track into later childhood and adulthood (Madruga et al., 2012; Mikkila et al., 2005; Robinson et al., 2007).

The consequences of breastfeeding versus formula-feeding is widely investigated (Agostoni et al., 2009), while knowledge about complementary feeding and a toddler's diet in relation to current body size and later health is still sparse (Agostoni et al., 2008). Going from milk to the family's food increases the complexity of the dietary pattern. In general, it is realised that the relation between diet and health cannot be fully characterised by a few nutrients or foods (Hu, 2002; Mente et al., 2009). Instead, a dietary pattern approach based on foods eaten at different ages during infancy and toddlerhood comprising the whole diet might grasp more of this complexity. The most common method to summarise the complexity of dietary patterns is the principal component analysis (PCA) (Smithers et al., 2011). PCA is an explorative, data-driven method which allows inclusion of many highly correlated foods simultaneously. PCA uses the correlations between the large numbers of dietary variables to identify latent dimensions in the data (Wold et al., 1987). However, few studies have characterised dietary patterns using PCA in young children from high income countries (Bell et al., 2013; Brazionis et al., 2012; Kiefte-de Jong et al., 2013; Kristiansen et al., 2013; Robinson et al., 2007; Sepp et al., 2002). To the best of our knowledge only three of these; an Australian study (Bell et al., 2013) and the two British studies Southampton Women Study (SWS) (Robinson et al., 2007) and Avon Longitudinal Study of Parents And Children (ALSPAC) (Brazionis et al., 2012) have investigated dietary patterns longitudinally in infancy and toddlerhood. Brazionis and colleagues investigated in ALSPAC the development of dietary patterns at three ages (6, 15 and 24 mo, n=2169) and found two consistent dietary patterns over time (Brazionis et al., 2012). The dietary patterns were not examined for relations with anthropometry and metabolic risk factors; neither was individual tracking of dietary patterns investigated. However, in a later publication the relation between the dietary patterns and blood pressure at 7 years of age was investigated (Brazionis et al., 2013). They found that a less healthy diet at 2 years was associated with a higher blood pressure at 7 years. The relation between dietary patterns and body size or composition has only been investigated for dietary patterns at one time point during infancy and toddlerhood (Baird et al., 2008; Bell et al., 2013), not including the effect of development of dietary patterns across different ages. Furthermore, to our knowledge, the relation to metabolic risk markers is yet unexplored.

Firm characterisations of the development of dietary patterns during the first years of life as well as knowledge about the impact on key anthropometric and metabolic outcomes are important to support health guidance. The aim of this paper is to characterise the development in dietary patterns from 9 to 36 mo of age and to explore the association between the development in dietary patterns and body size, body composition and metabolic risk markers at 36 mo.

Materials and Methods

Study design and participants

Data were from a Danish longitudinal observational cohort study (SKOT I) which monitored children at 9 (+/-14 days), 18 (+/-30days) and 36 (+/-90 days) mo of age from year 2007 to 2010 and was previously described by Madsen and colleagues (Madsen et al., 2010). Mailed invitations were sent to 2,211 families randomly selected from the National Danish Civil Registry in the capital area. Inclusion criteria were singleton infants born ≥ 37 weeks of gestation without diseases expected to affect growth or food intake and with Danish speaking parents. Anthropometric assessments were performed at three visits accompanied by instructions in dietary assessment, background questionnaires and parental BMI assessments. The study protocol was approved by The Committees on Biomedical Research Ethics for the Capital Region of Denmark (H-KF-2007-0003).

Dietary data

The diet of each child was recorded by parents after an oral and written introduction for seven consecutive days at 9, 18 and 36 mo of age using a seven day food record method. This method has been shown to be valuable to estimate dietary intake in this age groups when comparing with double-labelled water (Gondolf et al., 2012). Portion sizes were estimated with household measures and a photo booklet, and were noted in a pre-coded food record. Intake of food items, energy and nutrients were calculated for each participant using the software General Intake Estimation System (GIES, version 1.000d, developed at National Food Institute, Technical University of Denmark) and the Danish Food Composition Databank (version 7; Søborg; www.Foodcomp.dk, visited May 2011) as described previously (Gondolf et al., 2012). Quality control was carried out by trained research staff before data were entered in the database. Possible over- and under-reporters of energy intake were identified based on Goldberg cut-off (Black, 2000) using the Schofield equations (Alexander et al., 2004) to estimate basal metabolic rate at 18 and 36 mo. At 9 mo possible over- and under-reporters of energy intake were identified based on the estimated daily energy requirement of 338kJ/kg Body Weight(BW)/day for both genders as an average between 6 and 12 mo estimates (Molander et al., 2013) and cut-off values of +/-46% (Wells and Davies, 1999).

The food group variables for the PCA model were the same for all three ages selected based on nutritional knowledge trying to cover most aspects of the official recommendations, nutrition evidence and typical toddler diet in Denmark. In total the PCA was based on 25 food groups which are named with a short, compressed description, such as '*FatsAnimal*' and '*SugaryDrink*'. The mean intake (g/day) of all food groups, except for breast milk, was divided by total body weight (BW, kg) for each participant at each age to make the contribution of each food to the whole diet comparable across different ages and requirements. Intake of breast milk was estimated as number of breastfeeding sessions per day according to interview with parents (in the categories: <1, 1-2, 3-5, 6-8, >9 times/day) and recoded as the mean of each interval. These categories (feedings/day) were used in the PCA while an estimate of 99 g/feeding (Michaelsen et al., 1994) was used to calculate g/kg BW/day for the bar plot of foods and in the calculation of total energy intake.

Anthropometry

Weight was measured without clothes to the nearest 0.1 kg using a digital scale (At 9 mo: Sartorius IP 65; Sartorius AG, Göttingen, Germany; at 18 mo: Lindeltronic 8000, Samhall Lavi AB, Kristianstad, Sweden; at 36 mo: Tanita WB-100MA, Tanita Corporation, Tokyo, Japan). Height at 36 mo was measured by a stationary digital height measurer (235 Heightronic Digital Stadiometer), which made readings to the nearest 0.01 cm. Height was performed in triplicates, and the average was used in analysis. BMI (kg/m^2) was calculated from weight and height. Child height and BMI were converted to z-scores using the WHO growth standard as reference and the software program WHO Anthro 2005 (World Health Organization, 2011).

Blood samples

Blood triacylglycerol, glucose, insulin, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), insulin-like growth factor 1 (IGF-I) and insulin-like growth factor binding protein 3 (IGFBP3) were measured in a blood sample (plasma, except glucose which were analysed in full blood) taken at the 36 mo visit after a two hour fast. Mean fasting time (±SD) was 174±36 minutes with the exception of 29 children, who fasted from the night before the examination. Composition of the last meal before fasting was recorded and analysed using the software programme Dankost (version 3000, Dankost Ltd, Copenhagen, Denmark). Insulin, IGF-I, and IGFBP3 were determined on an Immulite 1000 analyser (Diagnostic Products Corporation, USA); glucose was determined in EDTA on HemoCue (HemoCue Denmark, Denmark); and total cholesterol, LDL, HDL and triacylglycerol were determined on a Pentra 400 analyser (HORIBA ABX, 34184 Montpellier, Cedex 4, France). To estimate the IGF-I/IGFBP3 molar ratio, which is an expression of the bioactive fraction of IGF-I, the following conversion equivalents were used: 1 ng/mL IGF-I = 0.133 nM IGF-I and 1 ng/mL IGFBP3 = 0.033 nM IGFBP3. More details about blood sampling have been published elsewhere (Madsen et al., 2010; Madsen et al., 2011).

Body composition

Predictive equations for fat free mass and fat mass have previously been generated using bioelectrical impedance (Quantum III, RJL Systems, Michigan, USA), and height and weight from this cohort at 36 mo (Ejlerskov et al., 2014). This was used to calculate fat free mass index (FFMI) as fat free mass/height^2 (kg/m^2) and fat mass index (FMI) as fat mass/height^2 (kg/m^2).

Background questionnaire and parental BMI

A background questionnaire at 9 mo included questions about parental age at child's birth, total household income, educational level of parents (updated at 36 mo) and parity. Total household income was divided into < 800,000 DKK (~145,000 US$) or unknown and > 800,000 DKK per year. The mean household income for a Danish family with two parents and two children is 684,000 DKK per year (125,226 US$) (Statistics Denmark, 2011). Educational level was divided into more or less than medium academic education and parity was represented as one child or more children. The weight and height of the parents were measured at the 36 mo visit with the same equipment used for the children, and these measures were used to calculate BMI (kg/m^2). If weight and height measures from the 36 mo visit were not available, then self-reported values from questionnaires were used.

Statistical analyses

Categorical variables are represented as % (n) and groups with or without three complete diet registrations were compared by the chi^2 test. Continuous variables are represented as mean ± SD (n) and compared between the groups with or without three complete diet registrations by the unpaired *t*-test, together for boys and girls, while the descriptive presentation is divided by gender.

For the identification of dietary patterns, a PCA was carried out including food groups at 9, 18 and 36 mo in long format meaning that the data is structured so that each observation (person at specific time point) is considered as a row in the data matrix, Hence the same person appears in three rows in the matrix with the 25 food groups as variables (columns). This way, a PCA model of the data will provide an estimate of the underlying food patterns that are common over different time points. The score for each observation, on the other hand, will then indicate how much the person adhere to a particular dietary pattern at a particular time point. Thus, by assuming that a general set of latent dietary patterns exist over time, the dynamics of these dietary patterns can be monitored in the scores as a function of time. To normalise data for the PCA they were centred and scaled to unit variance (auto-scaling) (Esbensen et al., 2001). Orthomax, simplimax, verimax and manual rotation were tried but not used, since it did not optimise interpretation of the dietary patterns. Neither did it change the overall interpretation of the dietary patterns to exclude children with the highest total energy intake (>+3 SD) or exclude children partly breastfed at 9 mo whereby they were kept in the model. Naming of dietary patterns was based on subjective assessments of food groups with highest loadings within each principal component meanwhile taking the information of the whole gradient of the dietary pattern into account based on interpretability instead of an arbiter cut-off defining highest. The number of principal components was selected based on the first and largest change in the scree plot (Cattell , 1966). The score variables from the PCA for each principal component were changed into categorical variables; specifically, each child was categorised as above or below the mean score of the cohort for the given age. These three age-specific ranks of the child were combined across ages and placed the child in one of eight categories of development (referred to as "categories of development in dietary patterns") for each dietary pattern summarising the development in dietary patterns from 9 to 36 mo. The categorisation method is exemplified by the category Below-Above-Below (BAB) in **Figure 1**. Data were analysed in MATLAB R2010b using PLS_Toolbox version 7.3.1. Tracking of dietary patterns across the three ages (9, 18, 36 mo) was evaluated by a comparison of the actual observed number of children with the theoretical calculated number expected if there was no tracking. The number expected if there was no tracking is theoretically calculated as $p_{9mo}*p_{18mo}*p_{36mo}*n$ for the category Above-Above-Above (AAA) and as $(1-p_{9mo})*(1-p_{18mo})*(1-p_{36mo})*n$ for the category Below-Below-Below (BBB), where p=proportion of children above mean, and n=total sample size. If the observed number is higher than the theoretically calculated number the individual adherence to a dietary pattern at different time points are not independent and can be interpreted as tracking.

The relation between different categories of development in dietary patterns and body size (height z-score, BMI z-score), body composition (FMI, FFMI) and metabolic risk markers (glucose, insulin, total cholesterol, HDL, LDL, triacylglycerol, IGF-I, IGFBP3 and IGF-I/IGFBP3) were analysed by ANCOVA adjusted for potential confounders. All analyses were adjusted for sex, parity, parental BMI, parental age, parental education level and household income. Metabolic risk markers were further adjusted for fasting time and energy content in last meal. In the ANCOVA analyses, all variables of categories of development in dietary patterns (equal to the number of selected principal components/dietary patterns) were included in the initial model, and backward stepwise elimination was used to identify which of the dietary patterns were significant. Post hoc pairwise comparisons of the eight categories within a dietary pattern were adjusted for multiple testing using the single step method (Hothorn et al., 2008). All statistical tests were conducted in the statistical programming environment R version 3.0.2 (www.r-project.org). All p-values were evaluated at a 5% significance level.

Results

Subject characteristics

In the SKOT I cohort, 330 children were enrolled, including one child who was excluded before analysis because of late manifestation of a severe chronic disorder. More information about dropouts has been published previously (Madsen et al., 2010). The number of children with dietary data available was n=307 at 9 mo, n=267 at 18 mo and n=240 at 36 mo all of which were included in the PCA. Only complete dietary cases, meaning children with usable dietary records from all three ages (n=229, 50% girls), were included in the formation of categories of development in dietary patterns from 9 to 36 mo. Weights at 9 mo (p=0.03) and 18 mo (p=0.03) were significantly higher for children without three times of dietary records compared to children with all three dietary records, but these were the only characteristics shown in **Table 1** that were significantly different between children with and without complete dietary data.

Dietary patterns

The mean energy intake for children with all three dietary records was 397 kJ/kg BW/day (including estimate of energy from breast milk) at 9 mo, 408 kJ/kg BW/day at 18 mo and 353 kJ/kg BW/day at 36 mo. Of these children 1%, 4% and 15% were possible under-reporters and 16%, 6% and 1% were possible over-reporters at 9, 18 and 36 mo respectively. All these children were included in the analysis, as no major changes were observed in dietary patterns by exclusion. Large variations were seen in the percentage of children eating different foods in the record periods (**Table 2**). *WheatBreadNoWholegrain*, *RyeBread*, *Fruit*, *Vegetable*, *Fish*, *Meat*, *FatsAnimal*, *Cheese* and *Milk* were eaten by 75% or more at all three ages. Other food groups were only eaten by a fraction of children within the recording period, e.g. 19% received *SugaryDrink* at 9 mo. However, the percentage of children drinking *SugaryDrink* increased steeply and was 94% at 36 mo (of these e.g. 70% drank juice and 34% drank soda. Yet, the same child might contribute to both percentages). Likewise, a steep increase in the number of children eating *FastFood*, *SweetsCake*, *Chips* and *FruitNutSnack* were seen from 9 to 36 mo. An overview of the median intake of the different food groups is seen in **Figure 2.** *Vegetable*, *Fruit* and various types of milk constitute the biggest weight fraction of the diet at all ages. Infant formula was the main milk at 9 mo while cow's milk nearly constituted the total milk intake at 18 and 36 mo. *Porridge* was a common food at 9 mo and faded out until 36 mo of age. The median intake of *SweetsCake* and *SugaryDrink* was highest at 36 mo.

The general trend from Figure 2 is recognised in the main dietary patterns generated by PCA and visualised in **Figure 3A & B**. Numerical values of the loadings (**Table S1**) and mean scores per age (**Table S2**) are also available in the supplementary material. Figure 3 is a map of variation in intake of food groups between children. Three dietary patterns were identified. The main variation in food groups (18%), reflected in the first dietary pattern named "TRANSITION FOOD", is as expected the transition from baby food predominant at 9 mo (*Porridge*, *Formula*, *BreastMilk*) to the versatile intake of different more solid foods which can be eaten by the whole family at later stages. Another 8% of the variation in food groups was described in the second component, named "HEALTHY FOOD". This dietary pattern describes a gradient of food intake ranging from potentially unhealthy foods, like *SweetsCake*, *SugaryDrink* and *Chips*, with lowest loadings to healthy food groups, like *Fruit*, *Vegetable* and *Fish* with the highest loadings. The third dietary pattern, explaining 5% of variation, was named "TRADITIONAL FOOD" because this pattern differentiated children with highest intake of *FatsAnimal*, *Potato*, *Meat* and *WheatBreadNoWholegrain* from children with highest intake of foods such as *FruitNutSnack*, *RyeBread*, *BreakfastCerealsNoAddSugar* and *Fish*.

Categories of development in dietary patterns from 9 to 36 months

The number of children in each category of development in dietary patterns is shown in **Table 3.** The categories have been interpreted based on the naming of each of the three dietary patterns from Figure 3. From this table it is seen that lower adherence to the TRANSITION FOOD pattern than average at all ages (category BBB) was the most common development for this pattern. It was also common to stay either below or above the average of the cohort at all three ages (category BBB or AAA) for the HEALTHY FOOD and the TRADITIONAL FOOD pattern. This indicates tracking of dietary pattern and was further supported by the fact that the observed number of children in category AAA and BBB was higher for all three dietary patterns than the theoretically calculated numbers expected if no tracking was present.

Development in the TRANSITION FOOD pattern related to 36 months outcomes

Mean BMI z-score ($p<0.001$) and FMI ($p=0.006$) at 36 mo were significantly different between the eight categories of development in the TRANSITION FOOD pattern. The BMI z-scores at 36 mo were higher for children with lower adherence to the TRANSITION FOOD pattern than average at 18 and 36 mo irrespectively of intake at 9 mo (categories ABB, BBB) compared to children with higher adherence to the TRANSITION FOOD pattern than average at 36 mo (categories AAA, BAA, BBA) (**Figure 4**A). The same pattern was seen for FMI at 36 mo (Figure 4B), however not significantly for category BBA and BBB. The TRANSITION FOOD pattern was not associated with height z-score, FFMI, glucose, insulin, total cholesterol, HDL, LDL, triacylglycerol, IGF-I, IGFBP3 and IGF-I/IGFBP3 at 36 mo of age. The result in Figure 4 can also be viewed in the supplementary **Table S3**.

