Body composition at 3-years of age The influence of early growth, infant feeding and IGF-I

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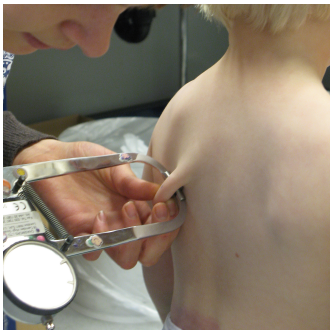
PhD thesis 2014

KATRINE TSCHENTSCHER EJLERSKOV





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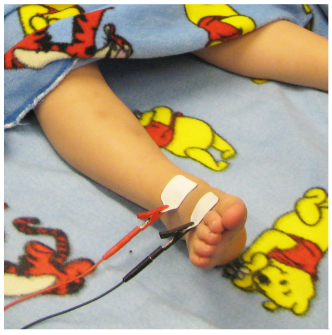

PhD thesis 2013

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Body composition at 3-years of age

The influence of early growth, infant feeding and IGF-I

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Body composition at 3-years of age - The influence of early growth, infant feeding and IGF-I

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Contents

Preface

The present thesis is the result of my Ph.D. study at the research unit for Pediatric and International Nutrition, Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark from October 2009 to September 2013. The thesis is based on data from the SKOT study, a large prospective cohort study that has followed 330 children from 9 months to 3 years. The SKOT study was supported by grants from *The Danish Directorate for Food, Fisheries and Agri Business* and is as part of the project *"Complementary and young child feeding (CYCF) - impact on short and long term development and health"*. I took part in the examinations of the children when they were 3 years of age. In the thesis I use data from all three examinations as well as information of weight at birth and 5 months.

Research contribution and work

The first year of my Ph.D. (October 2009 - October 2010) was mainly practical work in collaboration with my two Ph.D. colleagues, Line and Laurine – coordinating the study, performing child examinations, preparing examinations and materials, having family contact and introducing students to the examination procedures and data entering. I was also involved in creating and updating a homepage for the study, www.skot.life.ku.dk.

In fall 2010 and spring 2011, I helped my research group writing the protocol and had ethical approval at The Committees on Biomedical Research Ethics for the Capital Region of Denmark for the research project SKOT II – a sister study to SKOT but with inclusion criteria for the mothers to have a BMI above 30 in week 14 of pregnancy. I updated and ordered all necessary material (questionnaires etc.) for the study and I was highly involved in applying for project funding and presents for the participants. During the same period I was involved, but not primus motor, in data management and data processing of the SKOT data.

After my maternity leave (March 2011 – Jan 2012) I have focused on statistical analyses and on writing papers. I have been on several writing retreats together with 3 – 4 Ph.D. colleagues which have been both social and highly productive. I have had many scientific discussions with the co-authors of my articles and supervisors.

During my time as Ph.D. student I have had some teaching and supervising at the master course Nutrition, Growth and Development, and I have been co-instructor in a master course in the statistical software program STATA. Further, I have been the main supervisor of two bachelor students and co-supervisor for three master students.

Acknowledgements

I would like to thank the SKOT children and their families for their great involvement in the study. Especially after I have become a mother myself, I acknowledge the commitment these parents have felt for the study in their busy lives. Of course this involvement of the parents would not have succeeded if the project team had not been as caring and professional from the beginning of the study. I therefore thank Anja Lykke Madsen and the project staff involved in the recruitment of families and execution of the first examinations.

I have found these years academically enriching and the working procedure have suited me well, not the least because of the inner circle of really good colleagues that have enriched my work day when it tended to be dry and difficult work. Especially thank you Anni Larnkjær, Line Brinch Christensen, Louise Beltof Borup Andersen, Inge Rasmussen, Vivian Julia Anker, Signe Marie Jensen, Birgitte Hermansen and Julie Brønholm. The writing retreats in Hornbæk with Laurine Bente Schramm Harsløff, Karina Arnbjerg, Emma Louise Malchau Carlsen, Line and Louise have strengthened our collegial network in the last part of our Ph.D. studies and given us all new inspiration and energy for the run-up.

I wish to thank my two supervisors Kim Fleischer Michaelsen and Christian Mølgaard for constructive criticism, supervision and support during the whole process. You must have super-natural energy to be able to cope with all your work tasks and still be amazingly competent and pleasant. Also a great thanks to the two statisticians in our group – Signe Marie Jensen and Christian Ritz – without these clever brains, I would have been lost so many times on my way.

Finally, I thank my parents for their support all the way though the education system – a safe base and secure upbringing has been invaluable in my personal management of my studies. I thank my husband, Patrick and daughter, Sonja, for making our everyday so enjoyable – and I thank our parents for always lending a hand for babysitting in the many times of need during the process.

List of papers

The following three papers or manuscripts lay the foundation of the thesis. I refer to them as paper I, II and III. The first paper is methodical and explains how fat mass and fat free mass can be predicted in this 3-year-old population. These predictions lay the foundation for paper II and III. In paper II, I examined the relation between early growth and early nutrition on body composition at 3 years of age. In the third paper, I examined how IGF-I at 9 months and 3 years were related to body composition at 3 years and diet.

Paper 1

Katrine T Ejlerskov, Signe M Jensen, Line B Christensen, Christian Ritz, Kim F Michaelsen, Christian Mølgaard. Prediction of fat-free body mass from bioelectrical impedance and anthropometry among 3-year-old children using DXA.
In review at Scientific Research. Submitted 8th September 2013.

Paper 2

Katrine T Ejlerskov, Line B Christensen, Christian Ritz, Signe M Jensen, Christian Mølgaard, Kim F Michaelsen. Early growth patterns and early feeding: Impact on body composition at 3 years of age.
Ready for submission to American Journal of Clinical Nutrition.

Paper 3

Katrine T Ejlerskov, Anni Larnkjaer, Dorthe Pedersen, Christian Ritz, Christian Mølgaard C, Kim F Michaelsen. IGF-I at 9 and 36 months of age – relations with body composition and diet at 3 years.
Ready for submission to Growth Hormone and IGF-research.

Contribution to the papers

Paper 1

KTE was main responsible for data analysis and writing the manuscript. SMJ and CR supervised the quality standards of the statistical analyses. All authors contributed to interpretation of results and commented on drafts and approved the final version of the manuscript. KTE have primary responsibility for the final content.

Paper 2

KTE was main responsible for data analysis and writing the manuscript. SMJ and CR supervised the quality standards of the statistical analyses. All authors contributed to interpretation of results and commented on drafts and approved the final version of the manuscript. KTE have primary responsibility for the final content.

Paper 3

KTE was main responsible for data analysis and writing the first draft of the manuscript. CR supervised the quality standards of the statistical analyses. All authors contributed to interpretation of results and commented on drafts and approved the final version of the manuscript. KTE have primary responsibility for the final content.

List of abbreviations

BAZ: BMI-for-age z-score
BIA: Bioelectrical impedance analysis
BWZ: Birth weight z-score
DXA: dual-energy X-ray absorption
FFM: Fat-free mass
FFMI: Fat-free mass index
FFM_{DXA}: Fat-free mass measured by DXA
FFM_{pred}: Predicted fat-free mass
FM: Fat mass
FM_{cal}: Calculated fat mass
FM_{DXA}: Fat mass measured by DXA
FM %: Body fat percentage
FMI: Fat mass index
HAZ: Height/length-for-age z-score
IGF-I: Insulin-growth factor I
IGFBP-3: Insulin-growth factor binding protein 3
RMSE: root mean square error
RI: Resistance Index
SDS: Standard deviation scores
WAZ: Weight-for-age z-score
WFH: Weight-for-length/height z-score

Objectives

The overall objective for this thesis was to identify possible predictors of body composition at 3 years among 330 healthy Danish children from the SKOT cohort with a special focus on factors related to later obesity risk. This overall aim lead to the specific objectives mentioned below which are also the main aims for the three included papers:

I) The focus on body composition in terms of fat-free mass and fat mass brought along a need for better estimation of body composition in this age group. Thus, the first aim was to generate predictive equations for fat-free mass using bioelectrical impedance analysis and anthropometry – with DXA as reference method.

II) Secondly, being able to distinguish fat mass and fat-free mass in this group of 3-year-old children, I wanted to investigate if there were specific periods of growth in early childhood that were more important than others in terms of shaping later body composition. Moreover, it was of interest to see if the impact of growth in the sensitive periods or body composition at 3 years was modified by nutrition.

III) Finally, a focus on early growth and body composition naturally leads the attention on IGF-I – an important growth factor in early childhood believed to be programmed in early life by infant feeding but also influenced by current diet. Thus, the third aim was to examine how IGF-I levels at 9 and 36 months were related to body composition at 3 years of age and how IGF-I levels at 36 months was related to history of breastfeeding and current diet.

English summary

Background: Birth weight and high weight gain the first years of life is associated with later body composition and increased risk of obesity. A detailed description of body composition at 3 years of age and factors related to the distribution of fat and fat-free mass is interesting, since body composition build up in childhood to a certain degree will be retained later in life. At the same time, research have shown that increased tempo of growth including length gain is related to an early nadir in the BMI-curve with a subsequent increase in BMI (adiposity rebound) which is also associated with later increased adiposity risk. Growth in early childhood is highly related to the levels of insulin-like growth factor-I.

Objectives: Data used in this thesis is from the prospective cohort study SKOT which includes 330 children examined at 9, 18 and 36 months of age. The main objective with this Ph.D. study was to look at which factors were related to body composition at 3 years of age with a special focus on factors related to later obesity risk. A methodological objective was to be able to predict body composition by using bioelectrical impedance and anthropometry. Secondly, an objective was to identify growth periods with relation to body composition at 3 years and whether the impact of growth was modified by nutrition. A third objective was to examine the relation between IGF-I levels at 9 and 36 months and body composition at 3 years. Since the IGF-I level was expected to be affected by nutrition this was included in the analyses.

Results: In **paper I**, we showed that a simple model including bioelectrical impedance, height and weight was as good to predict fat-free mass as a more comprehensive model including the sum of subscapularis and triceps skinfolds as well. Prediction error for FFM was 3.0 % for both equations. Since fat mass constitutes a smaller part of the total weight, the relative prediction error was larger for fat mass (10.5 % in the comprehensive model and 12.0 % in the simple model). Results from **paper II** showed that birth weight and weight gain the first 0 - 5 months were strongly related to body composition at 3 years. No measures of adiposity (BMI, fat mass, fat mass index and skinfold thickness) were significantly associated to weight gain after 5 months of age. Two interesting effects modifications by full breastfeeding on the effect of birth weight and early growth on later fat mass index was found. With children that were fully breastfed for less than one months as reference group, we found that full breastfeeding for 4 - 5 months reduced the effect of early weight gain on fat mass index by 47 % ($p = 0.05$) while full breastfeeding for 6 months eliminated the effect of early growth on fat mass index ($p = 0.002$). Likewise, full breastfeeding for 6 months eliminated the effect of birth weight on fat mass index ($p = 0.002$). No effect modification was seen between breastfeeding and early growth on fat-free mass index at 3 years. In **paper III**, IGF-I concentrations at 9 and 36 months were positively related to height, weight, BMI, fat mass and fat free mass at 3 years, but not associated with percentage of body fat and fat mass index. A change in IGF-I from 9 to 36 months was positively related to fat-free mass and fat-free mass index but not with BMI, fat mass and fat mass index. Children breastfed at 9 months had lower IGF-I levels at 9 months but reached the same IGF-I levels at 36 months as infants not breastfed at 9 months. No associations were seen between IGF-I variables

at 36 months and current protein, milk or meat intake at 36 months but IGF-I variables were negatively associated with intake of fat and saturated fat expressed as energy percentages.

Discussion: The predictive equation that was generated in **paper I** enabled good predictions of fat-free mass and fat mass for 233 children compared with the 101 children with high quality DXA scans. Due to the age-dependent variation in the hydration level of fat-free mass we expect that the predictive equation can be used in other studies with a study population at 2 - 4 years of age. Result from **paper II** confirmed that birth weight and early weight gain are important for later body composition which has been shown in a large number of studies. A lack of studies has focused on whether the adverse effects of rapid weight gain are modified by diet. We found that full breastfeeding for 4 - 6 months had a considerable modifying effect on the effect of weight gain on fat mass index, and full breastfeeding for 6 months eliminated the effect of birth weight on fat mass index. Of course parental choices of duration of full breastfeeding and introduction to solids can easily be influenced by early growth patterns as well as related to their health behavior in general. However, the interactions are physiologically plausible and should be investigated in other study settings. Results from **paper III** showed that total and free IGF-I at both 9 and 36 months were positively related to most measures of anthropometry and body composition at 3 years. We found no clear associations between IGF-I levels and early development of obesity. It is possible that IGF-I levels are related to later risk of obesity through increased tempo of weight gain and linear growth leading to early adiposity rebound but this was not visible at 3 years. We had expected that intake of protein and cow's milk was positively associated with IGF-I values but this was not the case in this study. The negative associations between IGF-I levels and current intake of fat and saturated fat expressed as energy percentages could be related to the modulating effect of human milk on IGF-I, as human milk also contains high amounts of fat and saturated fat. However, the relation between diet, IGF-I and risk of early development of obesity is very complex and needs to be investigated further.