Development in the HEALTHY FOOD pattern related to 36 months outcomes

The mean height z-score (p=0.04), IGFBP3 (p=0.04), total cholesterol (p=0.02) and LDL (p=0.03) at 36 mo were significantly different between the eight categories of development in the HEALTHY FOOD pattern. Significant differences in cholesterol or LDL were seen when the categories of development in the HEALTHY FOOD pattern varied for 9 and 18 mo irrespectively of the intake at 36 mo. This trend was also observed at 9 mo for height and IGFBP3 irrespectively of intake at 18 and 36 mo. This is based on the following findings. Total cholesterol and LDL were higher for children eating less healthy than average at all three ages (category BBB) compared to children eating more healthy than average at the first two registrations, 9 and18 mo and less healthy than average at the last registration, 36 mo (category AAB) (Figure 4C, D). The height z-score was lower for children eating less healthy than average at both 9 and 18 mo and more healthy than average at 36 mo (category BBA) compared to children eating more healthy than average at 9 mo (category ABA) (Figure 4E). IGFBP3 at 36 mo was higher for children eating more healthy than average at 9 mo (category ABB) compared to children eating less healthy than average at 9 mo (category BBB) in groups who ate less healthy than average at both 18 and 36 mo (Figure 4F). The HEALTHY FOOD pattern was not associated with BMI z-score, FMI, FFMI, glucose, insulin, HDL, triacylglycerol, IGF-I and IGF-I/IGFBP3 at 36 mo of age.

Development in the TRADITIONAL FOOD pattern related to 36 months outcomes

None of the tested outcomes (height z-score, BMI z-score, FMI, FFMI, glucose, insulin, total cholesterol, HDL, LDL, triacylglycerol, IGF-I, IGFBP3, and IGF-I/IGFBP3 at 36 mo of age) were associated with the TRADITIONAL FOOD pattern.

Discussion

Three dietary patterns were identified: TRANSITION FOOD, HEALTHY FOOD and TRADITIONAL FOOD. When categorising children according to the course of development in each of these three dietary patterns over ages, the most common course among the children was to stay either above or below the average of the cohort at all three ages. BMI z-score and FMI at 36 mo were significantly different between categories of development in the TRANSITION FOOD pattern, while total blood cholesterol, LDL, height z-score and IGFBP3, at 36 mo differed significantly between the categories of development in the HEALTHY FOOD pattern. Especially, groups of children with lower adherence to the TRANSITION FOOD or lower adherence to the HEALTHY FOOD pattern than average at two or all three ages had higher BMI, FMI and metabolic risk markers and thereby seem to represent undesirable development in dietary patterns for toddlers.

Similarities of dietary patterns across populations and studies

As far as we know, only the ALSPAC publication by Brazionis and colleges (Brazionis et al., 2012) includes dietary records from different ages in the same PCA to look for development in dietary patterns and is therefore comparable to our study. However, they do not evaluate tracking at the individual level as we do. The main food groups contributing to high scores at our HEALTHY FOOD pattern (Figure 3A) seem to be comparable to the healthy pattern in ALSPAC (Brazionis et al., 2012), and low scores at our HEALTHY FOOD pattern resemble the less healthy pattern in

ALSPAC apart from a distinct contribution of either breast milk or formula in ALSPAC. This distinct contribution of breast milk and formula is probably caused by the timing of the first registration at 6 mo in ALSPAC compared to 9 mo in SKOT I.

Low scores in our TRANSITION FOOD pattern might be related with the infant guideline pattern identified in the SWS cohort for children at 6 and 12 mo, while high scores at our TRANSITION FOOD pattern might be related with their adult food pattern (Robinson et al., 2007). The SKOT I cohort showed both baby- and family/solid-foods more roughly in one dietary pattern instead of two because our PCA also included 18 and 36 mo diet records.

The naming of the TRADITIONAL FOOD pattern is based on a traditional Danish hot dinner with potato, sauce and meat which is represented in high scores for this pattern. Rye bread is also a traditional food for lunch in Denmark but might now be linked with modern life and progress as part of the New Nordic Diet concept (Mithril et al., 2013). A traditional food pattern has also been reported in other studies, e.g. in a Norwegian study of two year old children. The food cultures across the Scandinavian countries have a lot of similarities and the identified Norwegian traditional food pattern also resembles our pattern of a hot meal with potato, sauce and meat (Kristiansen et al., 2013). The TRADITIONAL FOOD pattern in SKOT I was not associated with any of the health outcomes even though low loadings at this pattern show similarities with high loadings in the HEALTHY FOOD pattern like *Fish* and *RyeBread*.

Tracking of dietary patterns

A higher proportion of participants in SKOT I kept the same position in PCA scores relative to the cohort mean from 9 mo until our last recording at 36 mo (categories AAA, BBB) than expected if the individual score values were independent over time (Table 3). This was true for all three dietary patterns. Maintaining the same course in dietary patterns at the individual level over time indicates tracking and was also observed in SWS from 6 mo to 12 mo (Robinson et al., 2007). Tracking of dietary patterns is presumably related to tracking of parental choices and routines at this early age. Tracking beyond 36 mo was seen in a Finnish study of dietary patterns in children 3-18 years of age at baseline with follow-up after 6 and 21 years (Mikkila et al., 2005). This indicates that children already at the age of complementary feeding can be categorised according to a more or less healthy dietary profile which might track for several years. But equally important to note; another group of the participants changed their adherence to the dietary patterns over time (categories AAB, BAA, BBA, ABB and in particular ABA and BAB).

Association between development in dietary patterns and body composition

The TRANSITION FOOD pattern primarily showed an age gradient in the development of diet from baby to family food. However, this pattern might be more than an age gradient because it was also associated with risk markers for later obesity. The unfavourable findings of higher BMI z-scores and FMI for children with lower adherence to the TRANSITION FOOD pattern at both 18 and 36 mo (categories ABB, BBB) are difficult to interpret but might be related to a less diversified dietary intake for these children. Another possibility is a higher degree of under-reporting for children with highest BMI and FMI. However, the estimated proportion of under-reporters is low compared to other studies (Burrows et al., 2010). We do not know of other studies investigating the association between longitudinal development of dietary patterns and body composition in toddlers. Nevertheless, a few studies investigated the association of dietary patterns at one age during toddlerhood or childhood and current or later body composition. The British SWS (Baird et al., 2008) and the Australian study (Bell et al., 2013) with toddlers 12 to 24 mo did not find any association between dietary patterns and current skinfold thickness or BMI z-score, respectively. In a follow-up at 4 years in SWS they investigated whether an association appears later in childhood (Robinson et al., 2009) and found that children eating most similar to infant guidelines at 12 mo had a higher lean mass index compared to children not eating according to infant guidelines, but no difference in FMI or BMI was found. Hereby, time for accumulation of dietary effects might be needed before an effect occurs, which might be a possible explanation for our finding at 36 mo in contrast to no association found in the British and Australian studies earlier in life. A healthy food pattern has been associated with lower BMI or fat mass in older children, 3-18 years of age (Mikkila et al., 2007; Shang et al., 2012; Wosje et al., 2010). In addition, including the whole profile of development in dietary patterns at 9, 18 and 36 mo in our analysis might contribute to making this early accumulating effect more clear.

Association between development in dietary patterns and metabolic risk markers

To our knowledge, no studies compare dietary patterns with biomarkers in children below 5 years, as was also pointed out in a recent review (Smithers et al., 2011), and few such studies have been carried out with children above 5 years and adolescents. We found an association between the HEALTHY FOOD pattern and the potential metabolic risk markers of later cardiovascular diseases, LDL and total cholesterol. Intake during the early period (9, 18 mo) tended to be most important for these associations because categories AAB and BBB differed at 9 and 18 mo, but not at 36 mo of age. The association between higher total cholesterol and LDL, and potentially unfavourable dietary patterns, found in SKOT I, was also seen in a Finnish study of children 3-18 years old (Mikkila et al., 2007) and among Chinese children aged 6-13 years (Shang et al., 2012). These associations are biologically plausible based on nutrients involved in the mechanism regulating the blood LDL level in toddlerhood (Cowin and Emmett, 2000). Considerable amounts of fibre, monounsaturated, and polyunsaturated fat are found in the foods with highest loadings (*Vegetable, Fruits, Fish, and FatsVegetable*) in the HEALTHY FOOD pattern and might contribute to lower LDL levels, while saturated fat and sugar are highly present in foods with lowest loadings (*SugaryDrink* and *SweetsCake*) and might contribute to higher LDL levels. In addition, the Finish and Chinese studies also found associations between dietary patterns and triacylglycerol, insulin, glucose and HDL, while we did not find any such associations.

Association between development in dietary patterns, height and IGFBP3

The association between the HEALTHY FOOD pattern and height in SKOT I cannot directly be confirmed by other studies examining dietary patterns, as only few studies have looked at this. SWS did not find any association between dietary patterns and height at 12 mo (Baird et al., 2008). However, the relation between dietary patterns and height might be more pronounced in food insecure settings compared to affluent cohorts like SWS or SKOT I. A case-control study comparing 7-year-old stunted and non-stunted children in Iran found that children with the highest intake of a carbohydrate-protein pattern, including highest intake of sweets, meat and dairy products, tended to be less likely to be stunted compared to children with the lowest intake (Esfarjani et al., 2013). We found in a previous cross sectional study of 2.5-year-old Danish children, where we did not examine dietary patterns, that height was positively associated with milk, but not with meat intake (Hoppe et al., 2004b). In SKOT I the milk intake is high compared to other food groups (Figure 2) and in the HEALTHY FOOD pattern milk has a positive although small loading (Figure 3A), which might be one of the reasons for the positive association with height. Especially, the diet at 9 mo seems important for the association with height when compared with 18 and 36 mo (category ABA versus BBA). The difference between the category with the lowest IGFBP3 (category BBB) and highest IGFBP3 (category ABB) for the HEALTHY FOOD pattern also indicated importance of 9 mo patterns. IGF-I is important for linear growth during childhood (Murray and Clayton, 2013) and the binding protein IGFBP3 regulates the level of active IGF-I and is therefore assumed to be negatively associated with growth, opposite to IGF-I (Murray and Clayton, 2013). However, we have in previous studies found that both IGF-I and IGFBP3 and the ratio IGF-I/IGFBP3 are stimulated by certain foods and diets (e.g. milk intake) in children (Hoppe et al., 2004a). Thus, milk intake might also contribute to the association between the HEALTHY FOOD pattern and IGFBP3, which may reflect a more general effect on the IGF system. To our knowledge, we are the first to investigate associations between IGF-1, the binding protein, and dietary patterns in childhood.

Strengths and limitations

The main strengths in our study are the longitudinal approach, not only looking at cross sectional dietary patterns, and the use of seven days' food diaries with thorough portion size estimations. Including longitudinal data in one PCA ensures that score values from different time points are compared precisely on the same dietary pattern; this approach is widely accepted in other fields using PCA (Wise et al., 1999; Wold et al., 1998) but has only been validated sparingly within child nutrition (Brazionis et al., 2012). Moreover, investigating the link to body size, body composition and metabolic risk markers strengthens the study.

It is a general limitation that PCA is not independent of subjective decisions when selecting content and number of food groups even though it is a data-driven method. This is especially worth noticing when condensing the dietary complexity extensively as we do in this study both across the whole diet and across different ages. We ended up with relatively few and broad dietary variables in the PCA to ease the visualisation and interpretation of data. Our relatively precise portion size estimation of different food groups is probably as important as differentiating between very specific food groups when looking at associations with body composition and risk markers of later diseases. Moreover, the categorisation method for development in dietary patterns used in this paper is not a validated method, which might be considered as another limitation and should be further investigated. The categorisation suggested here is one way of simplifying types of developments. Other more model based approaches including linear mixed models can be applied to address tracking (Mikkila et al., 2007; Smithers et al., 2013). However, this type of modelling induces a very particular type of tracking the validity of which needs close scrutiny. As our aim has been a mere description of potential tracking without imposing an array of potentially unrealistic assumptions we have deferred from using these modelling approaches. Our method has the advantage that it enables the inclusion of three time points in one simple variable describing the course of development in dietary patterns. In addition, our method focuses on archetypes rather than potentially less important minor individual differences. Lastly, these findings might not be representative for Danish children because the SKOT I cohort is a relatively small, homogeneous group with parents having a high socioeconomic status and all living in the capital area.

Conclusion

Development in dietary patterns can, in an informative manner, be characterised by PCA and related to toddlerhood body size, body composition and metabolic risk markers. The explorative method applied in this analysis seems to be able to handle some of the complexity in the understanding of child nutrition. The tracking of dietary patterns already from 9 mo for some children, together with changes in the course of development in dietary patterns for other children and the association with risk markers, even within a high socioeconomic and homogeneous population, emphasize the need for early and sustained promotion of healthy diets in the first years of life.

Key Messages

- Tracking of dietary patterns was observed already from 9 mo for a relative large group of children, together with changes in the course of development in dietary patterns for other children.
- Development of certain dietary patterns from 9 to 36 mo was associated with potential risk markers of later obesity and cardiovascular diseases.
- These findings, even within a high socioeconomic and homogeneous population, emphasise the need for early and sustained promotion of healthy diets in the first years of life.

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The authors declare that they have no conflicts of interest.

Statement of Authors' Contributions to Manuscript: KFM and CM designed research; KTE participated in project management and data collection; ET was responsible for calculation of dietary intake; LBBA analysed data and wrote the draft of the paper; CBP and RB supported decisions during data analysis. All authors reviewed, contributed to and approved the final version of the manuscript.

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Table 1 Characteristics of children with complete diet data at 9, 18 and 36 months divided by gender

Child	Girls[a]	Boys[a]
Exclusive breastfeeding, mo	3.7±2.0(115)	3.8±2.0(113)
9months		
Examination age, mo	9.1±0.3(115)	9.1±0.3(114)
Weight, kg	8.65±0.85(115)	9.34±0.95(114)
Length, cm	70.72±2.16(115)	72.77±2.33(114)
BMI, kg/m^2	17.3±1.5(115)	17.6±1.5(114)
18months		
Examination age, mo	18.0±0.6(115)	18.0±0.6(113)
Weight, kg	10.77±1.05(115)	11.50±1.12(114)
Length, cm	80.82±2.35(112)	82.73±2.84(114)
BMI, kg/m^2	16.5±1.4(112)	16.8±1.3(114)
36months		
Examination age, mo	36.4±1.0(113)	36.5±1.2(113)
Weight, kg	14.29±1.43(115)	14.88±1.53(114)
Height, cm	94.96±3.12(113)	96.55±3.58(113)
BMI, kg/m^2	15.9±1.2(113)	16.0±1.1(113)
Parents and Household		
Age, mother at birth, y	32±5(115)	31±4(113)
Age, father at birth, y	34±5(114)	34±6(113)
BMI, mother, kg/m^2	24.0±3.9(115)	24.1±4.4(114)
BMI, father, kg/m^2	26.1±3.8(114)	25.3±3.2(112)
Education mother		
At least medium academic education	76 (115)	75(114)
Education father		
At least medium academic education	68(111)	60(110)
Parity >1 at 9 mo	40(114)	41(113)
Household income >800.000DKK/y	38(115)	31(113)

[a] Education, household income and parity represented as % (n).
The rest of the variables are represented as mean±SD (n).

Table 2 Description of food groups and the percentages of children with intake > 0 g/day

Food group	Description	9 mo, % (n=307[a])	18 mo, % (n=267[a])	36 mo, % (n=240[a])
Porridge	Cereal gruel, porridge; homemade or ready-prepared	98	79	59
BreakfastCereals AddSugar	Sugar puffs and sugary cereals	2	14	23
BreakfastCerealsNoAddSugar	Oatmeal, muesli, cornflakes	14	73	90
WheatBreadWholegrain	Grainy bread, crisp bread	72	93	94
WheatBreadNoWholegrain	White bread, biscuits	76	98	97
RyeBread	Rye bread with and without seeds	75	99	100
PastaRice	Pasta, Rice	62	94	93
Potato	Potatoes boiled, baked, mashed or like potato salad	46	76	64
Fruit	Fresh fruit and berries, fruit porridge/soup/compote; homemade or ready-prepared	100	100	100
Vegetable	All vegetables eaten raw/cooked/mashed alone or in a dish	99	100	100
Fish	All fish and fish products eaten as sandwich spread or in a dish	85	90	92
Meat	All meat and meat products eaten as sandwich spread or in a dish, except poultry and fish	98	100	100
Poultry	All poultry and poultry products eaten as sandwich spread or in a dish	59	74	73
Egg	All egg and egg products eaten as sandwich spread or in a dish	74	98	100
FatsAnimal	Butter, spreadable butter, sauce made from butter	93	98	98
FatsVegetable	Oil, margarine, mayonnaise, remoulade, ketchup, low fat sauce	73	83	90
Cheese	All cheese and cheese products eaten as sandwich spread or in a dish	79	98	96
Milk	All milk and milk products, e.g. skimmed milk, semi-skimmed milk, full fat milk and yogurt, eaten alone or in a dish except human milk or infant formula	98	100	100
Formula	Infant formula	70	3	0
BreastMilk	Human milk from the mother	42	0	0
FruitNutSnack	Cereal bar, nuts, almonds, dried fruit and fruit spread, jam, honey, peanut butter, seeds, peanuts	62	96	98
Chips	Potato chips, popcorn	2	24	37
SweetsCake	Ice cream, chocolate, liquorice, soufflé, croissant, Danish pastry, cookies, cream cake, pancake, cream puff, mix of light/not light versions	27	88	99
SugaryDrink	Soda, juice, lemonade, chocolate milk, milkshake and yogurt drink, mix of light/not light versions	19	76	94
FastFood	Fried potato, French fries, hotdog, pizza, burger, spring rolls	51	88	92

[a] *BreastMilk*: n_{9mo}=310, n_{18mo}=289, n_{36mo}=270

Table 3 Categories of individual development in dietary patterns from 9 to 36 months according to the average PCA score at each age in SKOT I (n=229)