Conclusion and perspectives: This thesis represent a throughout examination of body composition at 3 years in a large group of healthy well-nourished Danish children. Birth weight and early weight gain were positively related with fat and fat-free mass at 3 years and the result indicates that the first five months of life is a sensitive period where it is possible to reduce the impact of high birth weight and rapid weight gain on later fat mass by continuing full breastfeeding for 4 - 6 months. The results therefore support the current recommendations for nutrition in infancy and are relevant in the discussion of early prevention of obesity. IGF-I was related to linear growth, fat mass and fat-free mass at 3 years but a relation between IGF-I and later obesity risk is speculative. A continuation of the SKOT cohort with a follow-up visit when the children have reached 7 - 8 years of age would be highly relevant and enable further elaboration on the findings presented in paper II and III. At this age most children have passed the adiposity rebound and a role for IGF-I on accelerated growth and age of the adiposity rebound could be further elucidated. Also a follow-up study of the same children would provide more knowledge of the relation between early growth and infant nutrition on later risk of overweight and obesity.

Dansk resumé (Danish summary)

Baggrund: Fødselsvægt og vækstmønster de første leveår har vist sig stærkt relateret til senere kropssammensætning og fedmerisiko. En nærmere beskrivelse af kropssammensætningen i 3-årsalderen, og hvad der har betydning for fordelingen af fedt- og fedtfri masse er interessant, da er en tendens til at den kropssammensætning, man udvikler i barndommen, i en vis grad bibeholdes senere i livet. Samtidig er der forskning, der tyder på, at øget vækstempo inklusiv højdetilvækst er relateret til et tidligt lavpunkt på BMI-kurven med efterfølgende tidligt opsving i BMI, hvilket også er associeret med øget fedmerisiko. Vækstfaktoren insulin-like growth factor-I (IGF-I) er relateret til tidlige vækstmønstre med større vægt og længdetilvækst, og er derfor interessant i denne sammenhæng.

Formål: Data, der er brugt i denne Ph.d. afhandling er fra det prospektive kohorte studie, SKOT, som inkluderer 330 børn, der blev undersøgt ved 9, 18 og 36 måneder. Hovedformålet med Ph.d. studiet var at se på hvilke faktorer, der relaterer sig til kropssammensætningen ved 3 år, specielt med fokus på faktorer relateret til senere fedmerisiko. Et metodisk formål var at kunne bestemme kropssammensætningen ved bioimpedans analyse og antropometri. Et andet formål var at identificere vækstperioder med betydning for kropssammensætningen ved 3 år samt at se om betydningen af en sådan vækstperiode for senere kropssammensætning kunne modificeres af kostindtag. Et tredje formål var at undersøge relationen mellem vækstfaktoren IGF-I ved 9 og 36 måneder og kropssammensætningen ved 3 år. Da IGF-I niveauet i tidlig barndom påvirkes af typen af ernæring, var variable for dette inkluderet i analyserne.

Resultater: I **paper I** fandt vi, at bioimpedans, højde og vægt var lige så gode til at prædiktere fedtfri masse som en mere omfattende model der inkluderede summen af subscapularis og triceps hudfolder. Prædiktionsfejlen for fedtfrimasse var 3 % for begge ligninger. Da fedtmasse udgør en mindre del af den totale vægt, blev den relative fejlprocent dog noget større for fedtmasse (10.5 % i den fulde model og 12 % i den simple model). Resultaterne fra **paper II** viste, at fødselsvægt og vægt øgning fra 0 - 5 måneder var stærkt relateret til kropssammensætningen ved 3 år. Ingen af fedmemålene (BMI, fedtmasse, fedtmasse indeks og hudfoldstykkelse) var relateret til vægt øgning efter 5 måneder. Der var to interessante interaktioner mellem amning og henholdsvis fødselsvægt og tidlig vægtøgning i forhold til fedtmasse indeks ved 3 år. Med børn, der var fuldt ammet i under en måned, som reference gruppe, fandt vi, at fuld amning i 4 - 5 måneder reducerede betydningen af tidlig stor tilvækst på fedtmasse indeks med 47 % ($p = 0.05$), mens fuld amning i 6 måneder helt fjernede effekten af tidlig tilvækst på senere tykkelse ($p = 0.002$). Ligeledes eliminerede fuld amning i 6 måneder betydningen af fødselsvægt for senere fedtmasse indeks ($p = 0.002$). Resultaterne i **paper III** viste, at IGF-I koncentrationerne ved 9 og 36 måneder var positivt relaterede til højde, vægt, BMI, fedtmasse og fedtfrimasse ved 3 år, men ikke fedtprocent eller fedtmasse indeks. En stigning i IGF-I niveau fra 9 til 36 måneder var associeret med BMI, fedtfrimasse og fedtfrimasse indeks, men ikke til fedtmasse ved 3 år. De børn, der var ammet ved 9 måneder havde lavere IGF-I niveau ved 9 måneder, men ved 36 måneder var de kommet op på samme IGF-I niveau som de børn, der ikke var ammet ved 9 måneder. Der var

ingen associationer mellem IGF-I variablene og indtag af protein, mælk eller kød ved 36 måneder, men IGF-I variablene var negativt associeret med indtag af fedt og mættet fedt udtrykt som energiprocenter.

Diskussion: Den ligning, der kom frem ved **paper I** muliggjorde god estimering af fedtmasse og fedtfrimasse for denne aldersgruppe – med estimeringer for 233 børn versus 101 børn med en høj kvalitet af DXA skanning. Grundet den aldersafhængige variation i hydreringsniveauet af fedtfrimasse vurderer vi, at den prædiktive ligning kan bruges i andre studier af børn mellem 2 og 4 år. **Paper II** bekræftede tidlig vækst som en vigtig periode i forhold til senere kropssammensætning, som fundet i mange andre studier, men bidrog yderligere med interessante resultater, der viste, at den ugunstige effekt af høj fødselsvægt og stor tidlig vægtøgning på fedtmasse indekset ved 3 år, blev modificeret i en gunstig retning af varigheden af fuld amning. Det er klart, at forældrenes valg om varigheden af amning og opstart er overgangskost let kan være influeret af tidlige vækstmønstre, samt at forældrenes valg af ernæring til spædbarnet kan hænge sammen med deres øvrige sundhedsadfærd. Interaktionerne er dog biologisk plausible og bør undersøges i andre studier også. **Paper III** viste, at IGF-I ved både 9 og 36 måneder var positivt relateret de fleste antropometriske mål og kropssammensætning ved 3 år. Der var ingen klare associationer mellem IGF-I niveauer og tidlig udvikling af fedme. Det er muligt, at IGF-I niveauer er relateret til senere risiko for fedme gennem en øget tilvækst af både vægt og højde, hvilket kunne lede til et tidligt opsving i BMI, men dette var ikke til at se ved 3 år. Vi havde forventet, at indtaget af protein og mælk havde været relateret til IGF-I niveauerne ved 3 år, men dette var ikke tilfældet i dette studie. Da modermælk også har et højt indhold af fedt og mættet fedt, og amning er associeret med lavere IGF-I niveauer, kunne den negative association mellem IGF-I variablene og indtag af fedt og mættet fedt være relateret til dette. Dog er relationerne mellem kostindtag, IGF-I niveauer og risiko for tidlig udvikling af fedme meget komplekse, og der er brug for mere forskning inden for dette emne.

Konklusion og perspektiver

Denne afhandling repræsenterer en dybdegående analyse af kropssammensætning ved 3 år i en stor kohorte af raske velnærede danske børn. Fødselsvægt og tilvækst 0 - 5 måneder var positivt relateret til fedt- og fedtfrimasse ved 3 år, og resultaterne tyder på at de første 5 måneder er en sensitiv periode, hvor det er muligt at reducere betydningen af høj fødselsvægt eller stor vægtstigning på senere fedtmasse ved at fortsætte fuld amning i 4 - 6 måneder. Resultaterne lægger sig herved op af de gældende anbefalinger for spædbarnets ernæring, og er relevante i diskussionen om tidlig forebyggelse af fedme. IGF-I var relateret til lineær vækst, fedtmasse og fedtfrimasse ved 3 år, men en relation mellem IGF-I og senere fedme er spekulativ. Det vil være yderst relevant at fortsætte SKOT kohorten med et opfølgningsbesøg ved 7 – 8 år. I denne alder vil de fleste børn have passeret lavpunktet i BMI-kurven, og vi ville hermed kunne få større indsigt i en mulig rolle for IGF-I niveauerne på accelereret vækst og alder for BMI opsvinget. Derudover kunne et opfølgningsbesøg give større indsigt i betydningen af tidlig vækst samt amningens indflydelse på relationen med senere risiko for overvægt og fedme.

1 Background

In continuation of the global obesity epidemic, global data show that the worldwide prevalence of overweight and obesity in children aged 0 - 5 years has increased from 4.2 % in 1990 to 6.7 % in 2010 and is extrapolated to be 9.1 % in 2020 (1). In Denmark, the prevalence of overweight and obese among 5- to 6-year-old children has been found to be 16.6 and 4.2 %, respectively (2). As seen in many other countries worldwide, there has been a steep increase in the prevalence of overweight among school children from 1947 – 2003 in Denmark (3). However, a study of 3- and 5-year-old Danish children found that mean BMI and the prevalence of overweight was stable from 1992 – 2001 (4).

Being overweight or obese in childhood is consistently found to track into later childhood and adulthood (5). A high degree of tracking of fat mass (FM) and fat-free mass (FFM) have been shown from 2 to 7 years (6) and from 4 to 9 years (7), with higher increase among those who acquired a high fat percentage early in life (6;7). Once overweight has established, treatment is difficult and the risk of relapse is high. Thus, childhood is regarded a critical period for the development of overweight and obesity and an important period for early prevention of obesity (1;8). The association between early rapid growth and later risk of obesity is well-documented (9;10). Risk factors have been associated with genetics, psychological disturbances, and the surrounding environment and often these factors are entangled. Further, animal studies have addressed the possibility that non-genetic factors can be transmitted from generation to generation, hereby increasing the risk of obesity in the offspring (11). Several studies have suggested an effect size of excessive infant growth on later obesity risk of around 20 % (12-14). With the perspective that obesity is a multifactorial disease, this effect size is substantial.

This background chapter will describe the relation between body composition at 3 years of age and early growth, and discuss a possible role of infant feeding practice and insulin-like growth factor-I (IGF-I) in this relation. Furthermore, selected methods for assessment of body composition in young children will be described.

1.1 Growth in infancy and early childhood

Growth in infancy and childhood is an important marker for health and disease (15). The growth velocity should be followed rather than single measurements since growth patterns are more informative than the exact placement at a single time point (15). By following weight, length, and weight-for-length or BMI over time, it is possible to monitor if the child follow the same percentile on the growth charts. Downward centile crossing (corresponding to a 0.67 change in z-score) is called ‘catch down growth’ and can indicate disease. Upward centile crossing is called ‘catch up growth’ or ‘rapid growth’ and are associated with later risk of adiposity (16). The term catch up growth is used if the growth acceleration is a response to foetal under-nutrition followed by a rich postnatal diet, or after a period of disease, that has delayed growth (17). Interestingly, early rapid weight gain is associated with increased risk of later obesity independently of birth weight (16).

Growth during infancy is characterized by a high velocity which is rapidly decreasing. During the first year of life a normal infant will gain weight more rapidly than length, with a peak in BMI

growth chart at around 8 months of age (**Figure 1**). At this point the length gain exceeds weight gain and BMI decrease. From birth until about 6 – 9 months of age, mean FM increases considerably. In a recent study of Ethiopian infants from a non-malnourished population mean FM increased by 9-fold from 0 – 6 months of age (from mean 0.22 kg at birth to mean 2.00 kg at 6 months), while FFM in the same period only increased 1.9-fold (from mean 2.88 kg at birth to mean 5.58 kg at 6 months) (18). From around 9 months to 5 – 6 years the percentage of body fat (FM %) decrease, after which FM % increase again (15). This is also within the age range where the BMI growth-curve begins to rise again, also referred to as the adiposity rebound (19).