Category	TRANSITION FOOD	n_{obs}[a]	n_{expt}[b]
AAA: Above-Above-Above	More TRANSITION FOOD than average at all ages	24	21
AAB: Above-Above-Below	More TRANSITION FOOD than average at 9+18 mo Less TRANSITION FOOD than average at 36 mo	29	-
ABA: Above-Below-Above	More TRANSITION FOOD than average at 9+36 mo Less TRANSITION FOOD than average at 18 mo	22	-
BAA: Below-Above-Above	More TRANSITION FOOD than average at 18+36 mo Less TRANSITION FOOD than average at 9 mo	26	-
BBA: Below-Below-Above	More TRANSITION FOOD than average at 36 mo Less TRANSITION FOOD than average at 9+18 mo	26	-
ABB: Above-Below-Below	More TRANSITION FOOD than average at 9 mo Less TRANSITION FOOD than average at 18+ 36 mo	29	-
BAB: Below-Above-Below	More TRANSITION FOOD than average at 18 mo Less TRANSITION FOOD than average at 9+36 mo	24	-
BBB: Below-Below-Below	Less TRANSITION FOOD than average at all ages	49	39

Category	HEALTHY FOOD	n_{obs}[a]	n_{expt}[b]
AAA: Above-Above-Above	More HEALTHY FOOD than average at all ages	47	29
AAB: Above-Above-Below	More HEALTHY FOOD than average at 9+18mo Less HEALTHY FOOD than average at 36 mo	23	-
ABA: Above-Below-Above	More HEALTHY FOOD than average at 9+36 mo Less HEALTHY FOOD than average at 18 mo	22	-
BAA: Below-Above-Above	More HEALTHY FOOD than average at 18+36 mo Less HEALTHY FOOD than average at 9 mo	33	-
BBA: Below-Below-Above	More HEALTHY FOOD than average at 36 mo Less HEALTHY FOOD than average at 9+18 mo	14	-
ABB: Above-Below-Below	More HEALTHY FOOD than average at 9 mo Less HEALTHY FOOD than average at 18+36 mo	17	-
BAB: Below-Above-Below	More HEALTHY FOOD than average at 18 mo Less HEALTHY FOOD than average at 9+36 mo	17	-
BBB: Below-Below-Below	Less HEALTHY FOOD than average at all ages	56	28

Category	TRADITIONAL FOOD	n_{obs}[a]	n_{expt}[b]
AAA: Above-Above-Above	More TRADITIONAL FOOD than average at all ages	32	26
AAB: Above-Above-Below	More TRADITIONAL FOOD than average at 9+18 mo Less TRADITIONAL FOOD than average at 36 mo	28	-
ABA: Above-Below-Above	More TRADITIONAL FOOD than average at 9+36 mo Less TRADITIONAL FOOD than average at 18 mo	24	-
BAA: Below-Above-Above	More TRADITIONAL FOOD than average at 18+36 mo Less TRADITIONAL FOOD than average at 9 mo	32	-
BBA: Below-Below-Above	More TRADITIONAL FOOD than average at 36 mo Less TRADITIONAL FOOD than average at 9+18 mo	25	-
ABB: Above-Below-Below	More TRADITIONAL FOOD than average at 9 mo Less TRADITIONAL FOOD than average at 18+36 mo	26	-
BAB: Below-Above-Below	More TRADITIONAL FOOD than average at 18 mo Less TRADITIONAL FOOD than average at 9+18 mo	20	-
BBB: Below-Below-Below	Less TRADITIONAL FOOD than average at all ages	42	31

[a]n: observed number of children in each category. The darkness of grey colour increase with n (Colour cut-off: n <22, 22-31, 32-41, >41). The categorisation was based on individual PCA scores of each child as either above or below mean score of the whole SKOT I cohort at the same age. [b]Theoretically calculated n expected if no tracking was present

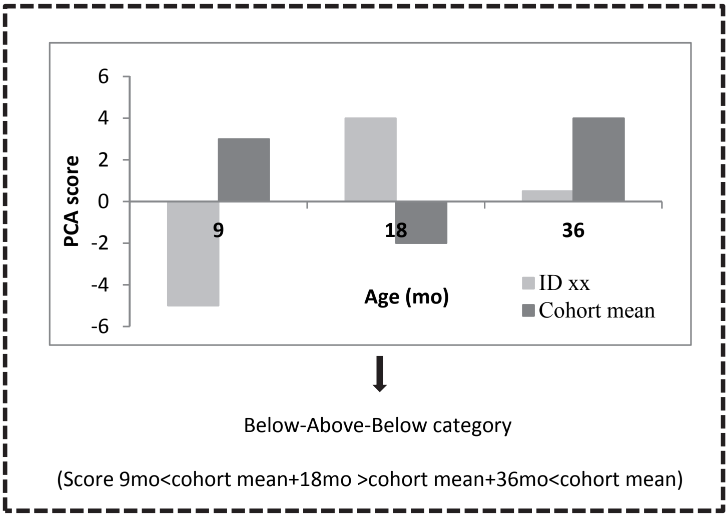
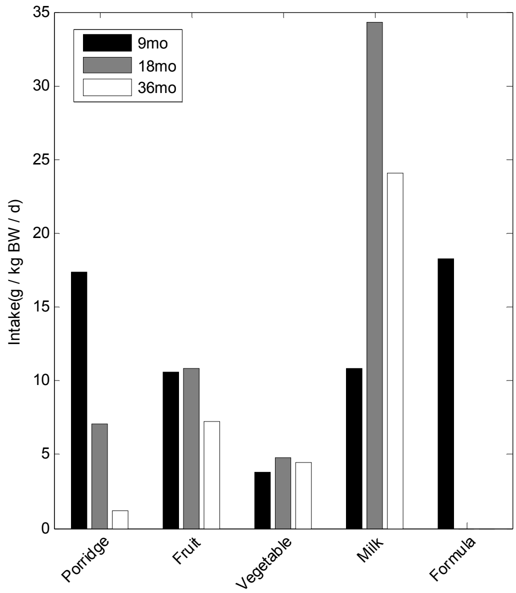


Figure 1 Exemplified illustration of the categorisation system for the fictive child ID xx
Child xx ends up in one category for each dietary pattern
(not necessarily the same category)

A



B

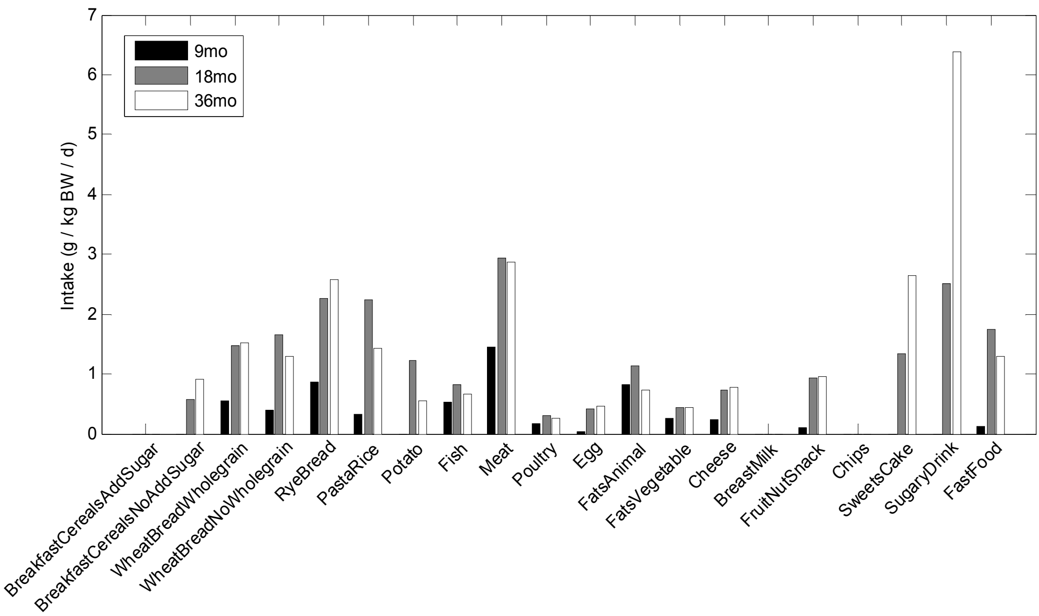


Figure 2 Median intake of food groups

A: Foods of highest intake. **B**: Foods of lower intake. BW: Body weight. n_{9mo}=307, n_{18mo}=267, n_{36mo}=240 except for *BreastMilk*: n_{9mo}=310, n_{18mo}=289, n_{36mo}=270. Food groups with median intake =0 illustrate that less than 50% of the children had an intake of this food group.

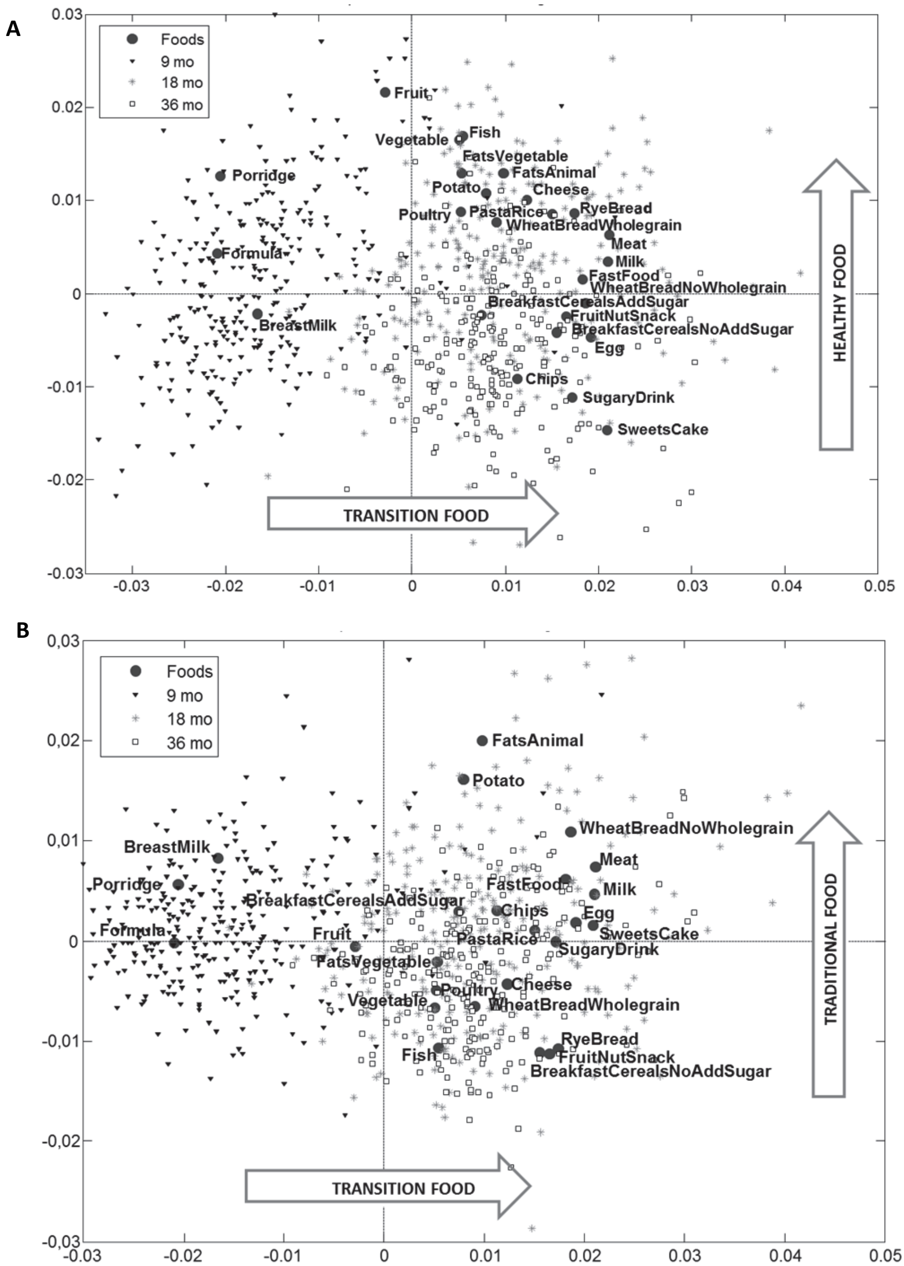


Figure 3 Dietary patterns in the SKOT I cohort

A: PCA bi-plot of the TRANSITION FOOD and HEALTHY FOOD patterns. **B**: PCA bi-plot of the TRANSITION FOOD and TRADITIONAL FOOD patterns. The foods causing the patterns are indicated with ●. Participants (scores) are indicated with ▼ at 9 mo, * at 18 mo and □ at 36 mo. Foods close to each other are correlated while participants placed close to a certain food variable (loading) have a high intake of this food and a lower intake of foods far away, relative to the rest of the participants in the SKOT I cohort. Input variables for the PCA was intake of foods (g/kg BW/day), n_{9mo}=307, n_{18mo}=267, n_{36mo}=240 except for *BreastMilk* (feedings/day): n_{9mo}=310, n_{18mo}=289, n_{36mo}=270. Note that the scale shown on the axes in this plot is arbitrary because the scores and loadings have been individually scaled to fit in the same coordinate system. The same TRANSITION FOOD pattern is shown both in A and B.

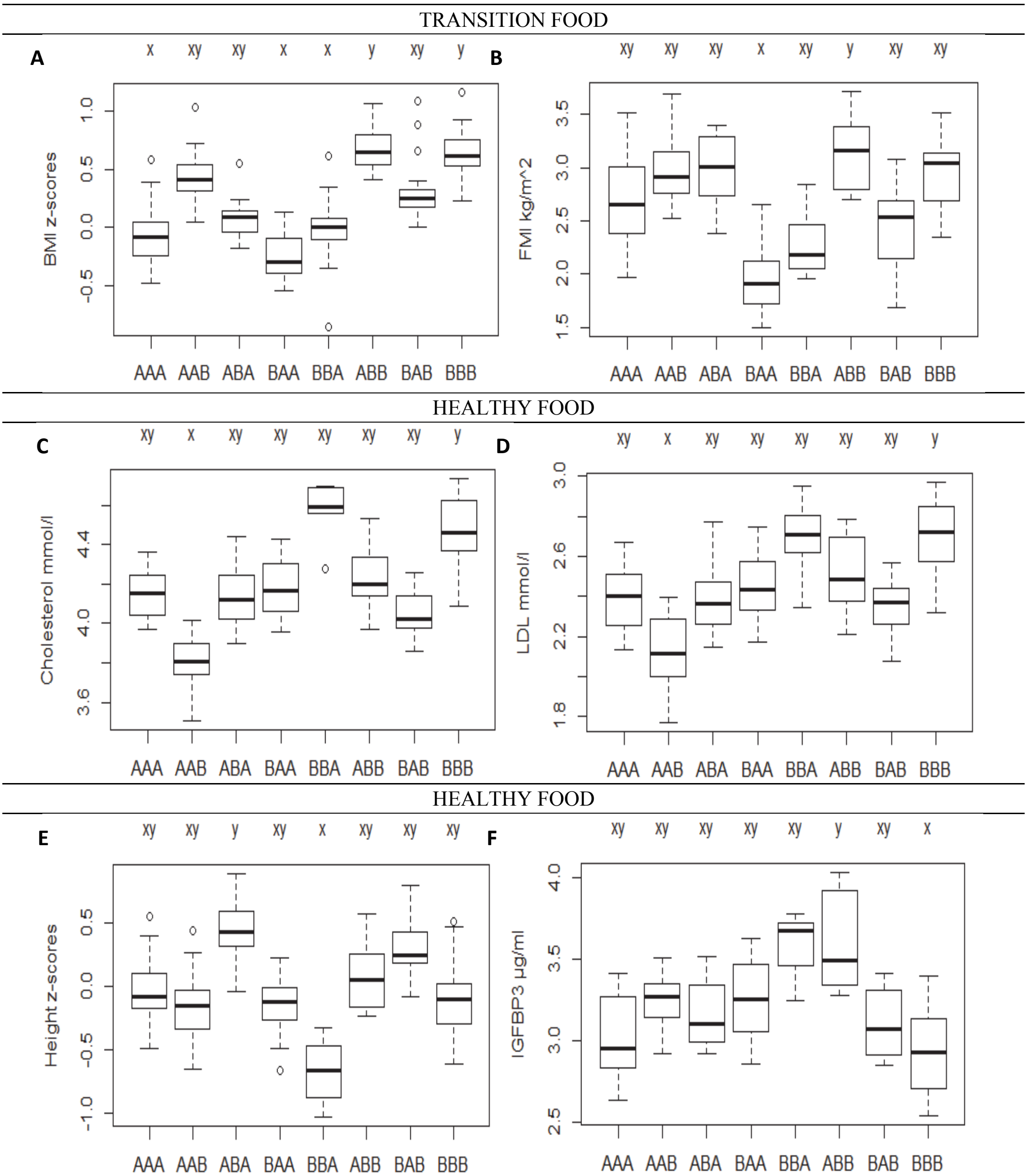


Figure 4 Differences in body size, body composition and metabolic risk markers at 36 months between the categories of development in the dietary patterns TRANSITION FOOD and HEALTHY FOOD

A, B: BMI z-scores and FMI respectively in the eight categories of development in the TRANSITION FOOD pattern.
C, D, E, F: Total blood cholesterol, LDL, height z-scores and IGFBP3 respectively in the eight categories of development in the HEALTHY FOOD pattern. Pairwise comparisons are based on ANCOVA. Letters below the boxplot (A, B) refer to the different categories of development in dietary patterns (Table 3). Significant different levels in the dietary categories are indicated with different letters (x or y) above the boxplot. Boxes indicate the interquartile range around the median and are extended by lines of +/-1.5 * interquartile range (or maximum/minimum, if these are within 1.5* interquartile range) and single more extreme values are indicated with dots.