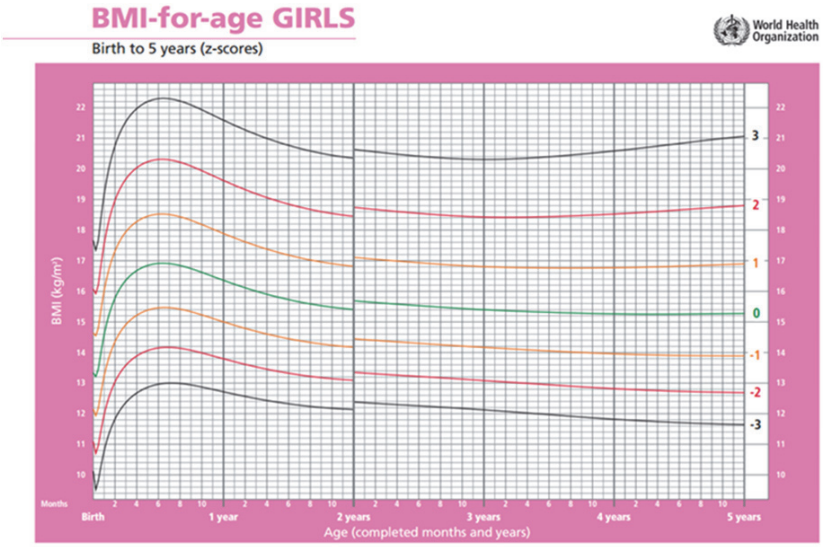

Figure 1. BMI-for-age growth standards (girls) from 0 – 5 years of age by WHO (http://www.who.int/childgrowth/software/en/)

1.1.1 Birth weight and relation with later body composition

It is now well accepted that exposures *in utero* can program the metabolic profile of the infant and affect the risk of chronic disease in adult life (20;21). Birth weight is an indicator for growth in the uterus, although it gives no information on foetal growth patterns and the distribution of FM and FFM (22).

Extremes in both ends of the birth weight distribution are associated with increased risk of obesity and metabolic disease (20;21). However, there are some indications high birth weight in fact programs more FFM than FM (23;24), a distinction not apparent when using BMI as the measurable parameter. Especially low birth weight could be speculated to program a smaller proportion of FFM later in life (23). In industrialised countries several studies have found birth weight positively related to later FM although not all studies are consistent (24). In Denmark, we have seen an increased prevalence in high birth weight since 1973 (25) and two studies based on 2760 and 5580 Danish children showed that high birth weight is a predictor for later overweight determined by BMI (2;4). Approximately twice as many children were born with birth weight above 4 kg in 2003 compared to

1973 (girls: 7.8% in 1973 versus 14.6% in 2003; boys: 13.2 in 1973 versus 22.1% in 2003) (25). However, mean birth weight has not increased from 1992 – 2001 (4).

1.1.2 The growth acceleration hypothesis

Increased tempo of growth in early childhood is related to higher risk of early adiposity rebound and later adiposity (9;10;19;26). Besides being associated with later risk of obesity, rapid post-natal growth is positively associated with early menarche (27), cardiovascular disease and several components of the metabolic syndrome hereunder insulin resistance, high concentration of low-density lipoprotein cholesterol, and high blood pressure (28;29). The growth acceleration hypothesis suggests that early rapid growth during infancy programs the metabolic profile of the infant to be susceptible to obesity and the other components of metabolic syndrome (28). The underlying mechanisms driving the correlation between early rapid weight gain and later risk of obesity are still not fully understood. Genetic predisposition, epigenetic differences, infant nutrition, insulin, and IGF-I has been proposed as possible candidates (11;26;30).

In this thesis, the term early growth is used to describe weight and length gain from birth and onwards, typically up till 2 years of age. The sensitive period lies within the first 1 – 2 years of life, and especially the first 6 months are considered crucial (26;29;31;32). Narrower windows have been suggested such as the first 3 - 4 months (13;29;33), and even the first 8 days have been associated with increased risk of overweight in later childhood (34). Young *et al.* (26) presents the OR for being overweight at 18 – 24 months according to birth weight above 4000 g, and excessive weight gain from 0 – 2, 2 – 4 and 4 – 6 months. High birth weight and excessive weight gain in each individual time interval were highly related to risk of overweight at 18 – 24 months of age, but weight gain from 2 – 4 months were significantly more influential than the other intervals.

The effect of weight gain in infancy on later body composition seems to depend on the study setting. In developing countries high infancy weight gain has been associated with later FFM rather than FM, while high infancy weight gain in industrialised countries is linked with both higher FM and FFM (24). Wells *et al.* (24) suggest that the differences between developing and industrialized countries could be explained by how close the infants are to the genetic potential for lean mass at birth. If the foetus has been able to accumulate lean mass close to its genetic potential, excessive infant energy intake might be stored as fat and not converted into lean mass. In contrast, infants who have not managed to accumulate lean mass according to genetic potential in the foetal stage, might be able to convert excessive energy directly to lean mass and hereby circumvent growth deficits. In industrialised countries it has been suggested that allocation of energy intake to FFM becomes increasingly difficult from late infancy onwards. Thus, life *in utero* and infancy may be a critical window for programming of adult FFM (24;35).

Rapid infant growth is also positively related to later FFM and height (17;32;35;36). Besides a positive association with later obesity determined by BMI, weight gain from 0 – 6 months was positively associated with greater height and lean mass at 9 years of age in a cohort of Brazilian boys (36). A possible link between increased tempo of growth, including linear growth, accelerated maturation and later risk of obesity has been suggested (37). This will be further elaborated on in section 1.1.3 and 1.3.3.

1.1.3 Age of adiposity rebound and later obesity risk

The age at adiposity rebound is characterised by the point where the BMI curve begins to rise again after a fall of BMI in late infancy and early childhood. The age of adiposity rebound is regarded as a risk marker for later obesity risk, with an early adiposity rebound increasing the risk (19;38). To assess the age at adiposity rebound it is necessary to monitor how BMI changes over time and find the age of minimum BMI (19). The normal age for the adiposity rebound is around 6 years of age, while early adiposity rebound is seen as early as 2 to 3 years of age (19). As **Figure 2** illustrates, early adiposity rebound can be associated with low or normal BMI prior to the rebound with subsequent high increase in BMI (19). This BMI pattern is different from the growth pattern showing a tracking of high BMI or FM from early childhood onwards.

Early adiposity rebound has been related to more advanced bone age in 7-year-old boys (38) and earlier menarche in girls (38;39). A prospective study from New Zealand including girls from 5 to 9 years of age showed that the differences in BMI seen during the adiposity rebound were attributed to an increase in FM (40). Girls with adiposity rebound before 5 years of age had a 2-fold higher annual increase in FM compared to late rebounders (> 5 years of age), while no difference were seen for gain in lean mass.

It has been speculated that increased tempo of weight gain and linear growth in infancy can lead to early adiposity rebound (19;39;41). In a study from New Zealand, height rather than BMI at 3 years was associated with earlier adiposity rebound (39). Early adiposity rebound tended to be associated with higher BMI at the time of rebound and early adiposity rebound was associated with higher BMI at 18 and 26 years of age (42).

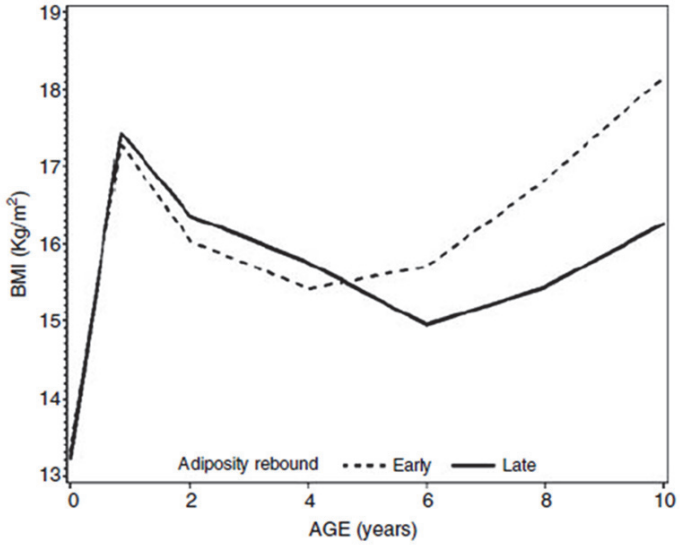

Figure 2. BMI growth patterns from 0 – 10 years according to age at adiposity rebound. Dashed line = adiposity rebound at 4 years. Solid line = adiposity rebound at 6 years. Early adiposity rebound is associated with normal or lower BMI before the adiposity rebound and higher BMI after the adiposity rebound (reprinted from Rolland-Cachera *et al.* 2006 (19))

1.2 Infant feeding practice

Infancy is characterized by pronounced nutritional and developmental changes. Nutritionally, the feeding procedure changes from intrauterine nutrition via the placenta to milk feeding, complementary feeding and finally family foods. Recommendations in many countries and from WHO are exclusive breastfeeding the first 6 months, introduction to complementary feeding around 6 months, and gradual transition to family foods around 9 to 18 months of age (43-45). In the complementary feeding period partial breastfeeding is recommended (43;45). A further discussion of these recommendations is beyond the scope of this thesis.

While overfeeding and excessive weight gain in infancy is recognised as risk factors for obesity later in life (9;46), the role of breastfeeding, formula feeding, and complementary feeding, is still debated. Meta-analyses and systematic reviews have shown a weak but consistent protective effect of breastfeeding with regards to obesity in childhood (47) as well as in adulthood (48;49), however, no effect was seen on mean BMI (50). Conclusions are to some degree hampered by the many potential confounding factors related to the duration of breastfeeding (51), which many observational studies have only partly accounted for (52). Infant feeding patterns believed to be healthy are likely to be linked with a responsive maternal feeding style and other health-promoting behaviours that will track beyond infancy (52). Moreover, the definition of breastfeeding is relevant for interpretation of data, but little consistency is seen for classification of breastfeeding in scientific studies (46). While some studies conclude on the effect of having ever or never been breastfed, other studies focus on different durations of exclusive breastfeeding or full breastfeeding – with different cut-offs or ages in question. The physiologic responses and the underlying mechanisms expected to explain any correlations, are in many cases poorly supported by be classification of breastfeeding used. To complicate things further, some variations in the composition of human milk is seen between women, which is affected by gestational weight gain (53), maternal obesity, and maternal diet (26). These are some of the reasons why optimum duration of exclusive and partial breastfeeding, and age of introduction to solids with regards to later risk of obesity and general health remains widely debated (52;54-56). The possible influence of early feeding types on later body composition will be described in the following sections as sketched out in **Figure 3**.

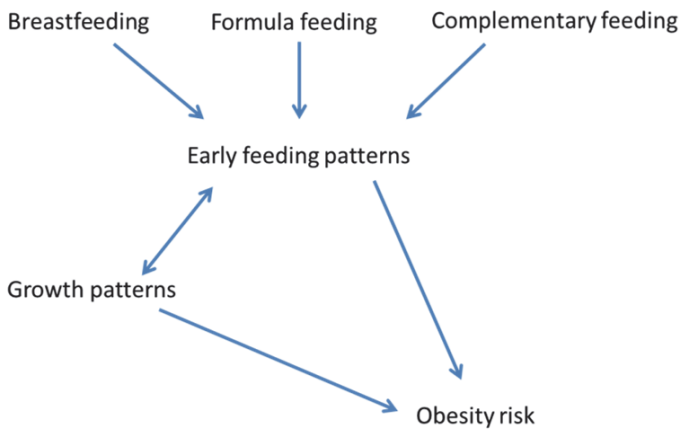

Figure 3. Possible interaction between early feeding and early growth and later risk of obesity.

1.2.1 Breastfeeding and formula feeding – relation with later body composition

Growth in infancy is highly dependent on nutrition. Formula-fed infants have a different growth pattern than breastfed infants, showing a generally slower weight gain the first months but higher weight gain hereafter (57;58). A recent systematic review and meta-analysis showed significant differences in FM with formula fed infants being fatter at 12 months (59). However, the differences in body composition trajectories between breast and formula fed infants changed over time with breastfed infants having higher FM at 3 - 4 months and 6 months and a trend towards lower FM at 12 months compared to formula fed. Thus, high FM at some ages in infancy might not determinate later adiposity risk but may have "an unknown biologic function" (59). Gale *et al.* (59) speculate that the shift from higher adiposity to lower adiposity at 12 months of age in breastfed infants compared with formula fed infants, could support a programming effect of early feeding on metabolism, glucose metabolism, or appetite regulation. Two randomised controlled trials support a role of nutrition on infant weight gain and later risk of obesity, which is independent of genetic inheritance. Infants randomised to high protein formula as opposed to low protein formula until 12 months of age have been shown to have increased weight gain and weight at 2 years of age (60), and infants born small for gestational age who received nutrient enriched formula instead of control formula until 6 – 9 months of age was shown to have higher FM at 5 – 8 years (61).

While most evidence for a protective effect of breastfeeding on later risk of overweight have BMI as outcome, studies examining the effect of breastfeeding on later FM and FFM are less consistent. Three newer prospective cohort studies have seen reduced FM at later ages among children, who were breastfed as infants (51;62;63), while three have not found a difference according to breastfeeding in infancy (64-66). Data from the ALSPAC cohort showed an inverse association between duration of breastfeeding and FM measured by DXA among 4325 children aged 9 to 10 years of age (51). Children, that had been breastfed for 6 months or more, had the lowest odds for being in the highest decile of FM at 9 – 10 years of age. Adjustment for confounders attenuated the relation between breastfeeding and some of the measures of FM but not all (51). Breastfeeding for 6 months or more reduced the risk of obesity but mean BMI was not reduced. In a prospective birth-cohort-study, including 569 children, Robinson *et al* (62) found that infants breastfed for 12 months compared with infants never breastfed, had lower FM at 4-years of age after control of possible confounding factors. Further, a healthy complementary food pattern was associated with higher lean mass at 4 years of age. Another prospective cohort study including 216 children found that being breastfed for more than 4 months compared with never breastfed, was associated with lower FM at 9-years-old girls but not among boys after adjustments for possible confounders (63). Among the studies not showing a protective effect of breastfeeding, a DXA study of 313 5-year-old children found no differences in FM between "ever" and "never" breastfed children, and no effect on FM was observed when introducing complementary foods before or after 4 months of age (64). A limitation of this study is that information on early feeding was collected retrospectively at 3 years of age and the classification "ever" or "never" breastfed is very crude. In a prospective cohort study by Butte *et al.* (65), differences in body composition were seen between 40 exclusively breastfed infants and 36 exclusively formula fed infants (both from 0 – 4 months of age) the first 12 months, but the differences levelled out from 12 to 24 months of age. However, the sample size in this study was small and the power may have been insufficient to detect a difference. In a Swedish study by

Tulldahl *et al.* (66), 192 adolescents with information on early feeding were DXA scanned at 17 and 18 years of age. Information on early feeding was collected retrospectively and the participants were grouped as breastfed in more or less than 3 months. No significant differences occurred between the two feeding groups. The authors note that at the time the study was conducted, it was recommended to begin the complementary feeding period around 3-4 months (67), which is under suspicion of being an independent risk factor for overweight (section 1.2.2).

Some infants still gain excessive weight the first 6 months despite being breastfed. These children have also been found to have increased risk of obesity (12;17). However, there are some indications that breastfeeding can modify the impact of excessive weight gain on later risk of obesity. A small retrospective study with 129 children, found that those who had been exclusively breastfed for 6 months were less likely to be overweight at 6 – 8 years of age despite high infant weight gain (> 8.15 kg from 0 – 2 years of age) (68), and preliminary data from the PROBIT study have shown stronger relationship between early rapid weight gain and obesity at 6 – 7 years among children who had been exclusively formula fed from 1 months of age compared to other infants (69). In the DONALD study, duration of full breastfeeding for ≥ 4 months was found to modify the effect of rapid growth on FM % at 2 years of age among 71 children with rapid weight gain from 0 – 2 years of age (70). Other results from the DONALD study have demonstrated modifying effects of breastfeeding on FM at 2 years of age for boys born by overweight mothers (71).

There are many plausible behavioural and physiological reasons for why the protective effects seen by breastfeeding in many observational studies could be real and not due to confounding effects (72). Advocators for this relation explain the protective effect through the composition of human milk, differences in infancy growth, body composition, protein intake, hormonal regulation, better appetite regulation, and self-regulation of energy intake (30;46;72). Critics highlight a high degree of confounding, residual confounding, and insufficient adjustments in epidemiologic studies (51;73;74), and call attention to a large cluster randomised breastfeeding promotion intervention study in Belarus, finding no effect of the breastfeeding intervention on risk of obesity at 6 – 7 (75) or 11 years of age (76).

1.2.2 Complementary feeding

Breastfeeding, formula feeding, and complementary feeding practices are three highly related components and it can be difficult to distinguish which component is responsible for certain effects. The potential role of the diet in the complementary feeding period for later body composition and risk of obesity, has received comparatively less attention compared to breastfeeding (62;64;67;70).

The shift from infant feeding to the family diet is characterized by a considerable increase in protein intake and reduction in fat intake (77;78). Especially high intake of protein in this period is under suspicion of leading to higher BMI and body fat percentage in later life (79-82). This is further described in section 1.2.3. There is no evidence for a convincing association between fat intake in the complementary period and later markers of obesity (83). Furthermore, recent findings from an observational study showed an inverse relation between fat intake at 2 years of age and subscapularis skin fold thickness and FM at 20 years of age (78).

The impact of the complementary feeding patterns on later body composition is complicated to define and an issue less investigated. In a prospective birth cohort study in Southampton including 1740 children, infants with dietary patterns most similar to infant feeding guidelines (high frequencies of fresh fruit and vegetables, home prepared foods, and breast milk), gained more weight and fat assessed by skinfold thickness from 6 – 12 months and infants with lower quality of complementary foods (highest frequencies of breads and processed foods) gained less weight in the same period. The results were independent of milk feeding, age of introduction to solids, maternal education, parity, and smoking during pregnancy (84). Other results from 569 children from the same cohort showed that a healthy dietary pattern at 12 months was associated with higher lean mass but not with FM at 4 years of age (62). The healthy dietary pattern was characterised by high consumption of fruit and vegetables, cooked meat and fish, and a high share of home-prepared foods. However, children fed according to the infant guidelines were also more likely to have been breastfed for a longer period.

A simpler approach is whether the timing of introduction to solid foods affects risk of obesity. Even though the findings are inconsistent and heterogeneous (55;56), there are some indications that introduction to solid foods before 4 months of age (< 17 weeks) is associated with increased adiposity later in life (85) and that each month delay of introduction from 2 – 6 months can lower the risk of overweight as adult (67). The reason why timing should be important is not clear. Early introduction to solids has been associated with country, gender, parental education, marital status (86), maternal smoking (86;87), high birth weight and weight at 6 weeks (87), maternal pre-pregnancy BMI (67), younger maternal age (87;88), higher energy intake at earlier ages (86;89), a lower quality of food in the complementary feeding period (90), and early food preferences could influence later eating habits (91). Thus, it is possible that the age of introduction to solids is linked to other traits related to unhealthy behaviour and the development of overweight. Another explanation could be that children that are introduced to complementary foods early for other reasons have greater appetites, which lead their parents to begin the complementary feeding period earlier to meet their energy demands.

Formula-fed infants generally are introduced to solid food earlier than breastfed infants (91;92). The effect of complementary feeding on growth and energy intake have been shown to be more evident in formula-fed compared to breastfed infants (85;91). Huh *et al.* (85) found that non-breastfed children (n = 279), who had begun the complementary feeding period before 17 weeks, had a six-fold higher risk of later obesity measured at 3 years compared with children who had been breastfed for at least 4 months (n = 568). These results were adjusted for weight gain between birth and 4 months, and should therefore be independent of early growth. This finding could be related to the different feeding pattern observed in formula-fed infants compared with breastfed. Formula-fed infants have been shown to consume larger volumes per feed and per day (93), and might have a reduced ability to self-regulate their energy intake even in later infancy (94).

Most work on timing of the complementary feeding period and obesity risk is made on observational studies (55;56), which cannot distinguish physiologic factors from decisions made by the mother, or advices given by the health care worker. The few randomised studies on the effect of time of introduction to complementary feeding on later adiposity risk have not been able to demonstrate an effect (95;96). A study from Honduras randomly assigned 152 infants, who were exclu-

sively breastfed at 4 months to a) exclusive breastfeeding until 6 months, b) introduction to solids at 4 months while continuing breastfeeding, or c) control. There were no differences in weight-for-length z-scores between groups from 16 to 26 weeks of age (95). In another intervention study (96), 165 infants were randomised to be introduced to solids at a) 3 months (either commercially prepared or parent's choice) or b) at 6 months (either commercially prepared or parent's choice). There were no demands to the type of milk feeding prior to the intervention, but during the intervention the infants were formula-fed along with the weaning diet. There were no differences in protein and fat intake between the early introduction group and the late introduction group. Anthropometry and body composition (DXA scan) were measured at 3, 6, and 12 months and no differences in growth or body fat percentage were seen between the groups. However, for both intervention studies the follow-up period were short and the power could be insufficient to see an effect.

There is some evidence that the effect of early introductions is dependent on the age at which adiposity is assessed. In support of a programming or delayed effect of early introduction to solids is results from the Dundee infant feeding study, a prospective cohort study. Early introduction to solids (< 15 weeks) was not associated with higher weight at 2 years of age compared to later introduction to solids (n = 455) (97), but at 7 years the children who had been introduced to solids early, had higher BMI and body fat percentage (n = 412) (98). Others have also found a delayed effect of age of introduction to solids at 10 years (99) and 42 years of age (67). In both these studies, no relation was seen with breastfeeding. However, no difference in FM and lean mass at 5 years of age was seen between infants weaned before or after 4 months of age (64).

1.2.3 The protein-adiposity hypothesis

High protein intake has been associated with increased growth velocity in infancy (60), and early rapid growth is associated with increased risk of obesity in later life (9;10). In observational studies a high protein intake in infancy or early childhood has been associated with increased BMI or risk of obesity at later ages (79;80;82;100;101). However, in a Danish study, protein intake at 9 months was positively correlated with height and weight, but not with body fat percentage at 10 years (102). Further, data from 772 children in the ALSPAC cohort did not show a relation between protein intake at 18 months of age and timing of the adiposity rebound (103). As most studies of early protein intake and later risk of obesity is observational, causality has not been proven. A maximum acceptable level of 14 energy percentage of protein has been suggested for 12-24 months old children (104).

The protein-adiposity hypothesis proposes that a high protein intake during infancy and early childhood increase the risk of obesity (82;104;105). IGF-I has been suggested as the underlying mechanism for the association between early protein intake and growth, because intake of protein are found to stimulate IGF-I secretion (106;107) and IGF-I promotes cell proliferation, protein synthesis, longitudinal growth (108), and differentiation of adipocytes (109).

The protein-adiposity hypothesis has recently been investigated by the European Childhood Obesity Programme study (CHOP), a large multi-centre randomised controlled trial (60;106). The study included 1090 healthy formula-fed infants randomly assigned to receive high-protein formula (infant formula: 2.9 g/100 kcal; follow-on formula: 4.4 g/100 kcal) or low-protein formula (Infant

formula: 1.8 g/100 kcal; follow-on formula: 2.2 g/100 kcal). In addition, a group of exclusively breastfed infants (first 3 months, n = 588) was included as an observational control group. At 6 months of age, free and total IGF-I levels were approximately 40% higher in the high-protein group than in the low-protein group with no difference in IGF-binding protein (IGFBP)-3. Breastfed infants had approximately 60 % lower IGF-I compared to those getting the formula with low protein (106). The growth patterns of the low-protein group were slower than the high-protein group and more similar to the breastfed infants (60). The children were followed until 24 months of age. At both 12 and 24 months of age the BMI were higher in the high-protein group compared with the low-protein group, while no differences were seen for linear growth (60). This large randomised controlled trial provides evidence, that reducing protein intake the first year of life, have an effect on weight-for-length and BMI at 2 years of age.

1.3 Insulin-like growth factor I, growth and body composition

Regulation of growth is complex and involves many hormonal interactions including growth hormone, IGF-I, insulin and sex hormones, acting differently at different age stages (107;108;110). The infancy, childhood and puberty growth model (108;110) suggests that growth in these three periods is promoted by three systems acting differently. The components are additive and partly overlapping (**Figure 4**).

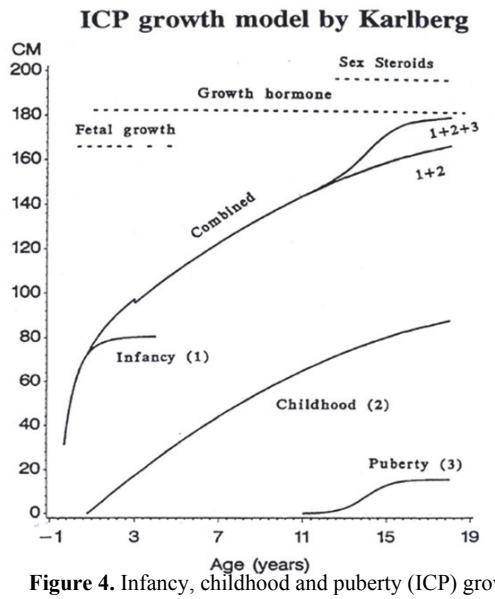

Figure 4. Infancy, childhood and puberty (ICP) growth model (reprinted from Karlberg *et al.* (110).