Supplementary material:

Table S1 Loadings for input variables in the PCA based on dietary intake at 9, 18 and 36 months of age for children from the SKOT I cohort

	Dietary patterns (% variance explained)		
Foods (input variables for PCA)[a]	TRANSITION FOOD (18%)	HEALTHY FOOD (8%)	TRADITIONAL FOOD (5%)
Porridge (g/kg BW/d)	-0.276	0.249	0.140
BreakfastCerealsAddSugar (g/kg BW/d)	0.100	-0.045	0.072
BreakfastCerealsNoAddSugar (g/kg BW/d)	0.208	-0.081	-0.276
WheatBreadWholegrain (g/kg BW/d)	0.122	0.151	-0.162
WheatBreadNoWholegrain (g/kg BW/d)	0.250	-0.021	0.271
RyeBread (g/kg BW/d)	0.234	0.172	-0.268
PastaRice (g/kg BW/d)	0.202	0.168	0.027
Potato (g/kg BW/d)	0.107	0.212	0.399
Fruit (g/kg BW/d)	-0.039	0.426	-0.014
Vegetable (g/kg BW/d)	0.068	0.326	-0.166
Fish (g/kg BW/d)	0.074	0.334	-0.263
Meat (g/kg BW/d)	0.284	0.123	0.182
Poultry (g/kg BW/d)	0.070	0.174	-0.122
Egg (g/kg BW/d)	0.257	-0.093	0.046
FatsAnimal (g/kg BW/d)	0.132	0.254	0.494
FatsVegetable (g/kg BW/d)	0.071	0.254	-0.052
Cheese (g/kg BW/d)	0.166	0.197	-0.105
Milk (g/kg BW/d)	0.282	0.068	0.116
Formula (g/kg BW/d)	-0.281	0.085	-0.004
BreastMilk (feedings/d)	-0.222	-0.044	0.204
FruitNutSnack (g/kg BW/d)	0.221	-0.049	-0.279
Chips (g/kg BW/d)	0.152	-0.181	0.075
SweetsCake (g/kg BW/d)	0.280	-0.289	0.039
SugaryDrink (g/kg BW/d)	0.231	-0.221	-0.001
FastFood (g/kg BW/d)	0.245	0.030	0.152

BW: body weight, [a]n_{9mo}=307, n_{18mo}=267, n_{36mo}=240, except for *BreastMilk*: n_{9mo}=310, n_{18mo}=289, n_{36mo}=270

Table S2 Mean scores according to age for each dietary pattern identified in a PCA based on dietary intake at 9, 18 and 36 months of age for children from the SKOT I cohort

Age	TRANSITION FOOD mean ±SD (n)	HEALTHY FOOD mean ±SD (n)	TRADITIONAL FOOD mean ±SD (n)
9 mo	-2.2±1.3(307)	0.30±1.42(307)	0.25±0.95(307)
18 mo	1.4±1.3(267)	0.38±1.41(267)	0.15±1.36(267)
36 mo	1.3±1.0(240)	-0.81±1.17(240)	-0.49±0.98(240)

Table S3 Differences in body size, body composition and metabolic risk markers at 36 months between categories of development in the dietary patterns TRANSITION FOOD and HEALTHY FOOD

I)	TRANSITION FOOD					
Compared	**BMI z-score**			**FMI, kg/m²**		
categories[a]	Est. diff.[b]	95% CI[c]	p[d]	Est. diff.	95% CI	p
AAB-AAA	0.55	-0.17, 1.27	0.28	0.30	-0.61, 1.21	0.97
ABA-AAA	0.12	-0.64, 0.88	1.0	0.13	-0.96, 1.22	1.0
BAA-AAA	-0.13	-0.85, 0.58	1.0	-0.63	-1.69, 0.43	0.58
BBA-AAA	0.070	-0.682, 0.823	1.0	-0.30	-1.35, 0.74	0.98
ABB-AAA	0.78	0.07, 1.50	***0.021***	0.49	-0.52, 1.49	0.80
BAB-AAA	0.40	-0.33, 1.14	0.70	-0.49	-1.54, 0.56	0.82
BBB-AAA	0.72	0.07, 1.36	***0.017***	0.24	-0.74, 1.21	0.99
ABA-AAB	-0.43	-1.17, 0.31	0.63	-0.17	-1.15, 0.80	1.0
BAA-AAB	-0.68	-1.37, 0.01	0.053	-0.93	-1.88, 0.02	0.059
BBA-AAB	-0.48	-1.20 0.23	0.44	-0.61	-1.51, 0.30	0.42
ABB-AAB	0.23	-0.46, 0.93	0.97	0.18	-0.74, 1.10	1.0
BAB-AAB	-0.15	-0.86, 0.56	1.0	-0.79	-1.74, 0.15	0.17
BBB-AAB	0.17	-0.45, 0.79	0.99	-0.067	-0.90, 0.77	1.0
BAA-ABA	-0.25	-0.99, 0.48	0.96	-0.76	-1.86, 0.34	0.39
BBA-ABA	-0.051	-0.80, 0.70	1.0	-0.43	-1.49, 0.63	0.90
ABB-ABA	0.66	-0.07, 1.40	0.11	0.36	-0.74, 1.46	0.97
BAB-ABA	0.28	-0.48, 1.04	0.95	0.62	-1.69, 0.45	0.61
BBB-ABA	0.60	-0.07, 1.26	0.11	0.11	-0.90, 1.11	1.0
BBA-BAA	0.20	-0.50, 0.90	0.99	0.32	-0.70, 1.35	0.97
ABB-BAA	0.92	0.23, 1.60	***<0.01***	1.1	0.07, 2.16	***0.029***
BAB-BAA	0.53	-0.18, 1.25	0.30	0.14	-0.94, 1.21	1.0
BBB-BAA	0.85	0.24, 1.46	***<0.01***	0.87	-0.12, 1.85	0.13
ABB-BBA	0.71	0.01, 1.41	***0.042***	0.79	-0.19, 1.78	0.21
BAB-BBA	0.33	-0.41, 1.07	0.86	-0.19	-1.20, 0.83	1.0
BBB-BBA	0.65	0.02, 1.28	***0.039***	0.54	-0.33, 1.42	0.54
BAB-ABB	-0.38	-1.10, 0.33	0.72	-0.98	-2.02, 0.07	0.082
BBB-ABB	-0.066	-0.67, 0.54	1.0	-0.25	-1.17, 0.67	0.99
BBB-BAB	0.32	-0.33, 0.96	0.80	0.73	-0.23, 1.68	0.27

Table continued next page

Table S3 continued

II)

	HEALTHY FOOD											
Compared	**Total Cholesterol, mmol/l**			**LDL, mmol/l**			**Height z-score**			**IGFBP3, µg/ml**		
categories[a]	Est. diff.[b]	95% CI[c]	p[d]	Est. diff.	95% CI	p	Est. diff.	95% CI	p	Est. diff.	95% CI	p
AAB-AAA	-0.41	-1.02, 0.20	0.43	-0.33	-0.85, 0.19	0.52	-0.11	-0.80, 0.57	1.0	0.076	-0.53 0.68	1.0
ABA-AAA	0.0086	-0.60, 0.61	1.0	-0.00025	-0.52, 0.52	1.0	0.41	-0.26, 1.09	0.56	0.17	-0.43 0.76	1.0
BAA-AAA	0.039	-0.50, 0.58	1.0	0.076	-0.39, 0.542	1.0	-0.11	-0.71, 0.48	1.0	0.19	-0.34 0.71	0.96
BBA-AAA	0.42	-0.36, 1.21	0.72	0.28	-0.39, 0.96	1.0	-0.65	-1.50, 0.21	0.28	0.42	-0.35 1.19	0.69
ABB-AAA	0.10	-0.54, 0.74	1.0	0.15	-0.40, 0.70	0.99	0.13	-0.61, 0.86	1.0	0.57	-0.05 1.20	0.097
BAB-AAA	-0.094	-0.82, 0.63	1.0	0.00094	-0.62, 0.62	1.0	0.25	-0.51, 1.01	0.97	0.050	-0.64 0.74	1.0
BBB-AAA	0.30	-0.18, 0.78	0.55	0.27	-0.14, 0.69	0.46	0.0025	-0.52, 0.53	1.0	-0.11	-0.58 0.36	1.0
ABA-AAB	0.42	-0.27, 1.10	0.56	0.33	-0.26, 0.92	0.66	0.53	-0.26, 1.31	0.44	0.093	-0.58 0.77	1.0
BAA-AAB	0.45	-0.19, 1.09	0.38	0.41	-0.14, 0.96	0.31	-0.00035	-0.72, 0.72	1.0	0.11	-0.51 0.74	1.0
BBA-AAB	0.83	-0.01, 1.67	0.056	0.61	-0.11, 1.34	0.16	-0.53	-1.46, 0.39	0.64	0.35	-0.48 1.18	0.90
ABB-AAB	0.51	-0.21, 1.24	0.37	0.48	-0.14, 1.10	0.26	0.24	-0.59, 1.08	0.99	0.50	-0.22 1.21	0.39
BAB-AAB	0.32	-0.49, 1.12	0.93	0.33	-0.36, 1.02	0.82	0.36	-0.50, 1.22	0.90	-0.026	-0.80 0.74	1.0
BBB-AAB	0.71	0.12, 1.30	***0.0075***	0.60	0.10, 1.11	***<0.01***	0.12	-0.54, 0.78	1.0	-0.19	-0.77 0.39	0.97
BAA-ABA	0.030	-0.60, 0.66	1.0	0.076	-0.47, 0.62	1.0	-0.53	-1.25, 0.19	0.33	0.018	-0.60 0.64	1.0
BBA-ABA	0.41	-0.42, 1.25	0.79	0.28	-0.43, 1.0	0.93	-1.061	-1.99, -0.13	***0.014***	0.25	-0.56 1.07	0.98
ABB-ABA	0.094	-0.64, 0.83	1.0	0.15	-0.48, 0.78	1.0	-0.28	-1.13, 0.56	0.97	0.41	-0.31 1.13	0.66
BAB-ABA	-0.10	-0.90, 0.69	1.0	0.0012	-0.68, 0.68	1.0	-0.16	-1.03, 0.70	1.0	-0.12	-0.89 0.65	1.0
BBB-ABA	0.29	-0.29, 0.87	0.78	0.27	-0.22, 0.77	0.69	-0.41	-1.07, 0.25	0.54	-0.28	-0.85 0.29	0.79
BBA-BAA	0.38	-0.41, 1.18	0.82	0.21	-0.47, 0.89	0.98	-0.53	-1.41, 0.34	0.57	0.24	-0.54 1.02	0.98
ABB-BAA	0.064	-0.61, 0.73	1.0	0.072	-0.50, 0.65	1.0	0.24	-0.54, 1.02	0.98	0.39	-0.27 1.04	0.60
BAB-BAA	-0.13	-0.88, 0.62	1.0	-0.075	-0.72, 0.57	1.0	0.36	-0.44, 1.16	0.86	-0.14	-0.85 0.57	1.0
BBB-BAA	0.26	-0.26, 0.78	0.79	0.20	-0.25, 0.65	0.87	0.12	-0.47, 0.70	1.0	-0.30	-0.80 0.21	0.60
ABB-BBA	-0.32	-1.20, 0.56	0.95	-0.13	-0.89, 0.62	1.0	0.78	-0.21, 1.76	0.24	0.15	-0.71 1.02	1.0
BAB-BBA	-0.52	-1.45, 0.41	0.68	-0.288	-1.08, 0.52	0.96	0.90	-0.091, 1.89	0.11	-0.37	-1.27 0.52	0.90
BBB-BBA	-0.12	-0.87, 0.63	1.0	-0.0096	-0.65, 0.63	1.0	0.65	-0.17, 1.48	0.24	-0.53	-1.27 0.20	0.33
BAB-ABB	-0.20	-1.03, 0.63	1.0	-0.15	-0.86, 0.57	1.0	0.12	-0.79, 1.03	1.0	-0.53	-1.32 0.27	0.46
BBB-ABB	0.20	-0.43, 0.82	0.98	0.13	-0.41, 0.66	1.0	-0.13	-0.85, 0.59	1.0	-0.69	-1.30 -0.070	***0.018***
BBB-BAB	0.39	-0.32, 1.11	0.69	0.27	-0.34, 0.88	0.87	-0.25	-1.00, 0.51	0.97	-0.16	-0.84 0.51	1.0

I) TRANSITION FOOD, II) HEALTHY FOOD, [a]Representing pairwise comparison of the eight categories of development in dietary patterns across 9, 18 and 36 mo of life, A=PCA score Above the mean score for each age, B= PCA score Below the mean score for each age. [b]Estimated difference in the outcome between the two categories compared. [c]Familywise 95% confidence interval adjusted for multiple testing. [d]Post hoc pairwise comparisons of the eight categories adjusted for multiple testing using the single step method.

4.4 Paper IV: The effects of water and dairy drinks on dietary patterns in overweight adolescents

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Current status (Oct. 2014): Submitted to Public Health Nutrition

Tables and figures can be found at the end of the manuscript

The effects of water and dairy drinks on dietary patterns in overweight adolescents

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Abstract

Objective: The aim of the present study was to investigate the effects of increased water or dairy intake on the overall diet in overweight adolescents on energy, nutrients, foods and dietary pattern levels.

Setting: Danish research centre.

Subjects: 173 overweight adolescent participants in the MoMS study.

Design: Participants were randomly assigned to consume 1L/day of skim milk, whey, casein (here combined as the dairy group) or water (water group) for 12 weeks. The rest of the diet could be eaten *ad libitum,* and dietary intake before and during the intervention was recorded by four-day food diaries. Dietary patterns were identified by principal component analysis.

Results: A decrease in the dietary pattern CONVENIENCE FOOD was observed during the intervention both in the water ($p = 0.0004$) and dairy ($p < 0.0001$) groups. This included a decreased intake from the food group *SugaryDrink*. Total energy intake (diet + test drink) decreased in the water group ($p = 0.007$) but was unchanged in the dairy group ($p = 0.88$) during intervention.

Conclusion: An extra intake of fluid seems to favourably affect the rest of the diet by decreasing the intake of convenience foods, such as sugar-sweetened beverages. The choice of a fluid with low energy content seems important when considering the total energy intake in these overweight adolescents and it supports the present recommendation about the promotion of water consumption. The evaluation of this intervention study was reinforced by including dietary patterns identified by PCA.

Keywords: water, intervention, dietary patterns, adolescents

Introduction

Plain water should be promoted as the main source of fluid for children. This is the recommendation by the Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)[1], and it is also the official recommendation in countries such as Denmark[2]. Water contains no energy but might contribute to a feeling of fullness. Water might therefore stabilise or reduce total energy intake by decreasing total energy density[3-5] and could potentially contribute to one's ability to maintain or to lose weight[6], as indicated in studies of adults; however, evidence is mostly based on studies of a single meal and evidence related to children is sparse.

The recommendation on water consumption from ESPGHAN continues by stating that plain water should be promoted *instead* of sugar-sweetened beverages[1]. However, little is known about compensation in the rest of the diet when children in non-restricted settings increase their water consumption. The few studies that have investigated this compensation[7-9] could not confirm the hypothesis. To our knowledge, no studies have analysed overall changes in dietary patterns when increasing water intake. Therefore, we wanted to investigate the effects of increased intake of different test drinks on the diet, including multiple dietary levels. The aim of this paper was to describe the diet of overweight Danish adolescents in the Milk Components and Metabolic Syndrome (MoMS) intervention study, both at energy-, nutrients-, foods- and dietary-pattern levels and to investigate how the diet changed during intervention, according to intervention groups. The MoMS study comprised four intervention groups whose participants drank 1L/day of either water, skim milk, whey or casein. This analysis is a secondary explorative analysis of the MoMS data, for which the intervention effects on body weight and biomarkers for metabolic syndrome were the main outcomes. Furthermore, the MOMS study provided an opportunity to compare the effects of water intake, with three different dairy drinks, on the diet.

In addition to the public health aspect of investigating a dietary intervention effect on dietary intake, this paper also contributes with a unique methodological approach. An increasing number of observational studies during the last two decades have improved our understanding of dietary complexity by reporting dietary patterns, most commonly by principal component analysis (PCA), instead of single-nutrient or food intake in adolescents [10-17]. However, this paper is, as far as we know, the first to evaluate the effects of an intervention study in adolescents by identifying dietary patterns with PCA. This method has been used in an intervention only once in children, specifically in toddlers[18].

Experimental methods

Study design and participants

This paper is based on the MoMS intervention study, with 12–15 year-old adolescents (N = 203) with an habitual milk and yogurt intake ≤ 250mL/day and who were overweight, as defined by the International Obesity Task Force (age-and sex-adjusted BMI corresponding to an adult BMI > 25kg/m^2)[19]. In the MoMS study, the participants were randomly assigned to one of four intervention groups: either 1L/day of skim milk, whey, casein or water for 12 weeks. The nutritional composition of the test drinks has been published elsewhere[20], and all dairy drinks had the same protein content (3.5g/100g). Exclusion criteria were smoking, recent consumption of antibiotics and chronic diseases. The participants were recruited during the years 2008–2010 by sending a postal invitation to all adolescents living in the Copenhagen area who were born during the years1995–1998, based on an extraction from the National Danish Civil Registration. The adolescents registered their diets before the intervention (week 0) and in the last week of intervention (week 12). Other measurements, such as anthropometry, blood samples, physical activity and blood pressure, were also carried out. To have a control group receiving no intervention, data were also collected for a subgroup of the participants 12 weeks before the intervention, but these data will not be mentioned further in this paper, as the dietary changes during the intervention period was the focus here. More details of the study can be found in Arnberg et al.[20]. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Scientific Ethics Committees of the Capital Region of Denmark (H-A-2008–084) and registered at clinicaltrials.gov (NCT00785499). Informed written consent was obtained from all participants.

The mean intake of the test drinks as a percentage of the planned intake was 95% for water, 92% for skim milk, 91% for casein and 87% for whey, based on a compliance booklet or counting of leftovers, as earlier reported[20]. All investigators and participants receiving a dairy product were blinded regarding the specific dairy product consumed. Blinding of water was not possible.