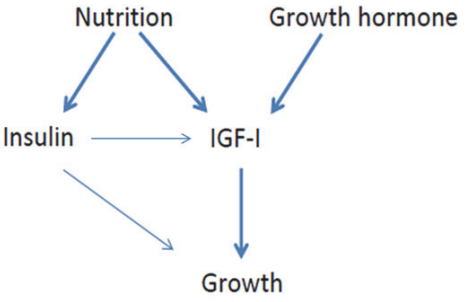

Figure 5. Growth in infancy is influenced by growth hormone and nutrition acting through insulin and IGF-I (108;121).

In foetal stage growth is mainly dependent on IGF-I, IGF-II and insulin while infancy growth is highly regulated by nutrition, influencing insulin and IGF-I but also growth hormone and thyroxin (108) (**Figure 5**). Childhood growth is regulated by the growth hormone – IGF-I axis and thyroid

hormone, and IGF-I becomes less dependent on nutritional regulation (108), although there is some evidence showing that especially protein and milk are still able to stimulate IGF-I in later childhood (111-114). The childhood growth phase emerges around 6 months of age and is main responsible for growth regulation from 3 years until puberty onset (Figure 4)(108;110). In teenage years, increased production of androgens in boys and oestrogens in girls leads to increase in growth hormone secretion and serum IGF-I and increased growth velocity (108;110). The focus here will be on IGF-I in early childhood.

IGF-I levels changes according to age and especially in the first year of life the pattern is complex. New data suggest that IGF-I levels decline from about 2 months to about 6-8 months, and thereafter increase again, hereby following the inverse pattern of change in BMI in the same period (**Figure 6**) (115). From 8 – 9 months IGF-I levels increase gradually until puberty (116). Girls have higher IGF-I concentrations than boys in infancy and during childhood (117-119) despite that they have lower growth velocity, while boys have higher IGF-I values during late puberty (120). In adults, no significant sex difference was seen for IGF-I concentration (116).

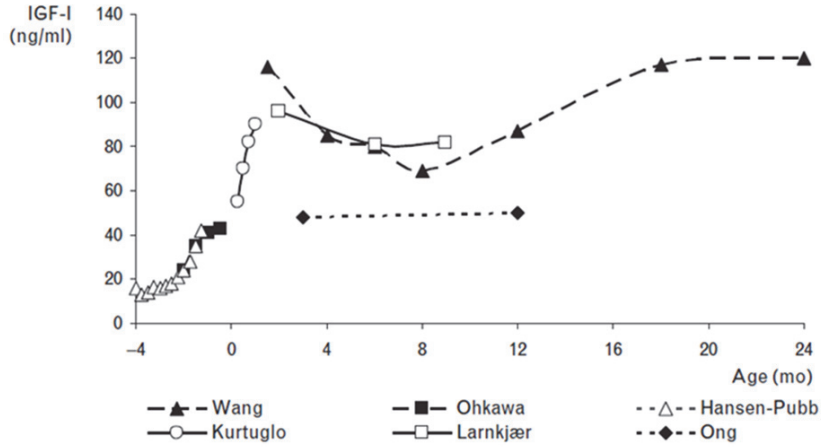

Figure 6 Insulin-like growth factor (IGF-I) concentrations from 0 – 24 months – a uniting of selected studies according to age (reprinted from Larnkjær *et al.* 2012 (115))

1.3.1 IGF-I regulation and function

The two main types of IGF's are IGF-I and IGF-II. Both are single-chain polypeptide hormones that share approximately 50% of the amino acid sequence with insulin (108). Growth hormone stimulates the production of IGF-I which is mainly produced in the liver and to a less extend in the peripheral tissues and the growth plates of bones (108). Insulin may also mediate IGF-I release from the liver (121). IGF-I production is the main mediator of the mitogenic and anabolic effects of growth hormone (108), and act on most cell types stimulating protein synthesis, cell proliferation and longitudinal growth (108) but also differentiation of adipose tissue especially in visceral fat cells (109). There are two variants of the IGF-receptors. Receptor type I has a structural similarity with the insulin receptor and a high affinity with IGF-I but also binds to insulin and IGF-II. Recep-

tor type II has higher affinity for IGF-II. More than 95 % of the circulating IGF-I is bound to IGF-binding proteins (IGFBP's) and form stabile complexes which prolong the metabolic half-life for active IGF-I and regulates the availability. There are six variants of IGFBPs but most IGF-I (≈ 90 %) is bound to IGFBP-3 (108;122). Therefore, the molar ratio IGF-I to IGFBP-3 is often used as a measure that reflects the bioavailability of IGF-I (113;123).

1.3.2 IGF-I – influence by nutrition

Optimal growth is dependent on adequate nutrient supply (124) and there is reasonable agreement that cow's milk has a stimulatory effect on linear growth in children, although the effect is likely to differ with age and nutritional state (125;126). The stimulating effect of cow's milk on growth could be due to the amino acid composition of proteins, bioactive peptides, minerals (e.g. calcium, phosphor and magnesium), vitamins, bovine IGF-I or the combination of these (125). IGF-I levels are also influenced by dietary factors (107;115;126), and although IGF-I become less dependent on nutritional regulation during childhood (108), protein and especially cow's milk intake have been shown to stimulate IGF-I secretion in later childhood (106;113;114;127).

As written in section 1.2.1, formula fed and breastfed infants have different growth patterns (57;58). This difference can at least partly to be mediated by IGF-I, since breastfeeding is associated with lower IGF-I concentrations in infancy (106;118;128). Earlier finding from the SKOT cohort showed a negative dose-response relationship between intensity of breastfeeding at 9 months and IGF-I concentrations at 9 months (123). Differences in the protein content of breast milk and formula milk is likely to be a main responsible factor for differences in IGF-I concentrations in infancy (106;126) , although it is speculated that breast milk has a modulating effect on IGF system beyond that of protein (115). In a large randomised multicentre trial comparing infant formula with high or low protein content (further described in section 1.2.3), IGF-I at 6 months was approximately 40 % higher in the high-protein group compared to the low-protein group while the group of breastfed children had approximately 60 % lower IGF-I compared to the low-protein group (106).

High IGF-I concentrations in infancy and early childhood have been associated with lower IGF-I concentrations later in life (111;120;126) and there is some indications that the relationship between breastfeeding and IGF-I reverse later in childhood with higher IGF-I concentrations among children who had been breastfed as infants when compared to infants with no or short duration of breastfeeding (111;120;126). In the ALSPAC study, being breastfed in infancy was associated with higher levels of total IGF-I at 7 – 8 years (111). A Danish study with 109 children from the Copenhagen cohort study found an inverse association between IGF-I at 9 months and 17 years (120). Infants that were exclusively breastfed at 2 months tended to have higher IGF-I at 17 years of age compared to those not breastfed. Also cow's milk consumption in early childhood have been inversely associated with IGF-I concentrations later in life (129;130). This has led to the hypothesis that infant feeding have a long-term programming effect on the IGF-I axis through a resetting of the pituitary control of growth hormone on IGF-I levels as a response to high IGF-I in infancy or childhood (126). Interestingly, as reviewed in Martin *et al.* (126) breastfeeding has been associated with greater height, especially leg length in childhood and early adulthood in some studies, at least in males

(99;131) . This finding goes well with breastfed children having higher IGF-I concentrations at later ages.

Early programming of the IGF-I axis could in theory underlie the associations seen between early life events and risk of life style diseases in adult life (30;111). Low IGF-I concentrations in adult life have been associated with increased risk of ischaemic heart disease (132), cardiovascular disease and type 2 diabetes (133). At the same time, breastfeeding is also associated with reduced risk of type 2 diabetes and risk factors for cardiovascular disease (134). It is out of the scope for this PhD thesis to go further into the relations between IGF-I and life style diseases in later life. In disfavour for the theory of early programming of the IGF-I axis by type of infant feeding, new results from the PROBIT study showed no effect of a breastfeeding intervention prolonging duration of breastfeeding on IGF-I concentrations at 11 years, and there were no difference in IGF-I concentrations at 11 years between infants exclusively breastfed for 3 to less than 6 months compared with 6 months or more (76).

1.3.3 IGF-I effect on body composition

The stimulating effect of protein on IGF-I in infancy and the many studies linking early high protein intake with rapid growth and later risk of obesity (section 1.2.3) have led to speculations that early modulation of the IGF system plays a role for the increased risk of obesity at later ages (37;105;115).

High IGF-I concentrations in infancy are associated with greater weight and height gain from 0 – 9 months, thicker skinfolds and higher BMI at 9 months (123). However, the same study found that high IGF-I levels at 9 months were related to a decrease in BMI from 9 – 18 months due to increased length gain. Ong *et al.* (118) also found IGF-I concentration at 3 months to be positively associated with weight gain prior to the measurement (from 0 – 3 months) but only to length gain in the months succeeding (3 – 12 months of age) leading to negative relation with BMI in this period. Likewise, Socha *et al.* (106) found IGF-I concentration at 6 months highly positively correlated with weight-for-length z-scores from 0 – 6 months but not with subsequent change in weight-for-length from 6 – 12 months. These results indicate that IGF-I concentrations are especially important for early weight gain while the effect of high IGF-I is more related to linear growth at later ages and seem to question a role of IGF-I for later risk of overweight. Ong *et al.* (118) suggest that IGF-I in infancy is important for lean mass and length gain, while insulin or another component stimulates FM. Other studies have found high levels of IGF-II and leptin (119) or low levels of IGFBP-II (135) closer related to adiposity that IGF-I.

On the other hand, the relation between linear growth, BMI trajectories and later risk of obesity is not simple. Accelerated growth of height, lean mass and FM have been observed in obese children (136), and overweight children tend to be taller than normal weight children until puberty (136;137). In a group of obese children, increased stature were only seen among those who had experienced rapid weight gain the first year of life (137). Height has been shown to be highly positively correlated with FMI, body fat percentage, leptin concentrations and insulin resistance in children aged 7 – 12 years and the children with highest linear growth from 7 – 12 years had greater increase in body fat percentage, leptin and insulin resistance (138). Overweight children have been found to

have higher or normal IGF-I levels compared with normal weight children (139;140). In a study from New Zealand, height rather than BMI at 3 years was associated with earlier adiposity rebound (39). Thus, it is possible that increased tempo of growth including length gain is related to early adiposity rebound and later adiposity (39;41) perhaps mediated by the IGF system (123). A follow up of the SKOT cohort would be of great value to explore the role of early IGF-I levels on adiposity markers in later childhood.

1.4 Assessing body composition in childhood

This section will give examples of how body composition can be assessed in childhood, and will not give a comprehensive overview of all available methods. The methods are selected based on their relevance to the present study. Further details of the methods applied in the SKOT cohort and used in the present thesis, will be given in the methods section.

1.4.1 BMI and weight-for-length

BMI and weight-for-length are two widely used indicators for body composition in children. The major difference between these two indices is that length is not squared in weight-for-length. For BMI, age- and gender-specific standard deviation scores (SDS) have been developed for from growth reference charts to facilitate comparison between these groups. Likewise, gender-specific SDS is often used for weight-for-length. The WHO growth standards were published in 2006 and are based on standardised measurements in children from 6 countries around the world, all born by non-smoking mothers willing to breastfed for at least 4 months and without socioeconomic constrains believed to influence growth (141). These standards are of great value since national wide growth references often are based on infants on mixed feeding types and with no restriction on smoking among mothers, both of which influence child growth (141). The growth standards represent normative growth patterns and from these, it is possible to calculate the distance from the median in SDS. Normal growth is defined to be within ± 2 SDS from the median, which is a definition based on statistics rather than influence on health outcomes. In monitoring a child's growth pattern, it is of interest that the child follows the same percentile over time (**Figure 7**). As described in section 1.1, a change in SDS ± 0.67 between two points in time, has been found clinically relevant and are often used as cut-off value for "catch-down" and "rapid growth" between 0-2 years (16). The number 0.67 SDS corresponds to the difference between centile lines on standard infant and childhood growth charts (2^{nd}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, and 98^{th} centile line) and can be clinically interpreted as upward or downward centile crossing (16).