Dietary data

The participants were asked to consume 1L/day of the test drink throughout the day and to eat their usual diet *ad libitum* during the entire study period. The dietary intake, excluding the test drink, will be referred to as the 'background diet'. Dietary intake before the intervention and during the last days of the intervention was recorded for four consecutive days (preferably, one weekend day and three weekdays). For the diet record, a pre-coded food diary was completed each day. Portion sizes were estimated in household measures accompanied by a booklet with 12 series of food photographs. This method of diet recording has previously been validated [21;22]. Data were analysed at the individual level using the general intake estimation software system (GIES, version 1.000d, developed at National Food Institute, Technical University of Denmark) and the Danish Food Composition Databank (version 7; Søborg; www.Foodcomp.dk). The selection of food groups and nutrients in this paper is based on Danish official dietary recommendations[2] and current trends in human nutrition and obesity research. Food groups are named with a short, compressed description, such as *'FatsAnimal'*, *'LowFatMilk'* or *'SugaryDrink'*. The estimated nutrient intake, including the test drink, was adjusted for compliance by multiplying with the proportion between the individual's actual compliance and full compliance. Possible over- and under-reporters of energy intake were identified based on the Goldberg cut-off[23] and used the Schofield equation[24] to estimate basal metabolic rate where a light activity level was assumed.

Anthropometry

Height was used as the mean of three measurements at a wall-mounted digital stadiometer, which made readings to the nearest 0.01 cm (235 Heightronic Digital Stadiometer, Quick Medical and Measurement Concepts). After an overnight fast and emptying the bladder, participants, wearing underwear and a T-shirt, were weighed to the nearest 0.1kg on a digital scale (Tanita BWB600, Tanita). Weight gain was calculated in kg by subtracting weight before intervention from weight in the last week of intervention. BMI (kg/m^2) was converted to z-scores (BAZ) using the software program WHO AnthroPlus and the WHO growth reference(25).

Statistical analysis

Energy, nutrients and dietary pattern scores were presented as mean intake, while food intake was not normally distributed and was presented as median. Dietary patterns were displayed by PCA, with food variables from before and during intervention together in long format (intake before and during intervention for each participant constituted different rows in the data matrix) and normalised by auto-scaling using MATLAB R2010b and PLS Toolbox, version 7.3.1. PCA disclose latent patterns in multifactorial issues, such as the whole diet. An individual dietary score value was assigned for each dietary pattern to each participant before and during the intervention. The naming of these latent dietary patterns was based on food groups with the highest loadings within each principal component. The number of principal components was selected based on a clear change in the scree plot(26). Orthomax rotation was tried but not used, since it did not optimise interpretation of the dietary patterns.

Changes in diet during the intervention were evaluated by one-way ANOVA on paired differences of intake during intervention minus intake before intervention at the levels of energy, nutrients, foods and dietary patterns. The use of paired differences eliminates the need for adjusting for confounding factors because each child was compared only with her/himself. At the energy and nutrient levels, the analyses were repeated, including the contribution from test drinks. Post hoc pairwise comparisons of the different intervention groups were executed and adjusted for multiple testing using the single-step method(27). Intervention groups were investigated, both as four subgroups (skim milk, casein, whey and water) and as two groups with and without an energy contribution (dairy group/water group), pooling the skim milk, casein and whey subgroups. The statistical programming environment R version 3.0.2 (www.r-project.org) was used in the analysis of dietary changes. All p-values were evaluated at a 5% significance level. Power calculation for the MoMS study has previously been reported(20).

Results

Characterisation of participants

Two hundred and three participants were enrolled in the MoMS project, but 10 participants withdrew before the start of the intervention. Dietary records, both before and during intervention, were available from 173 participants and are included in this paper. Characteristics of these participants are presented in Table 1, which shows a higher weight gain in the dairy group compared to the water group during the intervention. The differences in weight gain have previously been reported[20].

Characterisation of diet and change in diet according to intervention group

For the most part, changes in the water and dairy groups (but not those in the dairy subgroups) during the intervention are reported. Additional analyses of changes within each of the three dairy subgroups and a descriptive overview of all diet variables have been placed in the online supplementary material (Tables S1A & S1B).

<u>Energy</u>

The total energy intake for the 173 participants was 7997 ± 2384 kJ/d before intervention and 6663 ± 2290 kJ/d during intervention, excluding the test drink. Energy intake, excluding the test drink, was lower during intervention than before, both in the water group ($p = 0.01$) and dairy group ($p < 0.0001$). However, when dividing the dairy group into subgroups, this was significant only for skim milk ($p = 0.0007$) and whey ($p = 0.0001$) but not casein ($p = 0.16$). When including the energy contribution from test drinks, the lower energy intake during intervention compared to that before intervention was significant only for the water group ($p = 0.007$) but not for the dairy group ($p = 0.88$) (Figure 1A,B).

The estimated proportion of under-reporters was 34% before and 55% during the intervention (including the test drink), respectively, and the estimated proportion of over-reporters was 1%, both before and during the intervention (including the test drink). However, all possible under- and over-reporters were included in all analyses in this paper, as excluding over-reporters did not change the overall results, and excluding under-reporters would seriously affect the power.

<u>Nutrients</u>

During intervention, the percentages of total energy (E%) from fat ($p = 0.19$), protein ($p = 0.70$) and carbohydrates ($p = 0.52$) did not change from the background diet in the water and dairy groups (Figure 1C,D). When including the test drink, the fat E% decreased ($p < 0.0001$), while protein E% increased ($p < 0.0001$) in the dairy group, but it remained unchanged for the water group, both for fat E% ($p = 0.24$) and protein E% ($p = 0.91$). The carbohydrate E%, including the test drink, did not change ($p = 0.57$) in the water and dairy groups.

<u>Foods</u>

Some foods were eaten by only a small proportion of the participants during the registration periods, which gave skewed distributions of the intake. Therefore, the percentages of the adolescents who ate the different food groups are shown in Table 2, which also includes descriptions of the different groups. A general trend was observed of a recorded lower intake of foods per kg body weight (BW) during intervention than that before intervention (Figure 2A,B). The intake of *Vegetable*, *FatsAnimal*, *LowFatMilk* and *SugaryDrink* was significantly lower, both in the dairy and water groups during intervention, while *BreakfastCerealsNoAddSugar*, *Meat*, *FatsVegetable*, *Cheese*, *FruitNutSnack* and *FastFood* were significantly lower during intervention only in the dairy group but not in the smaller water group. When divided into the four intervention subgroups, the casein group differed from the other groups by having no significant change in food groups during intervention; additionally, the overall decrease in *SugaryDrink* was significant only for the skim milk group ($p = 0.0005$) (Supplementary Table S1B).

Dietary patterns

Three dietary patterns appeared by PCA. Based on the loading plots, these patterns are labelled CONVENIENCE FOOD, FAST FOOD and HEALTH-CONSCIOUS FOOD (Figure 3). CONVENIENCE FOOD explained 11% of the variation in food intake and was characterised by the highest intake of foods such as *FastFood*, *SugaryDrink*, *SweetsCake* and *Meat*. The FAST FOOD pattern explained 9% of the variation in food intake and was characterised by the highest intake of *FastFood* and *Cheese*, while the HEALTH-CONSCIOUS FOOD pattern explained 7% of variation in food intake and was characterised by the highest intake of *WheatBreadWholegrain*, *Fruit*, *BreakfastCerealsNoAddSugar* and *LowFatMilk*, and a low intake of *WheatBreadNoWholegrain*. Together, these patterns described 27% of the total variation in the participants' background diet. The CONVENIENCE FOOD pattern was the only dietary pattern showing a significant change during intervention. The change was seen for both the water group ($p = 0.0004$) and the dairy group ($p < 0.0001$), while no significant change in the FAST FOOD ($p = 0.81$) and the HEALTH-CONSCIOUS FOOD ($p = 0.27$) patterns was seen (Figure 2C,D). The participants had a mean score at the CONVENIENCE FOOD pattern that was higher before intervention than during intervention. This was also true for all intervention subgroups (Supplementary table S1B).

Discussion

The background diet before and during a dietary intervention with water or dairy test drinks was investigated for overweight adolescents at four dietary levels: energy, macronutrients, foods and dietary patterns. Comparing the diet before and during intervention showed several changes in the background diet. A decrease in the CONVENIENCE FOOD dietary pattern during the intervention was observed, both in the water and dairy groups together with a general trend of lower intake at the individual food-groups level, which was significant for *Vegetable*, *FatsAnimal*, *LowFatMilk* and *SugaryDrink*. This was also reflected in the lower energy intake from the background diet. These findings are especially interesting in relation to the current recommendation about drinking water to improve health.

Dietary intake

The energy distribution between macronutrients, both in intake before and during intervention, was within the recommended range (Carbohydrates: 50–60E%; Fat: 25–35E% and Protein: 10–20E%[28-30]). At the foods level, the adolescents ate less fruit and vegetables but, on average, more sugar-sweetened beverages than recommended[2]. Similar trends are seen in other Danish surveys[31;32]. The dietary pattern CONVENIENCE FOOD identified here is, to some degree, comparable with the dietary pattern PROCESSED, which was identified in a Danish national survey of 11–14 year-old adolescents[16]. They also identify a HEALTH-CONSCIOUS pattern, which seems to have only a few similarities with our HEALTH-CONSCIOUS FOOD pattern[16] . The dissimilarities might be related to different selection of food groups and a higher proportion of girls in the MoMS study. A distinct FAST FOOD pattern appears only in the MoMS study, but not in the national survey[16]; this might be because this study covers a selected group of overweight adolescents. A distinct FAST FOOD pattern has earlier been reported in a study of Brazilian obese adolescents[33], but also in a national survey of Danish adults[34].

Compensation in background diet during intervention

The water group showed a decrease in total energy intake during intervention and thereby indicates a long-term reducing effect of water consumption on energy intake, which supports results from acute test meals in adults[3;5]. Moreover, there was no significant change in total energy intake in the dairy group (including the contribution from test drinks) between the two dietary recording periods, which could be interpreted as sufficient compensation in background diet for the energy content in the test drink. This finding is supported by two studies that investigated a long-term intervention with dairy products[35;36], but which was contradicted by two short-term meal studies that showed a stable energy intake from the background diet and thereby an increase in total energy intake when including the contribution from sugary test drinks[37;38]. This could indicate a satiating effect of a protein-rich milk drink rather than a sugary drink; however, one study that tested both milk and sugar-sweetened beverage intake found an increase in total energy intake for both types of test drinks. Finally, the findings related to energy intake are challenged by the degree of under-reporting discussed later.

Data on foods and dietary pattern levels provide more information about the changes in the background diet. The CONVENIENCE FOOD intake decreased, including, for example, the food group *SugaryDrink* in both the dairy and water groups. This is in contrast to other intervention studies that have investigated the effects of increased water intake in children, which found unchanged intake or purchase of sugar-sweetened beverages[7-9]. However, our results support the assumption used in the ESPGHAN and official Danish recommendation[1;2]: increased water intake decreases the intake of sugar-sweetened beverages. The decrease in *SugaryDrink* was considerable, on average 0.9L/week. Dividing the dairy group into skim milk, casein and whey subgroups showed that only the skim milk group significantly decreased *SugaryDrink*, which might be because of reduced power due to relatively small groups. The CONVENIENCE FOOD pattern is not only characterised by *SugaryDrink* but rather by a range of different food groups, such as *FastFood*, *SweetsCake*, *FatsAnimal*, *FatsVegetable* and *Meat*, at approximately the same levels of loadings in the PCA. These other foods probably also contributed to the significant decrease in this pattern during the intervention. A noteworthy change in the background diet is the decrease in *FastFood* during the intervention, which also contributed to the decrease in the CONVENIENCE FOOD pattern, though this change was significant only in the dairy group.

The decrease in *LowFatMilk* in the dairy group is an expected finding because *LowFatMilk* is an obvious food to replace with the dairy test drink. Skim milk and casein would be the most obvious replacements in usual meals that include milk because of similar sensory characteristics. However, when subdividing the dairy group, the whey subgroup had a significant reduction in *LowFatMilk*, while the skim milk and casein subgroups were non-significant. This might be affected by the fact that all included participants were habitually infrequent milk drinkers, and the possibility of replacing milk would thereby be limited.

Change in dietary patterns during intervention

Only a few other studies have looked at dietary patterns in adolescence over time, and they were observational studies that had a follow-up period of several years[11;39;40]. Moreover, these studies compared dietary patterns based on separate PCAs for each time point and not dietary scores from different time-points in the same PCA as we do, which is an attempt to ensure that score values from the different time-points are compared precisely on the same dietary pattern. We have only found one intervention study that has taken advantage of the PCA method to evaluate the effects of a dietary intervention[18]. Yet, both the intervention and the age group differ considerably from the MoMS study. However, a firm investigation of the background diet at multiple levels, including dietary patterns, seems advantageous compared to the assumption of no change or similar changes for all intervention groups, e.g. only looking at energy intake. More insight into possible changes at the level of foods or dietary patterns during a dietary intervention study would increase our understanding of possible biological effects which might be due to, for example, less sugar-sweetened beverages rather than more water. Moreover, this insight would probably be valuable in decisions on optimal future community interventions, such as promoting water intake instead of prohibiting sugar-sweetened beverages.

Relation between dietary findings and health

The health outcomes investigated in the MoMS study have been reported previously[20;41;42] and show an increased BAZ in all dairy subgroups but no change in the water group after intervention compared to before intervention[20]. The increased BAZ in the dairy group was unexpected, as there was no change in total energy intake during the intervention. However, this might be due to an under-reporting of dietary intake; the under-reporting would possibly be amplified from first to second recording[43]. Thus, under-reporting seems to be a possible limitation in all intervention groups, as BAZ did not change in the water group despite a decrease in the reported energy intake, or it may take a longer intervention period before an effect on BAZ can be observed in the water group. The rate of under-reporting is higher for adolescents and for overweight individuals than it is for younger children and for normal-weight individuals; this is probably the explanation for the relatively high proportion of under-reporters in this study compared to that in other studies[44]. In a recent review, the proportion of under-reporters using similar dietary records was 19–41%[44] compared to 34% and 55% before and during intervention, respectively, in this study. Moreover, there is a risk of overestimating the proportion of under-reporters in a group of overweight/obese individuals due to lower metabolic activity in adipose tissue, thereby resulting in an overestimation of the basal metabolic rate[23]. However, the version of the Schofield equation, both including weight and height, used in this study has been recommended to minimise this overestimation in obese individuals[45].

No disadvantages of additional water intake in relation to body weight and risk of metabolic syndrome were shown in the MoMS study, and the advantages of additional dairy intake are unclear[20;41;42]. This again supports the recommendation of drinking water to maintain a healthy lifestyle in this study population. However, possible additional useful effects of milk intake on metabolic risk markers should continue to be investigated, including studies with smaller amounts of milk more comparable to official recommendations. Moreover, milk is an important contribution to a healthy Danish diet for adolescents to support bone health[32;36] and perhaps to decrease *SugaryDrink*, as indicated in the present study.

Strengths and limitations

The main strength of this study is the examination of multiple levels of the background diet. Especially, the inclusion of the whole dietary approach of well measured foods and dietary patterns increases the interpretation in relation to food-based recommendations. Moreover, the investigation was designed with no restriction of diet apart from the extra intake of the test drink each day for 12 weeks. This imitates how dietary changes may be adapted into usual life and supports free-living behaviour, which increases the interpretation of results in relation to public health.

The main limitation in this study is the under-reporting of dietary intake. There was an increase in under-reporting from the first to the second dietary record period, which has also been seen in other studies[43]. Moreover, the under-reporting might be differentiated and, unfortunately, most pronounced for potentially unhealthy snack-related foods[46] such as *SugaryDrink*. However, the degree of under-reporting does not differ between intervention groups, whereby the comparison of results between the intervention groups is likely to be relevant. However, the smaller sample size in the water group compared to the dairy group might explain some of the different findings in the two groups. A limitation related to PCA is the unavoidable subjective decisions incorporated into the definition of content and into the number of food groups even though it is a data-driven method. However, in this study, the investigation of the other dietary levels (energy, nutrients and food groups) supports the findings based on the dietary patterns. Finally, the participants were all overweight with a low habitual intake of milk, which could influence the generalizability to other groups of adolescents.

Conclusion

Consumption of extra fluid seems to have a favourable influence on the rest of the diet, as a decrease in the CONVENIENCE FOOD pattern was observed, including a decreased intake of the food group *SugaryDrink* during both water and dairy interventions. The choice of a fluid with low energy content seems important when considering the total energy intake in these overweight adolescents. These findings support the recommendation stating that plain water should be promoted as the main source of fluid for children, who should thereby reduce the intake of sugar-sweetened beverages. This conclusion is strengthened by the multiple dietary levels included, which also cover dietary patterns identified by PCA.

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Statement of Authors' Contributions to Manuscript: KFM and CM designed research; KA attended data collection and preparation; LBBA analysed data and wrote the paper; CBP and RB supported decisions during data analysis; ET supported nutrition evaluations. All authors reviewed, contributed to and approved the final version of the manuscript.

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Table 1 Characteristics of participants with complete diet data divided into intervention groups

	Water group			Dairy group			
N = 173	Mean	SD	n	Mean	SD	n	p†
Age‡, y	13.2	0.7	50	13.2	0.7	123	0.88
Weight‡, kg	66.9	8.5	50	66.8	10.0	123	0.63
Height‡, cm	162.9	7.6	49	163.1	7.9	123	0.95
BMI z-score‡||	1.8 (38% obese)	0.5	49	1.8(28% obese)	0.5	123	0.55
Weight gain§, kg	1.6	2.0	50	2.7	2.1	123	<0.001
Girls	64%		50	60%		123	0.71

†Significant differences between the water and dairy groups were tested by the Mann Whitney U test for continuous variables, and by chi-squared test for categorical variables.
‡At the examination before intervention.
§Weight during intervention–before intervention
|| Obese defined as BMI z-score > 2.