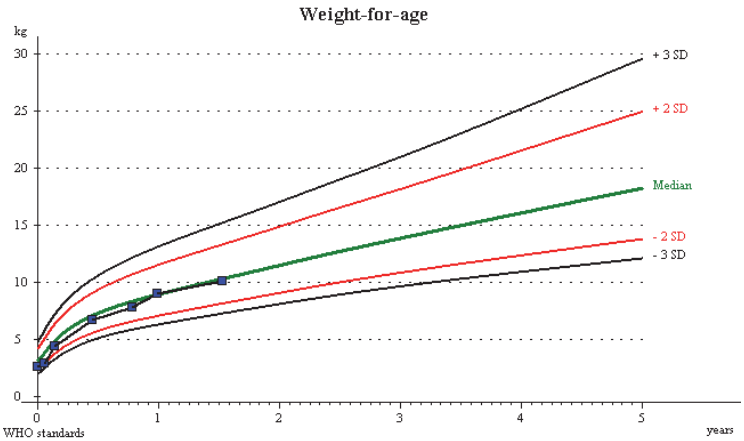

Figure 7. Example of an individual growth curve of weight according to age (http://www.who.int/childgrowth/software/en/).

The WHO cut-off values for being at risk of overweight, is defined as a BMI-for-age SDS (BAZ) above 1, where overweight is defined as BAZ between 2 - 3 and obese as > 3 BAZ above the median. In 2000, the International Obesity Task Force (IOTF) developed age- and gender-specific cut-off points for normal and overweight BMI (2 - 18 years) (142). These cut-off values are based on dataset from 6 large cross sectional growth studies from different countries around the world, and gives cut-off points for BMI for overweight and obesity in children, that correspond to the BMI cut-off values 25 and 30 used in adults.

The different ways of defining overweight results in different estimations of the prevalence of overweight and obesity in studies and populations over time. It is therefore of high important to state which reference is used as the basis for the current study and to use the same reference at later comparisons. In the papers, the prevalence of overweight and obesity in the SKOT cohort is determined according to both the cut-off values by IOTF and according to WHO growth standards.

1.4.2 Prediction techniques to estimate body composition

BMI is useful as crude indicator for adiposity in large settings, but is only a proxy for adiposity since large individual differences in FM and FFM exists within a given BMI (143). Estimations of FM and FFM in childhood enables more detailed information on the biology behind early development of obesity, the aetiology of life style diseases, and alterations in body composition e.g. in response to treatment of a disease or dietary interventions (144).

Precise predictions of body composition in young children are difficult to obtain. Dual Energy X-ray Absorptiometry (DXA), BOD POD based on air displacement plethysmography technology and deuterium dilution that gives a measure of total body water are supposedly the most appropriate techniques for use in 3-year-old children. Simple prediction of FM and FFM can be made from skinfold thickness and bioelectrical impedance analysis (BIA). Skinfold thickness provide information on regional fat stores and have proven relatively successful in ranking infants in terms of regional fatness, but becomes inaccurate when used to predict full body FM and FFM in individuals

(145-147). As discussed by Jensen *et al.* (148) caution should be made when assessing body composition from anthropometry. In their analysis of the predictive validity of anthropometric measures in the SKOT cohort, limitations were demonstrated, especially for predictions of FM and FFM, expressed as percentages of total body mass.

1.4.3 Bioelectrical impedance analysis

BIA gives information on whole body resistance and enables estimation of the two components, FFM and FM. BIA is a recognized method to estimate total body water in epidemiologic studies (145;149), although some inconsistencies have been found when used in young children (150). A recent systematic review (151) found BIA to be reliable in children but also susceptible to considerable measurement error. BIA is a practical method to estimate body composition as it is fast, inexpensive, portable and easy to use in young children (149).

The BIA instrument estimates total body water by sending an electrical impulse through the body and measure the resistance to the flow of the electric current which depends on the amount and distribution of FM and FFM (149). It is electrolytes in the body water that conduct the electrical current through the body and the volume of water is then estimated using the length of the subject and the resistance (152). FM is considered to be free of water. FFM is a collective designation of tissues including muscles, organs, connective tissue, and bones. Total body water is a sum of intracellular and extracellular water and constitutes a percentage of FFM (**Figure 8**). The hydration level of FFM varies according to growth, maturity, aging, ethnicity, disease, and obesity (145;149;153). Therefore, the conversion of total body water to FFM requires population and age-specific interpretation of the output. In adults total body water is assumed to be 73 % of FFM. In children, age- and gender-specific hydration factors are available from Fomon *et al.* (153). In 3-year-old children these have been found to be 77.9 % for girls and 77.5 % for boys (153).

Many predictive equations have been made using BIA to predict total body water or FFM in children and adults (151). However, few predictive equations are available for children in early childhood (2 – 4 years) (154-158). None of these studies provide validation of how well FM is predicted from the predicted FFM.

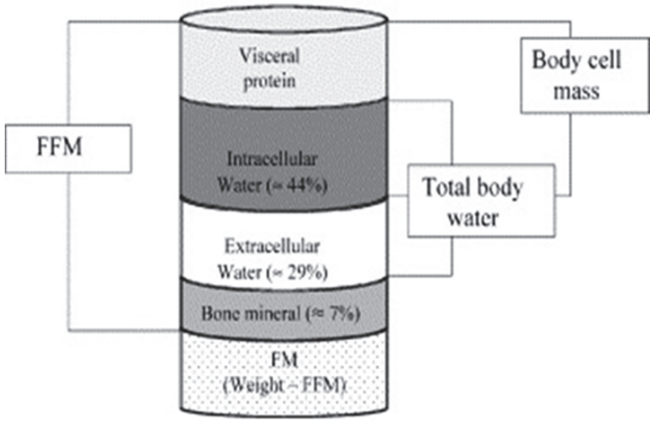

Figure 8. A schematic view of body composition compartments. In adults, intracellular and extracellular water constitutes 73 % of FFM (reprinted from Kyle *et al.* (152))

1.4.4 Dual Energy X-ray Absorptiometry

DXA provides information on body composition for whole body and regional composition of FM, lean tissue mass and bone mineral content. In this study, DXA was used as the reference method for body composition measurements of FM and FFM (lean tissue mass + bone mineral content). During a DXA scan, two X-ray tubes emit low doses of X-rays with different photon energies. According to the manufacturer of the DXA machine used in this study (Lunar Prodigy Advance, GE Healthcare, Madison, WI, USA), the radiation dose from each scan was maximum 0.0012 mSv. In comparison the normal background radiation in Denmark is 3 mSv per year, corresponding to 0.008 mSv per day. Thus, the radiation dose obtained from a DXA scan equals less than 4 hours of normal background radiation. The concept of the DXA technology is that photon attenuation is a function of tissue composition, and lean mass, FM and bones are supposed to be distinguishable by their X-ray attenuation properties (159). As with BIA, DXA measurements of FM and FFM are based on the assumption that the soft tissue is normally hydrated. Thus the DXA estimate is also vulnerable to changes in hydration of FFM (159).

1.4.5 Height adjusted indices for fat mass and fat-free mass

An approach to account for the natural variation in FM and FFM due to body size is to adjust FM and FFM for height instead of body weight. This enables assessment of changes in fatness independently of changes in FFM. In children, full adjustment for height does not always lead to the same coefficient as with BMI. Moreover, the coefficient for height in the fat mass index (FMI) can differ from the coefficient needed to fully adjust FFM for height in the fat-free mass index (FFMI) (160). Metcalf *et al.* (138) oppose to the fully adjusted height standardized expressions for FM and FFM based on findings from the prospective cohort study, EarlyBird. Here approximately 300 children were followed annually from 7 – 12 years. FM %, serum leptin concentrations and insulin resistance were used to test whether indices of fatness that had been fully adjusted for height were better markers of adiposity and the related metabolic disturbances compared to standard indices ($FM/height^2$). The results showed that standard FMI with height raised to the power of 2 were better associated with FM %, leptin and insulin resistance in both boys and girls compared with height-independent FM. The results from this study suggest a biologic relevance for height velocity in childhood with regards to adiposity risk (138) and relates to the discussion in section 1.3.3.

Another very common way to express whole-body fatness is to express FM as a proportion of weight (FM %). However, this measure for adiposity is criticized for being influenced by the relative amount of FFM (160). Wells and Cole (160) argue, that the expression of fatness as FM % will underestimate the absolute amount of gained FM in obese subjects since they have increased amounts of both FFM and FM. At the same time, the focus of weight loss intervention is successful reduction of fatness without compromising development of FFM. This aspect is not clear when evaluating fatness according to FM % (160).

2 Methodology

2.1 Overview of the SKOT study

The SKOT study was a prospective cohort study monitoring 330 healthy young children from 9 – 36 months of age. The overall objective with the study was *"to contribute to the scientific basis for dietary and life style strategies, policies and dietary guidelines to infants and young children in Denmark"* and with a special focus on prevention of obesity and diet related chronic diseases. The SKOT study was designed by Kim Fleischer Michaelsen, Christian Mølgaard, Anja Lykke Madsen and Lene Schack-Nielsen and all examinations took place at Department of Nutrition, Exercise and Sports, Frederiksberg, Denmark. Children were examined three times at age 9 months ± 2 weeks (in 2007 - 8), 18 months ± 4 weeks (2008 - 9) and 3 years ± 3 months (2009 - 10), respectively. The study protocol was approved by The Committee on Biomedical Research Ethics for the Capital Region of Denmark (H-KF-2007-0003).

The in-depth monitoring of growth and development in this important period in early childhood linked with detailed information of early feeding practice and family background is not only useful in relation to the objective of this study. It has great value as a basis of comparison with other studies. Recently, the SKOT study has got a sister study, SKOT II, which has exactly the same structure as SKOT but only includes children born by obese mothers.

2.2 Recruitment and maintenance

In 2007, 2 211 families from the Copenhagen were randomly selected through the National Civil Registry area and invited to participate in the study by letter. Eligible children for the study were healthy singletons born at term (week 37- 43) between August 2006 and September 2007 with no disease that could influence food intake or growth. Interested families were invited to an individual meeting at the Department of Nutrition, Exercise and Sports, Frederiksberg, Denmark. A total of 330 children (15 %) were included in the study, 312 (94 %) completed the 9 months examination, 291 (88 %) completed the 18 months examination and 263 (80 %) completed the 3-year examination (**Figure 9**). As an attempt to maintain the families in this comprehensive study, children received small gifts at each examination and received a birth day card at their 1st birthday.

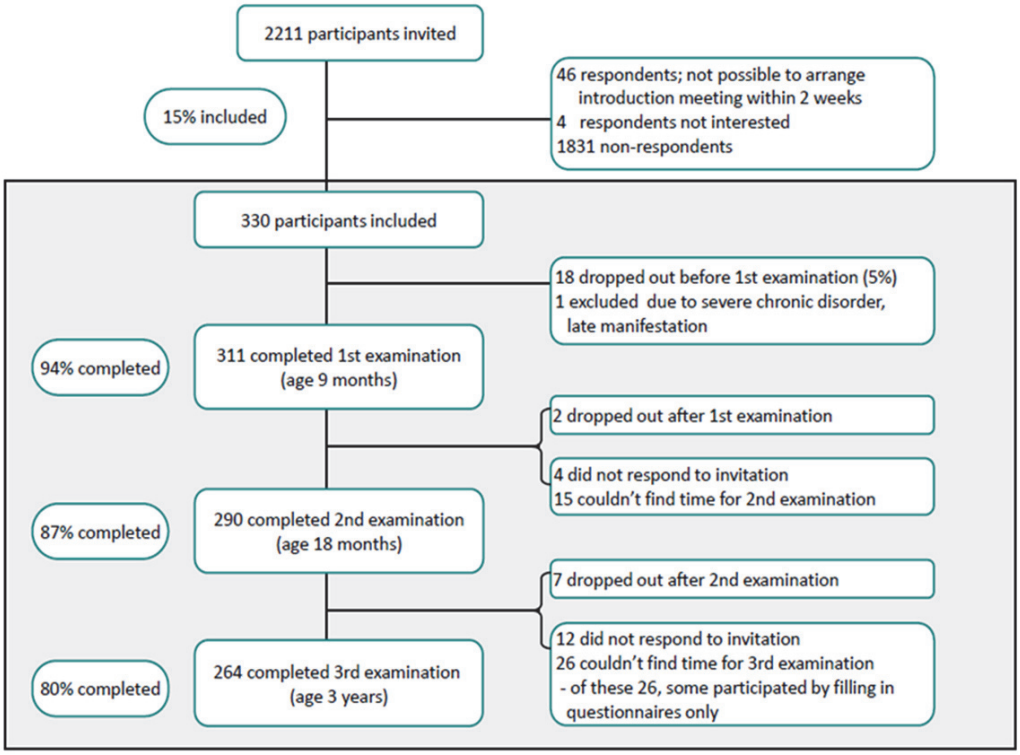

Figure 9. Participation and drop-out in the SKOT study (made by Line Brinch Christensen)

2.3 Examination details

A schematic overview of each of the three examination rounds can be seen in **Table 1.** Besides physical examinations of anthropometry and blood pressure at each examination, parents were asked to register the child's dietary intake for 7 days using validated pre-coded diet records (161). We got background information of infant feeding, motoric development, sleep patterns, household income, educational level etc. through interview and written questionnaires. At 9 and 36 months blood samples were taken for analyses of markers for metabolic syndrome, vitamin and mineral status, fatty acid composition in red blood cells and genotype. The 3-year examination was extended with BIA, psychomotor questionnaire 'Ages & Stages Questionnaires®' (ASQ-3™; Squires & Bricker 2009) developed for 36 months-old-children, a 7-day physical activity monitoring by the accelerometer Actigraph GT3X and a DXA scan.

Table 1. A schematic overview of the study

Age (months)		**9**	**18**	**36**
Recruitment/information meeting	X			
Examinations		X	X	X
Background interview		X	X	X
Anthropometry		X	X	X
Blood pressure		X	X	X
Faeces- and urine sample		X	X	X
Blood sample		X		X
Bio impedance analysis				X
7-day diet records		X	X	X
Questionnaire – general		X	X	X
Questionnaire – psykomotor		X	X	X
7-day physical activity monitoring				X
DXA scan				X

Additional information of birth weight and length and early growth was obtained from clinical charts (in Danish, "Barnets bog"). This information was registered along with the date of examination. At the 3-year examination the accompanying parent were measured for height and weight but otherwise this information was extracted from self-reported questionnaires. Information of the mother being pregnant was taken into account when using information on weight. Details of educational level were obtained by a structured interview at each examination and the information was merged to the longest obtained education at 3 years.

2.4 Anthropometry

Weight, length/height, recumbent length/sitting height, circumferences of arm, head and waist, and triceps and subscapularis skinfold thickness were measured at each examination. Children were barefooted and wore light clothing. Except for weight, all measurements were performed in triplicates, with the mean value used in analysis. This was done to minimise measurement errors.

At 9 months of age, naked body weight was measured to the nearest gram on a digital pediatric scale (Sartorius IP 65, Sartorius AG, Goettingen, Germany), and at 18 months on a digital scale (Lindeltronic 8000, Samhall Lavi AB, Kristianstad, Sweden). Recumbent length at both 9 and 18 months was measured to the nearest 0.5 mm on a digital measuring board (Force Technology, Brøndby, Denmark). At 3 years of age, weight was measured to the nearest 0.1 kg on a digital scale (Tanita WB-100MA, Tanita Corporation, Tokyo, Japan), while standing height was measured to the nearest 0.01 cm with head placed in Frankfurt plane by a wall-mounted digital stadiometer (235 Heightronic Digital Stadiometer, QuickMedical, Washington, United States). Mid-upper-arm circumference was measured on the non-dominant arm with a non-elastic band (Lasso-o, Child Growth Foundation, United Kingdom) at the mid-point between the olecranon process of the ulna and acromion process on the shoulder blade. The same mid-point point was used for measuring the triceps skinfold thickness while the subscapular skinfold was measured 2 cm below the inferior an-

gle of the scapula (the lower left shoulder blade). Both skinfolds were measured to the nearest 0.1 mm using a Harpenden skinfold caliper (Chasmors Ltd, London, United Kingdom). Standing waist circumference was measured horizontally at the navel to the nearest 0.1 mm with a non-elastic band (Lasso-o, Child Growth Foundation, United Kingdom). The same tape measure was used to measure head circumference to the nearest mm with the child's head in Frankfurt plane, just above the supraorbital ridges and over part of occiput hereby measuring the larges circumference. The measurements were conducted by well-trained observers following standardized procedures.

2.5 Bioelectrical impedance

At the 3-year examination, whole body resistance, reactance and impedance were measured using a single frequency (50 kHz) tetrapolar BIA Quantum III (RJL Systems, Michigan, USA) between right hand and right foot. Due to the blood sample, the child had been fasting approximately 2 hours prior to the examination. No restrictions were given with regards to physical activity and no request for bladder voiding was given before measurement. These factors might influence the hydration level but with the age of the participants and the burden for the parents in mind these factors were not prioritized.

During measurement the child was lying relaxed on an examination couch in light clothing, without metal or persons touching the skin. The signal electrode (LMP3 Diagnostic Tab Electrodes, Kendall, Covidien, Mansfield, USA) on the foot was placed over the distal portion of the second metatarsal (the base of the second toe). The signal electrode on the hand was placed above the metacarpophalangeal joint of the middle finger and not wrapped around the middle finger (proximal phalanx) as specified by the manufacturer, because the hands of the children were too small for this placement. The detecting electrode on the foot was placed at the anterior ankle on an imaginary line bisecting the medial malleolus and the detecting electrode on the hand was placed on an imaginary line bisecting the ulnar head as specified by the manufacturer. The procedure was performed twice with approximately 60 s between. Electrodes were not replaced. Mean values of resistance was used in the analyses.

2.6 Dual-energy X-ray absorptiometry

Whole body DXA scans were performed with a Lunar Prodigy Advance (GE Healthcare, Madison, WI, USA) using the software enCore, version 12.30. We did not require that the children had been fasting prior to the DXA scan but the parents were asked to take the child to the toilet before the scan if the child needed to empty the bladder. The children were scanned lying supine in light clothing without metal and with no or dry nappy. It was a challenge to keep the children lying still for 5 minutes during the scanning process. The quality of the scans varied with several not being suitable for use. All scans were subsequently assessed by one person to ensure consistency. This person manually went through all scans to see if the regions defined by the software were correct. The cut lines were adjusted if there were disagreement between the placement of the child and the software's definition of body regions.

DXA scans were divided into four categories:

1) Perfect scans: The child is lying still and it was possible to define the regions correct. The upper body is at a strait or almost strait line and arms and legs are clearly separated from the body.
2) Good scans with minor irregularities: The child might have had some movements but these were not considered to influence the output of the scan. No parts were considered missing or scanned more than once.
3) Scans with several irregularities: For these scans it could be difficult to assess whether some body parts were missing or scanned several times due to movements or misplacement of bodily parts.
4) Useless scans: Misplacement, considerable movements or parts of the body was missing.

2.7 Infant feeding practice

Information on duration of full and partial breastfeeding, age of introduction to complementary foods as well as type of complementary feeding was obtained by interviews at each examination. Full breastfeeding was defined as receiving only breast milk, water and vitamins but allowed exceptional bottle feeding e.g. if the child have been babysit for a single night (this changed the length of exclusive breastfeeding for 15 infants). Age of introduction to solids was registered as the earliest age in months at which the infant first received one of 19 food categories. Intensity of breastfeeding at 9 months defined as the number of meals per day was recorded as "no more", "0 - 2 times per day", "3 - 5 times per day" and "more than 6 times day" as in Madsen *et al.* (123).

2.8 Diet records

At all three examinations, children's diet were recorded for 7-days (minimum 4 consecutive days) by parents and caregivers in a pre-coded dietary questionnaire. The method has been validated against weighed dietary registration and double marked water in 9- and 36-months-old children (161). The parents were carefully introduced to the procedure at the examination – or as for the 9 months examination at the information meeting prior to the 9 months examination. Further, there were written instructions at the first page of each dietary record.

Each day the parents should register everything the child ate and drank on that particular day. Portion sizes were estimated by the parents and reported in household measures or according to a picture booklet with 12 picture series showing 4 - 6 different portion sizes. If the parent was not able to find a suitable pre-coded field, open fields were available for the parents to fill in. If the child attended day-care at the time for the dietary registrations, the day-care were asked to describe as detailed as possible what the child had been eaten on a separate sheet. Portion sizes should to be estimated in household measures. The parents were then to transfer these registrations to the pre-coded records.

The dietary records were sent to the National Food Institute, DTU and scanned for electronical preparation. The National Food Institute was responsible for processing of data, quality control and assessment of the nutritional content. The assessment of the nutritional content was produced by the software programme GIES (version 1.000 d, National Food Institute, Søborg, Denmark) based on data from the Danish Food Composition databank (version 7, National Food Institute, http://www.foodcomp.dk). Estimations of daily intake was based on the mean intake for the number of registered days and calculated at the individual basis. We received data on estimated intake per food group in grams, energy, micronutrients, and macronutrients. We also received data of energy and protein intake in different levels of food group details (e.g. a top-level food group "milk and dairy products" including estimated intake from recipes; a medium level food group "milk" and a detailed level "skimmed milk, low-fat milk or whole milk").

2.9 Blood samples

Blood samples were taken at the 9 months and 3 year examinations by medical laboratory technicians with experience in taking blood samples from infants and young children. Ahead of the examination parents had applied EMLA patches (AstraZeneca AB, Södertälje, Sweden) on the crook of the elbow which provided local anaesthesia of the skin. The EMLA patch was removed approximately 30 minutes before the blood sampling. Parents were instructed to let their child fast 1 ½ hour prior to the examination. This corresponded to approximately 2 hours at the time for the blood sampling. The content and time of last meal before fasting was recorded and analysed using Dankost (Dankost version 3000, Dankost Ltd, Copenhagen, Denmark).

A venous blood sample (6 ml) was collected in two test tube containing lithium-heparin and one EDTA vacuum tube and subsequently put on ice, centrifuged and plasma were stored at – 80 degrees Celsius. Plasma IGF-I and IGFBP-3 concentrations were determined by an automated, enzyme-labelled chemiluminescent immunometric assay performed on IMMULITE 1000 (Siemens Medical Solutions Diagnostics, Los Angeles, United States). The intra-assay variations for IGF-I and IGFBP-3 were 2.1 % and 3.1 %, respectively, while inter-assay variations were 7.2 % and 4.3 %, respectively. The IMMULITE system had a detection level of IGF-I at 25 ng/ml. At 9 months, 20 children had a value below this level and one child had a value below this level at 3 years. Samples with IGF-I values below the detection level were recoded with a value of 12.0 ng/ml.

2.10 Data processing and statistics

All data were entered twice by two different persons in Epidata. The two files were compared and discrepancies between the two versions were checked in a master file. From Epidata the master file was exported to STATA and processed to include label names, label codes and missing codes. A quality check of odd values and incoherent responses in the questionnaires was made for all files.

All statistics used in the articles are carried out using STATA version 11.0 (StataCorp LP, Texas, USA). The statistical tests for each individual paper are described in details in the statistical sections in the papers and will not be reproduced here.

3 Results and discussion

The following results and discussion section includes a short descriptive presentation of results from the 3-year examination in the SKOT cohort relevant to this thesis. Further, the most important results from the papers will be emphasized and discussed in relation to the literature. Results from earlier examinations will be included where relevant.

3.1 General results from the SKOT study

Out of the 2211 families invited to participate in the SKOT cohort, 330 families (15 %) accepted and were enrolled in the study. As described in the method section 2.2, 263 (80 %) completed the 3-year examination. The families who were enrolled in the study can be characterised by being highly motivated to participate. In general, the educational level was high compared to the general population. In total, 41% of the mothers and 43% of the fathers had a long academic education (> 4 years) compared with 7 % in the general population (162). Median family income was 649.999-700.000 DKK per year which is slightly higher than yearly income among families with children in the general population (614.000 DKK per year) (163). Thirty-four per cent of the families had a household income above 800.000 DKK per year. The characteristics of the participants are important since it is likely that the families, who chose to participate, share certain traits and in several ways not will be representative of the population in general. It is possible that the relative homogeneity of the SKOT cohort means that some of the extremes in dietary practices in infancy and at later ages as well as other behaviours are not represented. A report from 2009 showed that while the share of children who were still fully breastfed at 6 months was dependent on socioeconomic status (defined by occupation and job function) and province, it was not related to the parents educational level or household income (164). Unfortunately, information on socioeconomic status was not analysed in time for this thesis.

As seen in Figure 9 (Section 2.2) the drop-out rate was low among the participants when first included in the study. This is a great value for the quality of the study and is partly due to the well-resourced families and the care taken by the project staff to keep the participants in the study.

3.2 Characteristics of the study population

Anthropometric characteristics expressed as age- and gender-specific SDS from birth to 3 years are shown in **Table 2**. The weight-for-age SDS (WAZ) were above the WHO standards at all ages. Disregarding weight-for-length SDS (WFH) and BAZ at birth and 5 months, these values were also above WHO standards at 9, 18 and 36 months. The children in the SKOT cohort had considerably higher length-for-age SDS (HAZ) at birth and 5 months compared to the WHO growth standards. This resulted in significantly lower BAZ and WFH compared to WHO at birth and 5 months of age, while BAZ and WFH were higher than the WHO growth standards at 9, 18 and 36 months (Table 2). Length measures at birth and 5 months were obtained by general practitioners and health nurses using measuring tape and several studies have demonstrated that these measures often are overesti-

mated (165-167). Since the accuracy of the length measures at birth and 5 month was questionable, we chose to focus on early weight gain instead of BMI or WFH when birth and 5 months was included in the analyses. There is a risk of weight bias at 5 months due to measurement errors. Some children had several weight measures around 5 months of age sometimes revealing large with-in subject differences ± 500 g. The reason why some children had several measures at this age is unknown but is likely due to a visit from the health visitor at the same time as the 5 month immunization visit at the doctor. To be consistent, the weight measured closest to 152 days of age was chosen.