Table 2 Description of food groups and percentages of adolescents who ate from each food group during registration

Food group	Description	Before intervention % (n=173)	During intervention % (n=173)
Fruit	Fruit and berries, fresh, cooked, frozen, preserved	82	79
Vegetable	All vegetables eaten raw/cooked alone or in a dish	100	100
BreakfastCerealsAddSugar	Sugar puffs, sugary cereals	12	14
BreakfastCerealsNoAddSugar	Oat meal, muesli, cornflakes, porridge	54	50
WheatBreadWholegrain	Grainy bread, crisp bread	70	63
WheatBreadNoWholegrain	White bread/bun, biscuits	88	88
RyeBread	Rye bread with and without seeds	77	65
PastaRice	Pasta, rice	81	73
Potato	Potatoes, boiled, baked, mashed or prepared in potato salad	49	47
Fish	All fish and fish products eaten as sandwich spread or in a dish	62	66
Meat	All meat and meat products eaten as sandwich spread or in a dish, except poultry and fish	98	99
Poultry	All poultry and poultry products eaten as sandwich spread or in a dish	77	77
Egg	All egg and egg products eaten as sandwich spread or in a dish	94	93
FatsAnimal	Butter, spreadable butter, sauce made from butter	87	77
FatsVegetable	Oil, margarine, mayonnaise, remoulade, ketchup, low-fat sauce	74	63
Cheese	All cheese and cheese products eaten as sandwich spread or in a dish	96	90
HighFatMilk	Whole milk, cream, sour cream	12	13
LowFatMilk	Skim milk, semi-skim milk, buttermilk, yogurt	87	80
FruitNutSnack	Cereal bar, nuts, almonds, dried fruit and fruit spread, jam, honey, peanut butter, seeds, peanuts	64	56
Chips	Chips, popcorn	38	33
SweetsCake	Ice cream, candy, soufflé, Danish pastry, cookies, cream cake, pancake, cream puff, light/not light	97	92
SugaryDrink	Soda, juice, lemonade light/not light, cider	95	88
FastFood	Fried potato, French fries, hotdog, pizza, burger, spring rolls	75	70

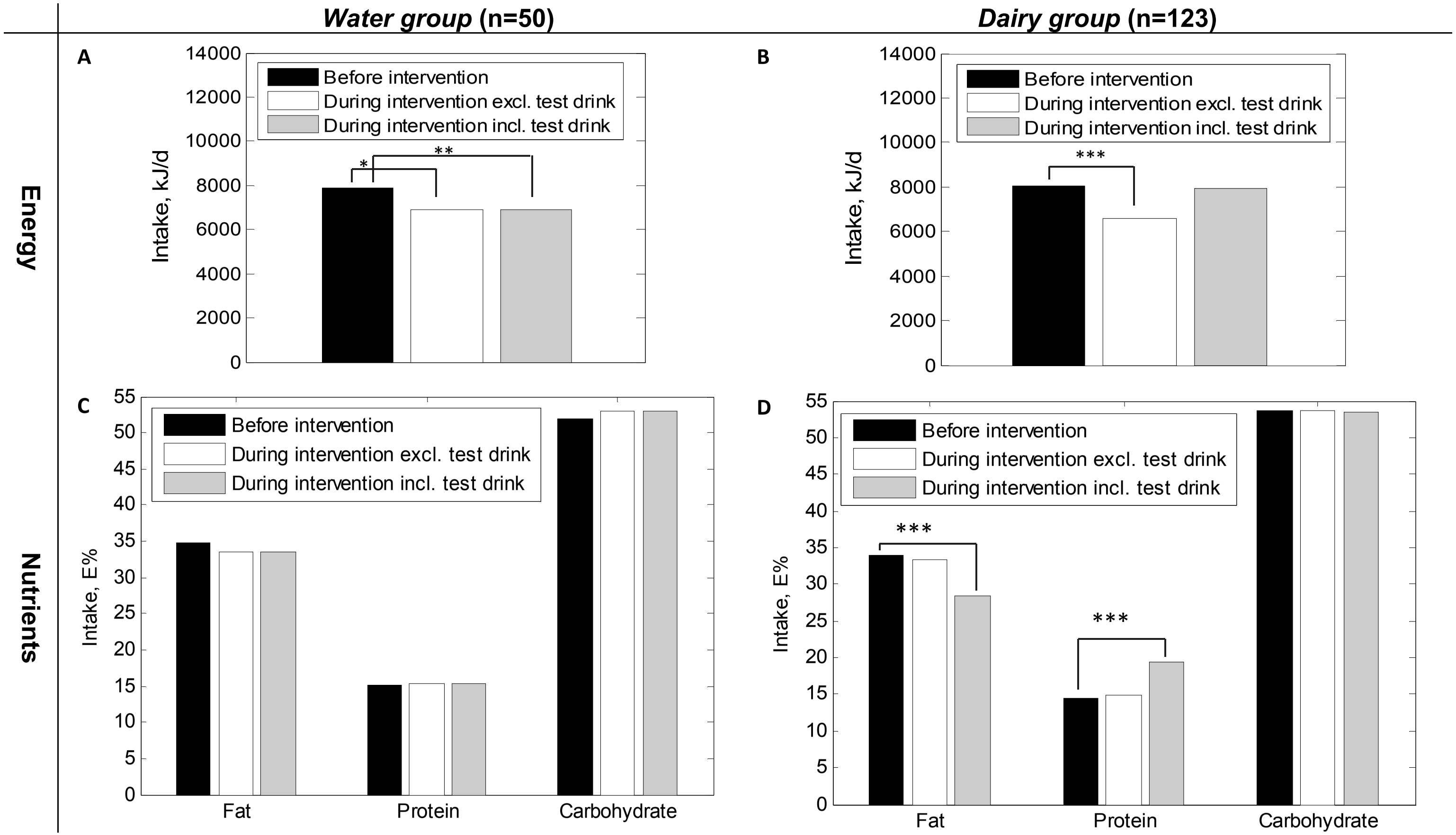

Figure 1 Intake of energy and macronutrients before and during intervention divided into water or dairy test drink
A: Mean intake of energy in the water group, **B:** Mean intake of energy in the dairy group, **C:** Mean intake of macronutrients in the water group, **D:** Mean intake of macronutrients in the dairy group. Significant differences between values before and during intervention are indicated with asterisks (P<0.001***, <0.01**, <0.05*) based on ANOVA followed by post hoc pairwise comparisons. If no asterisks are present, the difference was not significant.

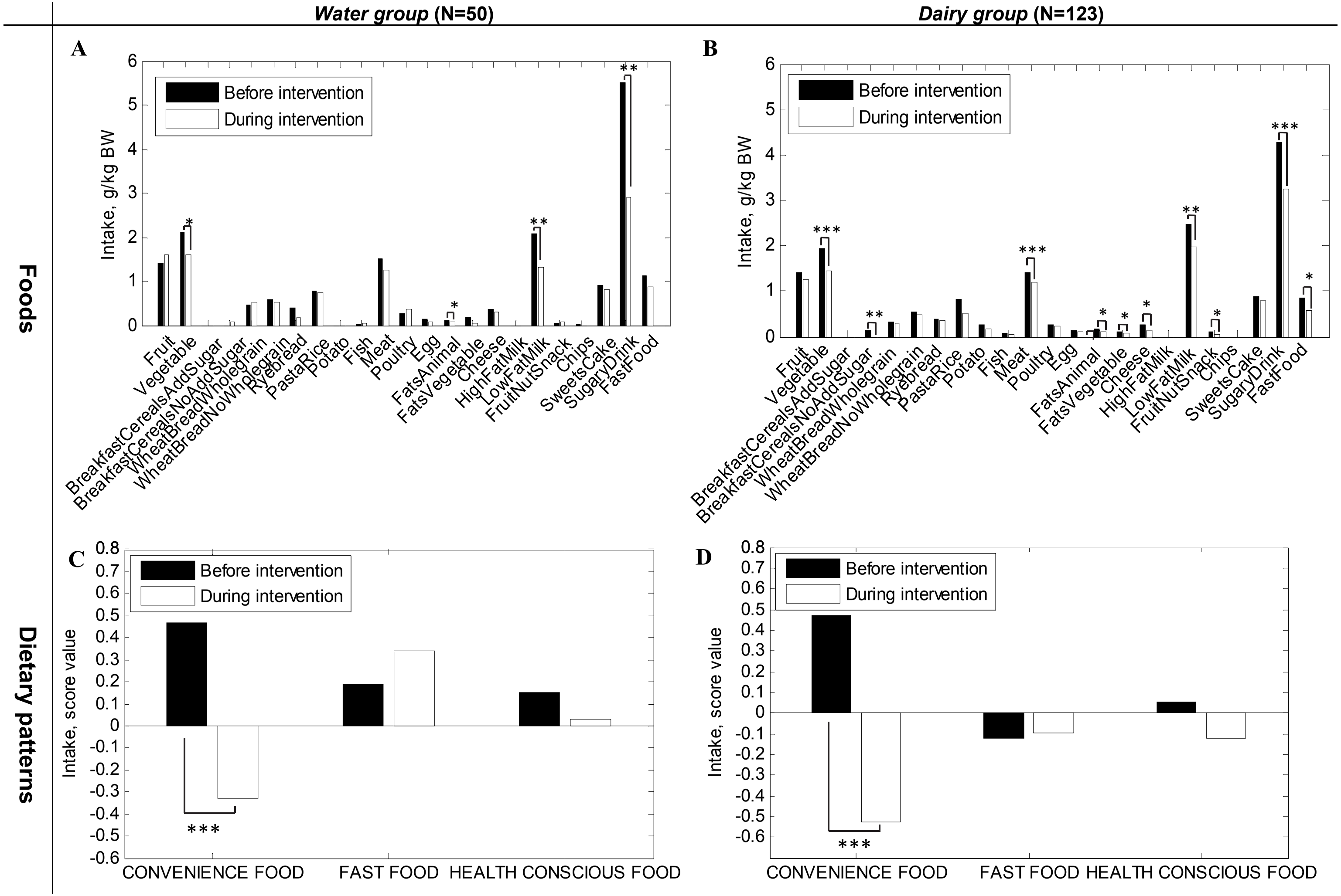

Figure 2 Intake of foods and dietary patterns before and during intervention divided into water or dairy groups. **A**: Median intake of foods in the water group, **B**: Median intake of foods in the dairy group, **C**: Mean intake of dietary patterns in the water group, **D**: Mean intake of dietary patterns in the dairy group. Significant differences between values before and during intervention are indicated with asterisks (P < 0.001***, < 0.01**, < 0.05*), based on ANOVA followed by post hoc pairwise comparisons. If no asterisks are present, the difference was not significant.

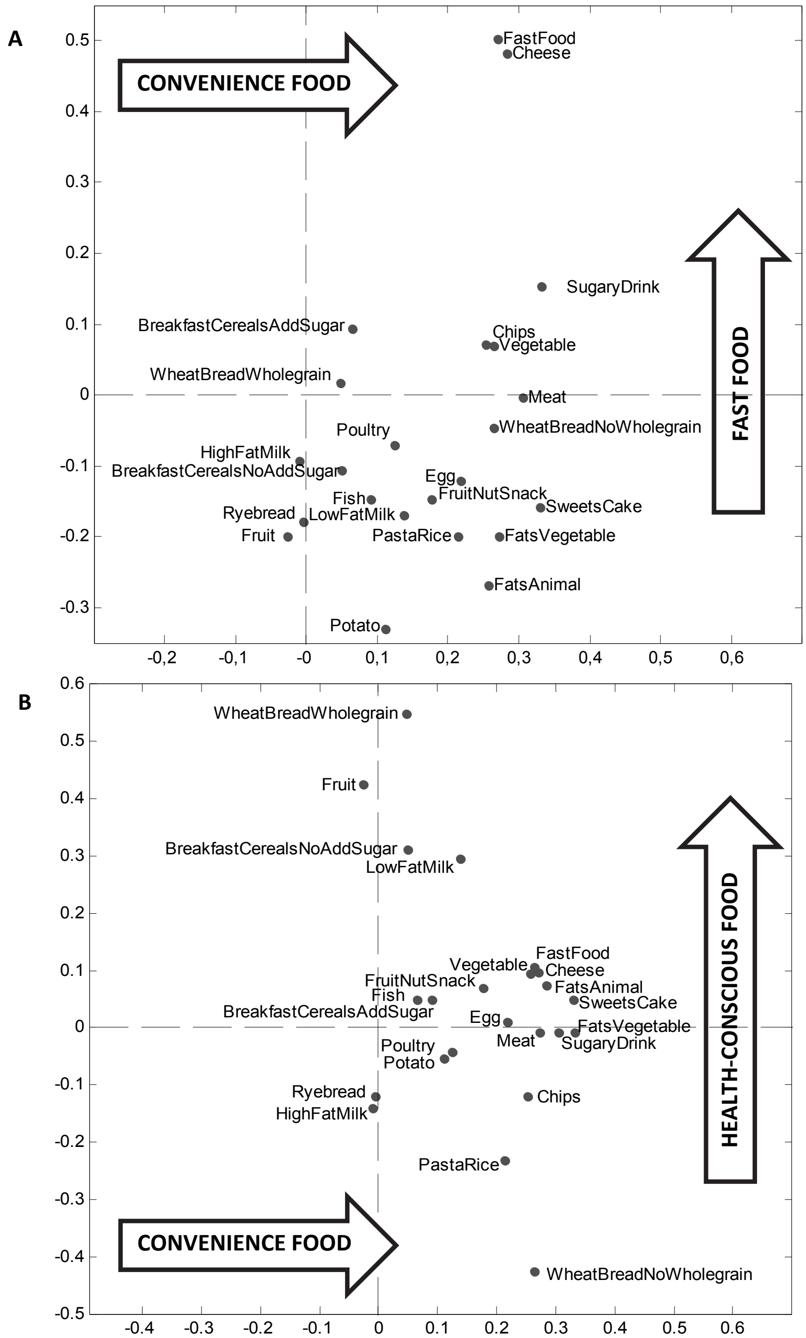

Figure 3 Dietary patterns based on both diet registrations before and during intervention.
A: PCA loading plot of CONVENIENCE FOOD and FAST FOOD patterns **B**: PCA loading plot of CONVENIENCE FOOD and HEALTH-CONSCIOUS FOOD patterns. PCA based on intake of foods (g/kg body weight/day) before and during intervention.

Supplementary material:

Table S1 Intake of energy, nutrients, foods and dietary patterns of subgroups in MoMS with indication of changes during intervention†

A) Before intervention	**Milk** (n=44)		**Whey** (n=43)		**Casein** (n=36)		**Dairy** (n=123)		**Water** (n=50)	
Energy	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy, kJ/d	7566.91	2276.10	8580.87	2892.11	7981.1	2198.39	8042.62	2505.35	7883.44	2073.39
Energy, kJ/d incl. test drink	----	----	----	----	----	----	----	----	----	----
Nutrients	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fat, E%	34.32	5.13	35.51	5.35	31.42	5.59	33.89	5.56	34.78	4.95
Fat, E% incl. test drink	----	----	----	----	----	----	----	----	----	----
Carbohydrate, E%	53.30	6.39	51.65	6.04	56.35	6.40	53.62	6.50	52.00	5.56
Carbohydrate, E% incl. Test drink	----	----	----	----	----	----	----	----	----	----
Protein, E%	14.44	2.34	14.73	2.65	14.43	2.44	14.54	2.47	15.10	2.11
Protein, E% incl. test drink	-----	----	----	----	----	----	----	----	----	----
Foods	Median	25;75 percentiles	Median	25;75 percentiles	Median	25;75 percentiles	Median	25;75 percentiles	Median	25;75 percentiles
Fruit, g/kg BW	1.00	0.41; 2.65	1.09	0.11; 2.36	1.67	0.86; 3.41	1.41	0.47; 2.95	1.43	0.49; 2.55
Vegetable, g/kg BW	1.72	0.96; 3.49	1.78	1.31; 2.68	2.26	1.59; 2.98	1.95	1.26; 2.91	2.10	1.59; 3.11
BreakfastCerealsAddSugar, g/kg BW	0	0; 0.01	0	0; 0	0	0; 0	0	0; 0	0	0; 0
BreakfastCerealsNoAddSugar, g/kg BW	0.10	0; 0.52	0.12	0; 0.61	0,19	0; 0.67	0,13	0; 0.59	0	0; 0.22
WheatBreadWholegrain, g/kg BW	0.34	0; 0.73	0.31	0.01; 1.05	0.33	0; 0.79	0.32	0; 0.84	0.47	0.15; 1.19
WheatBreadNoWholegrain, g/kg BW	0.50	0.12; 0.81	0.70	0.30; 1.12	0.57	0.28; 1.36	0.55	0.23; 1.03	0.60	0.33; 0.83
RyeBread, g/kg BW	0.40	0.16; 0.85	0.40	0.18; 0.91	0.44	0.13; 0.98	0.40	0.18; 0.92	0.39	0; 0.72
PastaRice, g/kg BW	0.66	0.06; 1.62	0.88	0.32; 1.41	1.00	0.23; 1.44	0.83	0.20; 1.44	0.76	0.45; 1.29
Potato, g/kg BW	0	0; 0.91	0.40	0; 0.91	0.30	0; 1.10	0.28	0; 0.96	0	0; 0.89
Fish, g/kg BW	0.01	0; 0.27	0.07	0; 0.30	0.23	0.00; 0.36	0.07	0; 0.31	0.03	0; 0.18
Meat, g/kg BW	1.46	0.92; 2.10	1.69	0.87; 2.20	1.39	0.79; 1.82	1.41	0.88; 2.03	1.50	1.02; 2.32
Poultry, g/kg BW	0.22	0; 0.77	0.27	0.03; 0.62	0.29	0.05; 0.61	0.27	0.03; 0.68	0.26	0.01; 0.77
Egg, g/kg BW	0.13	0.06; 0.21	0.14	0.04; 0.29	0.13	0.07; 0.26	0.14	0.05; 0.24	0.14	0.05; 0.28
FatsAnimal, g/kg BW	0.15	0.03; 0.28	0.27	0.07; 0.47	0.12	0.02; 0.37	0.18	0.04; 0.38	0.11	0.02; 0.39
FatsVegetable, g/kg BW	0.13	0; 0.29	0.15	0; 0.28	0.06	0; 0.25	0.12	0; 0.27	0.18	0.05; 0.29
Cheese, g/kg BW	0.29	0.06; 0.53	0.26	0.10; 0.55	0.22	0.05; 0.67	0.27	0.08; 0.56	0.38	0.19; 0.63
HighFatMilk, g/kg BW	0	0; 0	0	0; 0	0	0;	0	0; 0	0	0; 0
LowFatMilk, g/kg BW	2.19	1.29; 3.08	3.22	1.54; 4.03	2.44	1.64; 4.32	2.46	1.42; 3.90	2.07	1.29; 4.11
FruitNutSnack, g/kg BW	0.10	0; 0.24	0.08	0; 0.29	0.12	0; 0.38	0.10	0; 0.28	0.06	0; 0.14
Chips, g/kg BW	0	0; 0.06	0	0; 0.15	0	0; 0.10	0	0; 0.10	0.02	0; 0.16
SweetsCake, g/kg BW	0.86	0.45; 1.27	1.12	0.49; 1.95	1.10	0.47; 1.59	0.88	0.47; 1.64	0.90	0.60; 1.50
SugaryDrink, g/kg BW	5.04	3.05; 8.79	3.27	1.73; 10.03	5.45	2.61; 8.12	4.27	2.50; 8.72	5.50	3.06; 7.04
FastFood, g/kg BW	0.92	0.20; 2.43	0.91	0; 2.31	0.70	0.07; 2.69	0.85	0; 2.40	1.14	0.33; 2.46
Patterns	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CONVENIENCE FOOD	0.22	1.83	0.86	1.97	0.32	1.38	0.47	1.78	0.47	1.34
FAST FOOD	0.18	1.54	-0.34	1.75	-0.22	1.48	-0.12	1.60	0.19	1.49
HEALTH CONSCIOUS FOOD	0.11	1.37	0.02	1.31	0.02	1.01	0.05	1.24	0.15	1.41

Table S1 continued

B) During intervention	**Milk** (n=44)		**Whey** (n=43)		**Casein** (n=36)		**Dairy** (n=123)		**Water** (n=50) ‡	
Energy	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy, kJ/d	6135.65***	2180.26	6534,17***	1957.89	7140.80	2533.08	6569.16***	2236.19	6892.54*/*	2426.40
Energy, kJ/d incl. test drink	7571.88	2144,64	7960,80	2391.39	8383,12	2528.59	7945.28	2351.83	6892.54**/*	2426.40
Nutrients	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fat, E%	32.97	5.79	34,07	6.50	33.07	5.45	33.38	5.93	33.50	5.57
Fat, E% incl. test drink	28,40***	4.84	28.61***	6.28	28.01**	5.20	28.36***	5.45	33.50	5.57
Carbohydrate, E%	53.69	7.31	52,77	6.42	54.59	5.52	53.63	6.51	53.01	6.54
Carbohydrate, E% incl. Test drink	53.25	5.54	52.70	8.25	54.72	4.66	53.49	6.41	53.01	6.54
Protein, E%	15.33	3.73	14,73	2.86	14.42	2.34	14.85	3.07	15.27	2.13
Protein, E% incl. test drink	19.91***	3.69	19.27***	4.00	19.01***	3.11	19.43***	3.64	15.27	2.13
Foods	Median	25;75 percentiles	Median	25;75 percentiles	Median	25;75 percentiles	Median	25;75 percentiles	Median	25;75 percentiles
Fruit, g/kg BW	0.91	0.13; 2.63	0.59	0; 2.30	2.25	0.39; 3.38	1.26	0.24; 2.93	1.61	0.81; 2.96
Vegetable, g/kg BW	1.42*	0.69; 2.44	1.48	0.83; 2.29	1.43	0.82; 2.34	1.45***	0.74; 2.33	1.61*/NS	1.05; 2.64
BreakfastCerealsAddSugar, g/kg BW	0	0; 0	0	0; 0	0	0; 0	0	0; 0	0	0; 0
BreakfastCerealsNoAddSugar, g/kg BW	0	0; 0.38	0 *	0; 0.29	0.11	0; 0.42	0 **	0; 0.32	0.09	0; 0.43
WheatBreadWholegrain, g/kg BW	0.16	0; 0.64	0.41	0; 0.83	0.29	0; 0.82	0.29	0; 0.80	0.54	0; 1.13
WheatBreadNoWholegrain, g/kg BW	0.47	0.23; 0.95	0.57	0.20; 0.99	0.45	0.19; 1.00	0.49	0.22; 1.00	0.52	0.27; 1.04
RyeBread, g/kg BW	0.22	0.00; 0.71	0.31	0; 0.94	0.46	0.09; 0.99	0.35	0; 0.81	0.17	0; 0.55
PastaRice, g/kg BW	0.62	0.27; 1.25	0.40	0; 0.95	0.69	0; 1.28	0.52	0; 1.17	0.74	0.37; 1.28
Potato, g/kg BW	0	0; 0.85	0.25	0; 0.90	0.22	0; 0.82	0.17	0; 0.84	0	0; 0.51
Fish, g/kg BW	0.05	0; 0.27	0.05	0; 0.30	0.06	0; 0.25	0.06	0; 0.27	0.05	0; 0.33
Meat, g/kg BW	1.05*	0.71; 1.61	1.24*	0.74; 1.80	1.17	0.62; 1.80	1.21***	0.69; 1.70	1.27	0.79; 1.88
Poultry, g/kg BW	0.09	0; 0.40	0.24	0; 0.60	0.36	0.13; 0.57	0.24	0.01; 0.54	0.37	0.04; 0.51
Egg, g/kg BW	0.10	0.04; 0.22	0.10	0.04; 0.28	0.12	0.05; 0.32	0.11	0.04; 0.28	0.09	0.04; 0.23
FatsAnimal, g/kg BW	0.07	0; 0.25	0.17*	0; 0.34	0.08	0.01; 0.33	0.10 *	0; 0.29	0.07 */*	0.02; 0.20
FatsVegetable, g/kg BW	0.09 *	0; 0.19	0.10	0; 0.33	0.05	0; 0.15	0.09 *	0; 0.21	0.05 NS/**	0; 0.23
Cheese, g/kg BW	0.21	0.08; 0.48	0.12	0.06; 0.38	0.14	0.04; 0.36	0.15*	0.06; 0.41	0.29	0.08; 0.58
HighFatMilk, g/kg BW	0	0; 0	0	0; 0	0	0; 0	0	0; 0	0	0; 0
LowFatMilk, g/kg BW	1.97	0.28; 3.01	1.97**	0.08; 3.18	1.86	0.88; 3.56	1.97**	0.61; 3.18	1.30**/*	0.63; 3.09
FruitNutSnack, g/kg BW	0.02	0; 0.15	0.03	0; 0.14	0.11	0; 0.24	0.04*	0; 0.16	0.07	0; 0.18
Chips, g/kg BW	0	0; 0.03	0	0; 0.08	0	0; 0.06	0	0; 0.06	0	0; 0.10
SweetsCake, g/kg BW	0.76	0.27; 1.38	0.90	0.22; 1.44	0.79	0.51; 1.39	0.78	0.27; 1.40	0.80	0.40; 1.30
SugaryDrink, g/kg BW	2.77***	0.73; 5.11	3.54	2.10; 9.00	4.01	1.72; 7.55	3.23***	1.46; 6.65	2.91**/**	1.74; 5.33
FastFood, g/kg BW	0.53	0; 1.63	0.73	0.31; 1.54	0.47	0; 1.10	0.58*	0; 1.48	0.86	0; 2.25
Patterns	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CONVENIENCE FOOD	-0.79 ***	1.37	-0.32 ***	1.58	-0.45 **	1.40	-0.53***	1.46	-0.33***/***	1.43
FAST FOOD	0.13	1.50	-0.11	1.24	-0.35	1.11	-0.09	1.31	0.34	1.48
HEALTH CONSCIOUS FOOD	-0.08	1.25	-0.24	1.37	-0.04	1.08	-0.13	1.24	0.03	1.08

A: Dietary intake before intervention, **B**: Dietary intake during intervention. †Significant differences between values before (Table S1A) and during intervention (Table S1B) are indicated with asterisks (<0.001***, <0.01**, <0.05*) based on ANOVA followed by post hoc pairwise comparisons. If no NS or no asterisks the difference was not significant. ‡ Significance indicated before the slash is based on a model with intervention groups divided in two (water and dairy groups) and significance indicated after the slash is based on a model with intervention groups divided in four (water, whey, casein, milk).

5 Discussion

In this section the main findings from this thesis, about characterisation of dietary patterns, the degree of adherence to a dietary pattern over time and possible indicators and outcomes of different dietary patterns in childhood, are briefly summarised across the four papers found in section 4. This discussion is a supplement to the individual discussions included in each paper and will mainly include transverse topics and the relevance of findings. Finally, general strengths and limitations of the four papers are included.

5.1 Trends in the diet of different groups of Danish children

5.1.1 Summary of findings across Paper I, II, III and IV

In Paper I and II we identified the two dietary patterns; FAMILY FOOD (13%) and HEALTH-CONSCIOUS FOOD (9%) in 9 mo old infants in a pooled sample of SKOT I and SKOT II, while we identified the three dietary patterns; TRANSITION FOOD (18%), HEALTHY FOOD (8%), and TRADITIONAL FOOD (5%) in children from 9 to 36 mo of age from the SKOT I cohort in Paper III. Finally, in adolescents from 12 to 15 years of age, we identified three dietary patterns; CONVINIENCE FOOD (11%), FAST FOOD (9%), and HEALTH-CONSCIOUS FOOD (7%) in Paper IV. In all four studies the PCA analysis was based on more or less the same food groups covering the whole diet of the participants. The percentage of variation described by each pattern are given in brackets above showing that the dietary patterns all together explained 22-31% of the total variation in the dietary recordings across the four papers. Thereby the degree of explained variation is similar across the papers and comparable with other studies of dietary patterns in children collected in a review reporting an explained variation varying from 13% to 58% (26).

5.1.2 Generalisation of findings

Specific foods contributing to a healthy diet in one population might not be present in the food culture of another population whereas other foods will contribute to their healthy dietary pattern. The participants in this thesis are all Danish children and the exact food content of dietary patterns identified here might be more or less specific for the Danish food culture. However, the patterns seem comparable with findings in other Western countries (45;48;53;61) also identifying healthy patterns, traditional patterns and patterns related to the transition from baby food to family food (**Table 1** and **2**, section 1.3.2). This overall similarity across populations might indicate an advantage of analysing dietary patterns oppose to the intake of single foods but the associations with indicators and health outcomes might differ across cultural settings.

The generalisation of dietary patterns can also be affected by the age of the study sample as some dietary patterns might be relevant within a limited age group. For example the FAMILY FOOD or the similar TRANSITION FOOD pattern are explaining the largest single amount of variation both in Paper I, II and III and is a reflection of the special complementary feeding period in late infancy and early toddlerhood with the food groups *BreastMilk*, *Formula,* and *Porridge* presenting the lowest loadings in the analyses and a versatile group of family foods presenting the highest loadings. Children included in Paper I and II represent a narrow age span but still the FAMILY FOOD pattern explained the largest amount of variation in dietary intake. This indicates a high degree of variation between infants within this short age span and illustrates the rapid changes during the complementary feeding period and differences in feeding practice among families. Moreover, the degree of variation explained by a dietary pattern might also be age specific. For the patterns identified in adolescents in Paper IV it is worth noticing that the two patterns explaining the largest amount of variation, the CONVINIENCE FOOD and FAST FOOD patterns are potentially unhealthy patterns containing high loadings for foods related to snacking such as *SugaryDrink*, *SweetsCake,* and *FastFood*. In this age group snacking might be partly administrated by the adolescent itself. Contrary the HEALTH-CONSCIOUS FOOD pattern contains more meal related foods and main meals are probably still at this age controlled by the parents to a greater extent than snacking. Hereby the generalisation of the dietary patterns should be extrapolated to other age groups with caution.

Generalisation of the identified dietary patterns might also be affected by selection bias as people who choose to participate in nutrition research often have a healthier diet than the background population (133). Though, this does not necessarily mean that the dietary patterns identified in Paper III for SKOT I, which presumably have more health-conscious parents, would not appear in the background population but the adherence to the patterns probably differs as observed between SKOT I and SKOT II in Paper I. Hence, children in SKOT I presumably have higher scores on the HEALTHY FOOD pattern than an average child from the background population. Pooling the two SKOT cohorts as done in Paper II increases the variation in non-diet characteristics of the study sample and probably also the variation in diet and presumably resembles the background population to a higher extent. Lastly SKOT II as well as MoMS represents by design selected groups of either offspring of obese mothers or overweight adolescents themselves. It was speculated if the FAST FOOD pattern identified in MoMS was specific for these overweight individuals or a pattern especially pronounced for the age of the participants. Hereby the generalisation of findings to non-overweight individuals is questionable. However, the comparison of SKOT I and SKOT II did not support specific dietary patterns for offspring of obese mothers but rather identified different degree of adherence to the same set of dietary patterns as offspring of non-obese mothers.

5.2 Tracking of childhood dietary patterns

5.2.1 Summary of findings across Paper III and IV

In Paper III a large group of children had a course of development of dietary patterns staying either above or below the mean of the cohort at all included ages; 9, 18 and 36 mo. This was interpreted as tracking of dietary patterns from infancy into toddlerhood. Tracking was most evident in the HEALTHY FOOD pattern with the highest number of children maintaining the same course and less evident in the TRANSITION FOOD and TRADITIONAL FOOD patterns because of more equal numbers of children in the different categories of development of dietary patterns. In Paper IV development of dietary patterns over time was also investigated. However, the timespan was very short (12 weeks) and the participants were exposed to a dietary intervention whereby the term stability, rather than tracking, of dietary patterns is probably more suitable. The participants were asked to eat their normal diet *ad libitum* together with the test drink and as hypothesised the diet during the intervention period changed which was expressed as an unstable CONVINIENCE FOOD pattern but a stable FAST FOOD and HEALTH-CONSCIOUS FOOD pattern.

5.2.2 The relevance of tracking in dietary patterns

Dietary patterns in early life can be important for health later in life even if no consequences can be observed for current health of the infant because the introduced eating habits might follow the child into later childhood and adulthood and at that stage unhealthy eating patterns seem to have health implications (18). Moreover, tracking of dietary patterns from early life might contribute with accumulation of positive or negative effects on health as indicated by the findings in Paper III. Especially children with a lower adherence to the TRANSITION FOOD or the HEALTHY FOOD pattern at two or all three ages had higher BMI z-scores, higher fat mass index and higher levels of metabolic risk markers and thereby could represent undesirable developments of dietary patterns for toddlers. Tracking of dietary patterns is presumably related to tracking of parental choices and routines in infancy and toddlerhood but as the independence of the child increases, the tracking might to a greater extend reflect the inherited habits of the child (134). Adolescence seems to be a particularly instable period in relation to dietary patterns probably because of the increasing importance of social acceptability from peers (74;135) and this should be taken into account when interpreting the findings in Paper IV.

Tracking of dietary patterns seems evident and relevant. However, Paper III also shows a group of children changing course of dietary pattern over time both in a presumably healthier and unhealthier direction, which indicates that the adherence to dietary patterns from 9 to 36 mo is changeable. This is interesting from a prevention perspective, but from the findings in Paper III, which is based on an observational design, it is not known if it is possible to affect these different courses of dietary patterns by health interventions. Paper IV is one of the first intervention studies to be evaluated based on changes at the level of dietary patterns displayed by PCA and the intervention seemed to affect the dietary patterns in a healthier direction. However, Paper IV is based on short term stability of dietary patterns in adolescents and therefor findings cannot be extrapolated e.g. to infancy and toddlerhood.

To support our knowledge of development of dietary patterns over time it seems important to evaluate to what extent statistically significant unstable dietary patterns are clinically relevant. This probably also depends on the time span within which stability or tracking are investigated. Another interesting issue, which is not widely examined (74), is whether tracking of dietary patterns is stronger than tracking of nutrients or foods intake which was neither addressed in this thesis.

5.3 Indicators of childhood dietary patterns

5.3.1 Summary of findings across Paper I, II and IV

In Paper I some differences in the diet were observed between SKOT I and SKOT II suggesting that infants in SKOT II had a less healthy complementary diet than infants in SKOT I. These differences might be related to some of the main differences in parental characteristics between the two cohorts; especially SKOT II having higher maternal BMI and lower parental social class. This was further investigated in Paper II together with a wide range of other possible indicators. The explorative analyses of indicators of dietary patterns suggested that families with a special need for targeted support in relation to prevention of unfavourable offspring dietary patterns can be characterised by a high maternal BMI, a high number of children in the household and perhaps an immigrant/ descendant status. In Paper IV the focus on indicators for dietary patterns was not obvious. However, exploring changes in dietary patterns during the MoMS intervention could be regarded as an investigation of dietary advices as a possible indicator for the changes in dietary patterns. The advice of drinking either 1 L of water or dairy products a day seemed to affect the CONVINIENCE FOOD pattern.

5.3.2 The relevance of indicators in relation to prevention of unfavourable dietary patterns

It is relevant to investigate indicators of dietary patterns to comprehend how undesirable dietary patterns can be prevented. The identified significant indicators in Paper I and II are a mix of changeable (e.g. maternal BMI) and non-changeable (e.g. parental immigrant/descendant status) characteristics and suggests that prevention should aim partly on reducing e.g. high maternal BMI and partly on supporting obese mothers and parents with an immigrant status to follow the Danish nutrition recommendations. Fortunately, new parents are often highly motivated for life style changes which can improve the health perspective of the offspring (136). Therefor the finding of e.g. the very early association between high maternal BMI and undesirable infant dietary patterns might serve as motivation for changes in family food choices and other life style factors which with timely support might positively affect the family's (and thereby the child's) dietary patterns towards more healthy patterns and potentially also lead to maternal weight loss. Thereby, this would possibly be beneficial for maternal as well as offspring health.

In section 1.3.3 the limited and mixed evidence for the gender of the child and the paternal BMI as indicators for childhood dietary patterns was mentioned. However, gender and paternal BMI were not found to be independent indicators of infant dietary patterns by our analyses and the association might only be evident later in childhood. Gender differences in a healthy dietary pattern have been reported down to 18 mo of age (55).

It needs to be emphasised that quite a large amount of the variation in the score values of the dietary patterns in Paper II are unexplained proposing that other indicators (e.g. parental diet and detailed physical activity /sedentary behaviour measures) than those in focus here are relevant for the dietary patterns in infancy. In addition, a considerable amount of noise caused by limitations in the diet registration might contribute to the remaining variation. The relevance of the identified indicators might also be limited by the unanswered question of causality in this thesis. However, the standardised regression coefficient in Paper II indicates an internal ranking of the indicators and stresses the importance of e.g. maternal BMI.

If the MoMS intervention in Paper IV should contribute to the foundation of a broader public health dietary advice, a possible unique influence of the research setting and weight loss motivation should be taken into consideration. The same effect of increasing water consumption cannot necessarily be expected in another setting. The other water intervention studies referred to in Paper IV are more public health oriented and try to increase water consumption by increasing availability of water coolers, increasing awareness of health advantages and providing tools for behavioural changes through teaching (107-109), which are good public health practices but have not been able to affect intake or purchase of sugar-sweetened beverages in those settings.

5.4 Health outcomes of childhood dietary patterns

5.4.1 Summary of findings in Paper III

Paper III was based on data of dietary intakes in the SKOT I cohort at 9, 18 and 36 mo of age. Development in the dietary patterns TRANSITION FOOD and HEALTHY FOOD, but not TRADITIONAL FOOD, were associated with some of the investigated markers possibly related to obesity and cardiovascular diseases later in life even within this homogeneous population mainly from a high social class. BMI z-score and fat mass index at 36 mo were significantly different between categories of development in the TRANSITION FOOD pattern, while height-for-age z-score, blood IGFBP3, total cholesterol, and LDL at 36 mo differed between the categories of development in the HEALTHY FOOD pattern. Especially groups of children with lower adherence to the TRANSITION FOOD or HEALTHY FOOD pattern at two or all three ages had higher BMI z-scores, higher fat mass indices and higher levels of metabolic risk markers and hence, could represent undesirable development of dietary patterns for toddlers.

5.4.2 The relevance of dietary patterns for health outcomes

Strong morbidity endpoints for obesity and cardiovascular diseases are not possible to investigate in toddlers because they develop over years and first appear later in life. Though, possible markers for later morbidity appear years before an actual diagnosis can be determined and are therefore used in Paper III with an attempt to predict risk-groups. However, the use of risk markers increase the uncertainty of the relevance of findings, and the strength of the relation between marker and later morbidity is questionable. Even though Paper III reports explorative observational findings the question arises if these associations reflect causal relations and whether it represents clinical relevant differences or just show variations within the healthy spectrum.

It is not possible to define a cut-off below which a difference in risk markers at 36 mo is not clinically relevant. None of the categories of development of dietary patterns had a median BMI z-score above the definition of overweight defined by WHO as +2 z-scores (85). However, there was a median difference in BMI of nearly 1 z-score between the highest and lowest group and this might be of clinical relevance at the group level. High BMI already in toddlerhood is associated with higher risk of obesity in adulthood (137). The long term tracking of early fat mass, which possibly is a stronger predictor of adverse health outcomes than BMI (138), is less investigated, but seems to track from toddlerhood into childhood (139;140) and from childhood into adulthood (141). Tracking of blood lipids including cholesterol has been observed from toddlerhood to childhood (142), during childhood and from childhood into adulthood (143;144). An early higher cholesterol level, down to 4 years of age, has been associated with increased risk of cardiovascular diseases in adulthood (145;146). Moreover, intake of nutrients such as saturated fat, monounsaturated and polyunsaturated fat, fibre, and sugar (147) may be involved in the mechanism regulating the blood LDL level in toddlerhood. Considerable amounts of fibre, monounsaturated, and polyunsaturated fat are found in the foods with highest loadings (*Vegetable, Fruits, Fish, and FatsVegetable*) in the HEALTHY FOOD pattern and might contribute to lower LDL levels, while saturated fat and sugar are highly present in foods with lowest loadings (*SugaryDrink and SweetsCake*) and might contribute to higher LDL levels. This supports the biological plausibility of the identified associations. An association between development of dietary patterns and health outcomes might be stronger in the background population than reported for the homogeneous SKOT I cohort. Infants in SKOT I, who mainly come from a higher social class than the background population in Denmark, will presumably both eat healthier and have a better health profile than the background population as seen in other studies (133).

5.5 The multivariate data-driven dietary pattern approach – is it useful?

The data-driven dietary pattern approach might grasp more of the complexity in dietary intake than a single nutrient or food but simultaneously it holds a risk of being too wide and complex ending up as useless and incomprehensible. The reporting of dietary patterns in Paper I, II, III and IV showed a clustering of different foods which are potentially healthy and likewise potentially unhealthy foods. Clustering disclosed that children with the highest intake of different unhealthy foods tended to have higher intake of unhealthy food in general and this information is an advantage of the dietary pattern approach compared to a single food approach. Moreover, it is often claimed that dietary patterns might be stronger markers of disease risk compared to single nutrients and foods (11) but as mentioned in section 1.2 few studies have aimed to explore this assumption and it is also beyond the scope of this thesis. However, whether dietary patterns are stronger markers than single nutrients probably depends on e.g. the disease risk in question. Nevertheless, Paper III showed that dietary patterns in infancy and toddlerhood are associated with body size, body composition and metabolic risk markers which supports the assumption of dietary patterns being useful. Even though dietary patterns might be good markers of health outcomes it is still the combination of nutrients and other bioactive components in the food which carry the mechanistic effect of the diet and for biologic plausibility the dietary patterns therefore should be associated with nutrient intake. Such an association has previously been reported in other studies (148) but has not directly been investigated in this thesis. However, in addition to dietary patterns Paper I and IV also report intake at energy, nutrients and foods levels which supported the findings at the dietary pattern level. From this, dietary patterns seem useful in the investigation of indicators, but the combination with other dietary levels increases the interpretation of the results. Moreover, including multiple dietary levels is beneficial in the comparison with previous findings at nutrients and foods levels and for comparison with nutrition recommendations.

The PLS and RRR are related methods to the PCA method used in this thesis for identification of dietary patterns. These are less used within the nutrition area than the PCA method as mentioned in section 1.3.1. It is difficult to generalise the usefulness of PCA to other related methods but if aiming at a less explorative approach with the purpose of defining a dietary pattern which is by definition associated to specific health outcomes the PLS and RRR seem promising to apply e.g. in MoMS and the SKOT cohorts.

5.6 Strengths and limitations

The main strengths across the four papers in this thesis are: *First,* the whole-diet approach focusing on dietary patterns supported by other dietary levels all based on diary records with thorough portion size estimation is an advantage. Moreover, as many of the same food groups was used across the four papers as possible based on the assumption that they were of universal importance for all participants and to increase the possibility of comparing results. However, minor differences in food groups were used to acknowledge differences in age and focus of the studies. In addition, the presentation of the PCA plots within the papers, rather than tables, which is often used, made the interpretation and naming transparent and it seems beneficial in interpretation and communication of the results. *Second*, different time points were included in the PCA both in Paper III and IV which contributes to our understanding of development of dietary patterns. In all four papers food intake used in the PCA has been divided by the total body weight in an attempt to standardise different requirements which seems especially important when including different time points in the same PCA in early life because of rapid growth. Unfortunately it is not always evident which units are used in other studies and I am not aware of other studies dividing the food intake by total body weight before a PCA. Adjustment for total energy intake has been reported in adolescence though without large differences when compared with patterns based on foods in gram/day (37;61;65;67). *Third*, the association between infant dietary patterns and a wide range of possible indicators has been examined in Paper I and II including e.g. paternal characteristics which has been sparingly investigated previously. *Fourth*, the association between dietary patterns and obesity related outcomes has been investigated in Paper III including metabolic risk markers which to my knowledge has not previously been reported in toddlers. *Fifth*, Paper IV is presumably one of the first to apply the PCA approach to evaluate an intervention study at the level of dietary patterns. *Lastly*, the inclusion of different groups of Danish children varying in age and background characteristics is favourable because there are still only a limited number of papers published about dietary patterns in childhood.

The limitations across the papers can be divided into three main areas; the diet registration, the PCA method, and the challenge of measuring children. *Diet registration* is a challenging task holding the risk that the dietary records do not reflect usual intake because of over- or under-reporting due to e.g. difficulties in portion size estimation, recall bias, or behaviour caused by social desirability (149;150). This may also apply for parents making the registration on behalf of the child (150) as were done in SKOT I and SKOT II. Seasonal variation in food habits might also affect the degree of usual intake captured and especially for the 9 mo registrations the rapid change of diet during the complementary period give the term usual a narrow meaning. Moreover, valid portion size estimation is a particular challenge in infants and toddlers because the food is explored and tasted but not necessarily swallowed. An attempt was done to ease the diet registration in day care by providing the day care with a light version of the food diary. However, the staffs in day care are busy and the validity is likely to be lower for the meals eaten there (125). In MoMS the validity of the diet registration might be challenged by a possible increased under-reporting among overweight and obese individuals (150) and the fact that under-reporting might be differentiated between various foods with e.g. sugar-sweetened beverages being some of the most under-reported (149). This increased under-reporting among obese individuals was not distinct when comparing the estimated percentages of under-reporters in SKOT I (1%) and SKOT II (1%). Finally, amplification of under-reporting over multiple registration periods found in other studies (151) might also be a limitation when investigating dietary patterns over time in SKOT I and MoMS (Paper III and IV). However, efforts were done to refresh the method with the participants before each registration period.

Even though, the *PCA method* is a data-driven method it is not independent of subjective decisions and this might introduce some drawbacks. The pre-processing decisions include selection of content, number and unit of food groups (21;37) and whether to include different ages into one or several PCAs. Different degrees of condensation of food groups were tried to evaluate the robustness of the dietary patterns ending up with relatively few and broad food groups across the four papers, in comparison with other studies (26). This was done to ease the visualisation and interpretation of data but pose a risk of blurring important differences within a food group e.g. including both healthy and unhealthy alternatives. In Paper III and IV diet registrations from different time points were included in the same PCA when studying development of dietary patterns over time, which has not been done in most other studies. This might make the interpretation and naming of the dietary patterns, especially the TRANSITION FOOD pattern in Paper III, more troublesome. However, it ensures that score values from different time points are compared precisely on the same dietary pattern, which is in contrast to comparing one PCA for each time point. Using multiple PCAs holds a risk of comparing dietary patterns which are not totally comparable and thereby misinterpreting the equality of dietary patterns over time. In addition, the categorisation of children into different courses of development of dietary patterns in Paper III is rather rough as it only differentiates above /below the mean. However, the advantage of this method is that it enables the inclusion of three time points in one simple variable describing the course of development of dietary patterns focusing on archetypes rather than potentially less important minor individual differences. Nevertheless, some children might have a score value very near to the mean and it might seem random which category they fall into. However, taking the sample size into consideration it was inappropriate to make outcome analyses e.g. only including children with score values below the 25^{th} and above the 75^{th} percentile. Finding significant differences in outcomes between the different categories of development of dietary patterns indicate that dividing children into groups above/below mean might be usable.

A final limitation of the studies in this thesis is the *challenge of measuring* e.g. anthropometry of children, especially infants and toddlers, which do not always follow the same agenda as the research staff, whereby deviations from the standardised protocol arise increasing the risk of imprecise measures or missing measures. Imprecise measurements were decreased by performing many of the measurements in triplicates. Busy families and families with limited resources also influence the rate of missing values and drop outs and thereby decrease the generalisation of findings and decrease the sample size, which already is relatively small compared to other studies.

6 Conclusion

Dietary patterns in infancy, toddlerhood, and adolescence have been explored in this thesis both in relation to characterisation of the dietary patterns, to uncover possible indicators, and to investigate the associations with outcomes related to growth and obesity. A HEALTHY FOOD or a HEALTH –CONSCIOUS FOOD pattern were evident across all investigated age groups. Children with low scores in these patterns have a lower intake of presumably healthy foods than children with high scores. The dietary pattern named FAMILY FOOD or the similar TRANSITION FOOD pattern are distinctive for the diet during infancy and toddlerhood and explained the largest single amount of variation in diet data in this age group. These patterns can be interpreted as an age related transition from a milk based diet to the family's diet.

Identification of indicators for undesirable dietary patterns already in infancy enables an early targeted preventive support in families with these characteristics. Our explorative analyses suggest that these families can be characterised by a high maternal BMI, a high number of children in the household, and possibly a parental immigrant/descendant status. For instance, infants with obese mothers were found to have lower scores in a HEALTH-CONSCIOUS FOOD pattern including e.g. lower intake of *Fruit, Vegetable* and *BreastMilk*. Moreover, they had a higher percentage of energy intake from protein compared to infants in a cohort of mainly non-obese mothers from a higher social class. This suggests that infants of obese mothers tend to have a less healthy diet during the complementary feeding period.

The relevance of early promotion of a healthy diet is supported by the finding of tracking of dietary patterns from infancy together with the finding of an association between the development of dietary patterns and obesity related outcomes already in toddlerhood. Especially groups of children with lower adherence to the TRANSITION FOOD or HEALTHY FOOD patterns at two or all three of the investigated ages (9, 18 and 36 mo) had higher BMI z-scores, higher fat mass indices and higher levels of metabolic risk markers and hence, might represent undesirable development of dietary patterns for toddlers. In addition to tracking, a group of children change their adherence to the dietary patterns during toddlerhood, some in a healthier direction and others in a less healthy direction, which calls for a sustained promotion of healthy eating habits possibly throughout childhood.

In relation to the specific content of dietary health promotion our findings in overweight adolescents support the recommendation stating that plain water should be promoted as the main source of fluid for children instead of sugar-sweetened beverages. Specifically, we found a decrease in the CONVENIENCE FOOD pattern, including a decreased intake of the food group *SugaryDrink* during both the water and dairy intervention. Moreover, low energy content in the extra fluid seemed favourable when considering the total energy intake in these overweight adolescents.

The data-driven multivariate approach, PCA is the method used across all four papers in this thesis to identify dietary patterns covering the whole diet. By a condensation of the large amount of information from dietary records into a few latent patterns, this explorative method has been useful in an attempt to handle more of the complexity within child nutrition, than revealed when focusing on a single nutrient or food. PCA seems to be suitable both in observational and intervention designs, as well as for investigation of the development of dietary patterns over time. Even though PCA seems to be a useful method the findings have been strengthened by including multiple dietary levels covering energy, nutrients as well as foods intake. These analyses will hopefully contribute to the understanding of dietary patterns in childhood and inspire to further research and promotion of healthy eating habits.

7 Perspectives and future studies

This thesis rise a number of explorative hypotheses about early tracking of dietary patterns, indicators as well as outcomes of dietary patterns, and the possibility to affect dietary patterns in adolescents by a dietary intervention. To reinforce the findings in this thesis it is obvious to include data from the 18 and 36-mo examinations in SKOT II, when they become available. With these data the comparison of dietary patterns in SKOT I and SKOT II (Paper I) could be performed after the complementary feeding period to investigate if the dietary differences are more evident in toddlerhood than in infancy. It would also be interesting to explore if the indicators of each dietary pattern change during childhood as a follow up of Paper II e.g. if an association between paternal BMI and offspring dietary patterns will arise after infancy. The 36 mo data collection both in SKOT I and SKOT II includes accelerometer measures of physical activity which enables a further investigation of physical activity as an indicator for dietary patterns in childhood with a much more objective and sensitive measure than used in Paper II. Inclusion of non-diet factors such as physical activity within the PCA resulting in broader life style patterns rather than solely dietary patterns might also be worth investigating further in early childhood to reinforce the predictive strength of the relation to later risk of obesity.

The future availability of data from multiple time points from both SKOT cohorts also makes it possible to investigate the association between indicators and development of dietary patterns by applying the approach for categorisation of the children according to development of dietary patterns used in Paper III. In relation to growth and obesity outcomes the investigation based on the SKOT I cohort in Paper III could be retried within the SKOT II cohort to explore a possible influence of maternal obesity on these findings. In addition, the TOP study including the SKOT II mothers also contains a wide amount of data e.g. about maternal diet during pregnancy which remains to be linked to offspring diet data. With such a link the presumably mediating effect of maternal food choices for the association between maternal BMI and infant dietary patterns could be explored.

Moreover, a current attempt to continue the SKOT I study with a follow up at 8 or 9 years of age is highly relevant for the investigation of dietary patterns. This enables a further investigation of tracking of dietary patterns and an exploration of the associations with risk markers later in childhood to reinforce the relevance in relation to health. In addition, it would be beneficial to repeat the analysis of associations between dietary patterns and metabolic risk markers in toddlerhood in other cohorts than the SKOT cohorts, as this has not been investigated in early childhood previously to the best of my knowledge. The PCA approach has been the method applied in this thesis to display dietary patterns e.g. to be able to have a fully explorative approach towards the diet. However, other less applied chemometric methods like PLS and RRR could potentially also be beneficial e.g. when aiming at identifying dietary patterns which are associated with a specific health outcome as a data-driven alternative to diet indices.

In Paper IV the effect of the relatively controlled MoMS research intervention is compared with public health programs promoting water consumption. These studies did not report dietary data, did not use the broad dietary pattern approach, or were not able to show a reduction in sugar-sweetened beverages intake or purchase (107-109) contrary to MoMS. Therefore, it would be interesting to use thorough diet registration and investigation of dietary patterns in a public health program to further explore the evidence behind the recommendation stating that plain water should be promoted as the main source of fluid for children *instead* of sugar-sweetened beverages. In such an intervention possible differences in the change of dietary patterns according to e.g. BMI and age of the children should be investigated to increase the generalisation of findings.

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