A part of the explanation that many of the anthropometric measures are above the WHO standards is that the cohort includes infants that have only been breastfed for a shorter period while the WHO growth standards are developed in a population predominately breastfed the first 4 - 6 months. We have previously shown that infants that were breastfed at 9 months had WAZ and BAZ values closer to the median of the WHO growth standards at 9 and 18 months. Further, they had lower WAZ and BAZ values compared with children that were no longer breastfed at 9 months at both 9 and 18 months of age (123). However, at 3 years duration of full or partial breastfeeding was not associated with BMI, FMI or FFMI.

Table 2. Anthropometric characteristics in the SKOT cohort according to WHO z-scores.

	Mean	Range [min - max]	P-value
At birth (n = 262)[1]			
WAZ	0.52	-1.98 – 2.71	< 0.001
HAZ	1.44	-3.84 – 4.29	< 0.001
WFH	-0.96	-4.71 – 2.54	< 0.001
BAZ	-0.34	-3.99 – 2.63	< 0.001
At 5 months (n = 184)[2]			
WAZ	0.39	-2.00 – 3.29	< 0.001
HAZ	1.31	-1.12 – 4.21	< 0.001
WFH	-0.38	-2.69 – 2.89	< 0.001
BAZ	-0.46	-2.85 – 2.88	< 0.001
At 9 months (n = 262)			
WAZ	0.41	-1.62 – 3.00	< 0.001
HAZ	0.29	-1.94 – 2.84	< 0.001
WFH	0.42	-1.52 – 4.21	< 0.001
BAZ	0.33	-1.75 – 4.37	< 0.001
At 18 months (n = 253)			
WAZ	0.43	-2.02 – 2.58	< 0.001
HAZ	0.15	-2.91 – 3.20	0.013
WFH	0.49	- 1.86 – 3.00	< 0.001
BAZ	0.48	-1.69 – 3.14	< 0.001
At 36 months (n = 262)			
WAZ	0.22	-2.06 – 2.04	< 0.001
HAZ	-0.00	-2.09 – 2.46	0.97
WFH	0.30	-1.76 – 2.36	< 0.001
BAZ	0.29	-1.78 – 2.44	< 0.001

[1] Measured by midwifes at the hospital. [2] Measured by general medical practitioner. Data presented as mean [minimum - maximum]. Differences between WAZ, HAZ, WFH and BAZ in the study sample and the WHO standards were evaluated by means of one-sample *t*-tests. Only children who completed the 3-year examination are included. BAZ, BMI-for-age SDS; HAZ, height/length-for-age SDS; WAZ, weight-for-age SDS; WFH, weight-for-height/length SDS.

3.3 Gender differences in body composition and IGF-I at 3 years

For the SKOT children, there were no differences in BMI and waist circumference between boys and girls at 3 years, while highly significant differences were seen between genders for means of weight, height, triceps and subscapularis skinfolds, FM, FFM, FMI and FFMI (**Table 3**). Boys had more muscle mass and bone mass compared to girls, while girls had thicker skinfolds and higher FM. Girls had considerably higher IGF-I, IGFBP-3 and molar ratio of IGF-I/IGFBP-3 than boys (all, $p < 0.001$).

Table 3. Anthropometry, body composition and IGF-I at 3 years according to gender

	n	Girls	n	Boys	p-value
Anthropometry					
Weight (kg)	130	14.33 (1.50)	132	14.95 (1.50)	0.001
Height (cm)	130	95.0 (3.2)	132	96.6 (3.34)	<0.001
BMI (kg/m^2)	130	15.9 (1.2)	132	16.0 (1.1)	0.42
Waist circumference	126	50.2 (2.8)	123	50.4 (2.7)	0.67
Triceps skinfolds (mm)	122	9.4 [8.3;11.1]	119	9.2 [7.8;10.2]	0.011
Subscapularis skinfolds (mm)	125	6.6 [5.9;8.1]	120	6.0 [5.4;6.7]	<0.001
Body composition					
FM_{DXA} (kg)	49	2.74 (0.77)	50	2.30 (0.66)	0.003
FFM_{DXA} (kg)	49	11.60 (0.85)	50	12.43 (1.14)	<0.001
BMC (g)	49	428.9 (49.7)	50	452.6 (52.0)	0.02
FM_{cal} (kg)	125	2.71 (0.74)	116	2.28 (0.64)	<0.001
FFM_{pred} (kg)	125	11.64 (0.90)	116	12.67 (1.00)	<0.001
FMI (kg/m^2)	125	3.01 (0.79)	116	2.45 (0.65)	<0.001
FFMI (kg/m^2)	125	12.95 (0.58)	116	13.60 (0.62)	<0.001
IGF-I variables					
IGF-I (ng/ml)	115	85.3 [61.0;108.0]	114	59.8 [44.2;81.9]	<0.001
IGFPB-3 (µg/ml)	115	3.3 [2.8;3.8]	114	2.9 [2.6;3.4]	<0.001
IGF-I/IGFBP-3 molar ratio	115	0.10 [0.08;0.13]	114	0.08 [0.07;0.11]	<0.001

Data shown as mean (SD) or median [25th;75th percentile]. Compared by unpaired *t*-test or Wilcoxon rank sum (Mann-Whitney). BMC; Bone Mineral Content; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat-mass index. IGF-I, insulin-like growth factor-1; IGFPB-3, insulin-like growth factor binding protein-3. The IGF-I/IGFBP-3 molar ratio was calculated using the following equivalents for conversion (113): 1 ng/ml IGF-I = 0.133 nM IGF-I and 1 µg/ml IGFBP-3 = 33 nM IGFBP-3. FM_{DXA}, BMC and lean tissue mass was measured by DXA in a subsample of the children. FFM_{DXA} was calculated as lean tissue mass + BMC. FFM_{pred} and FM_{cal} was predicted from paper I. FMI and FFMI is derived from FM_{cal} and FFM_{pred}.

3.4 Infant feeding practice in the SKOT cohort

Information of breastfeeding duration and age of introduction to solids for the SKOT cohort has been published previously (123;168;169). As written in paper II, 68 % of the children were fully breastfed at 4 months while 43 % were partially breastfed at 9 months. **Figure 10** shows the gradual decrease in full and partial breastfeeding according to age (modified according to Madsen *et al*. (170)). Most children in the SKOT study were introduced to solids at 4 months of age or later. Five children (2 %) were introduced to solids earlier than 4 months, 133 (57 %) at 4 months, 63 (27 %) at 5 months and 32 children (14 %) at 6 months. Large differences in age of introduction to complementary foods are seen between countries (88) with a sizeable number being introduced to complementary feeding before the recommended age at 6 months and even before 4 months of age (87-90).

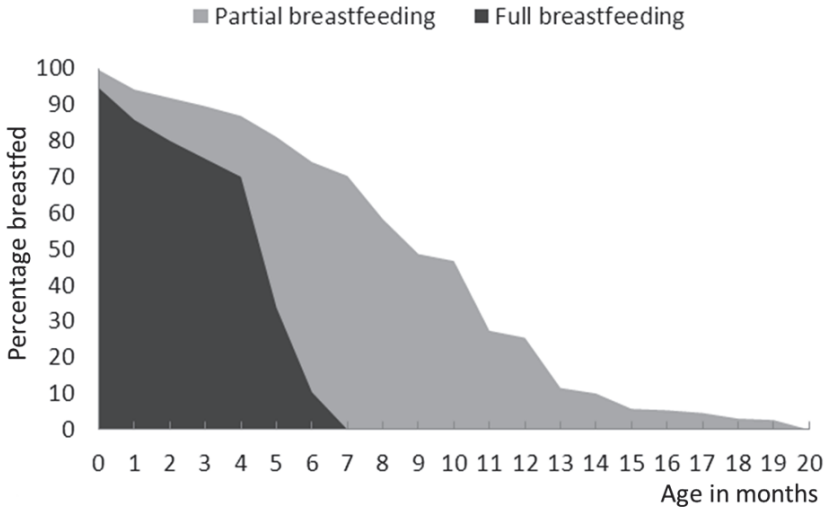

Figure 10. Duration of full and partial breastfeeding drawn as percentage breastfed infants from 0 – 20 months of age.

The duration of full breastfeeding and age of introduction to solids are by nature interdependent and it is not possible to disentangle from this observational study which factor is most influential on body composition at 3 years of age. As also seen in many other studies (87;88;90;91), in the SKOT cohort early termination of breastfeeding was associated with early introduction to solids ($p < 0.001$) (169). Moreover, differences were seen in the dietary registrations at 9 months according to if the child was partially breastfed or not. At 9 months, non-breastfed infants in this cohort had higher intakes of protein and lower intakes of fat in the complementary diet compared to partially breastfed infants (169). Thus, diet in infancy is correlated with patterns of food intake later which should be bared in mind when analysing possible relations between infant feeding and later body composition.

3.5 Main results and discussion - paper I

At the 3-year examination in the SKOT study, 189 children were DXA scanned. Out of these 101 (53%) was of good quality (category 1 or 2). The main result from paper I was the generation of two predictive equations for FFM derived from two multiple linear regression models, a comprehensive model (height^2/resistance (RI), six anthropometric measurements) and a simple model (RI, height, weight) with DXA as reference method. The prediction error of FFM_{pred} was 3.0 % for both equations (root mean square error (RMSE): 360 and 356 g, respectively). We chose to recommend the simple prediction equation since many large epidemiological studies do not have information on skinfolds and because skinfold measurements can be highly dependent on the examiner. From the simple predictive equation FFM_{pred} and FM_{cal} could be calculated:

$$FFM_{pred} = 327.2\ RI + 223.8\ weight + 76.8\ height + 417.6\ sex - 2784.4$$
$$FM_{cal} = (0.981\ weight + 0.374)* 1000 - FFM$$

FFM_{pred} and FM_{cal} are both in g, height is measured in cm, weight is the digital weight in kg and female was coded 0 and male 1.

The values of RMSE for FM_{cal} were 264.3 g in the full model and 303.4 g in the simple model. Due to the lower FM-total body weight ratio prediction errors for FM_{cal} were 10.5 % in the full model and 12.0 % in the simple model. This is an important point since many studies use the predicted values to calculate FM and estimate the degree of adiposity. The agreement between FFM_{pred}, FM_{cal} and DXA values was high on population level but the relatively wide 95 % limits of agreement for both FFM (LOA: - 852 g to 856 g) and FM (LOA: - 724 g to 725 g) indicated some predictive uncertainty at the individual level.

Five predictive equations for TBW or FFM are available for children in early childhood (2 – 4 years) (154-157;171) and application of these five predictive equations on the SKOT children revealed great variation in FFM predictions and inconsistency with FFM_{DXA}. The predictive equation by Rush *et al.* (171) was based on 77 DXA scans in 2-year old children born by mothers treated for gestational diabetes and provided good predictions for FFM compared with FFM_{DXA} for the children in the SKOT cohort. Thus, we were able to confirm the applicability of this predictive equation in this cohort of healthy 3-year-old Danish children. Since the predictive equation generated in paper I is based on a cohort characterized by healthy children and parents, we find that the predictive equations are relevant and contribute with extra knowledge of how body composition can be predicted in young children.

As shown in paper I, prediction equations of FFM that are made with DXA as reference method are at risk of miscalculating FM (digital weight - FFM) due to differences between DXA weight and digital weight. We chose to create an adjusted weight variable in the generation of the predictive equations since there was a deviation between DXA weight and measured weight at around 100 g. Although this difference do not sound alarming, a deviation of 100 - 200 grams is more influential in children since their total body weight is lower and the relative error is therefore higher compared to adults. Since FFM_{pred} would be calculated to be consistent with FFM_{DXA}, the difference between the digital weight and DXA weight would be transferred to FM_{cal}. In this group of 3-year-old children, FM consitutted approximately 2.5 kg and thus an error of 100 g would add an extra 4 % of uncertainty to the estimate.

An analysis of the potential bias for the predictions (magnitude of bias) showed a discrepancy between FFM_{pred} and FM_{cal} compared to DXA values. FFM_{pred} was under-predicted in leaner children and over-predicted among children with higher FM_{DXA} (magnitude of bias in the simple model: $\beta = 0.16$ g (0.06), $p = 0.004$). Conversely, FM_{cal} was over-predicted among the leaner children and under-predicted among children with higher FM_{DXA} (simple model: $\beta = -0.18$ g (0.05), $p < 0.001$). Likewise, the magnitude of bias of FFM_{pred} increased with increasing FM_{DXA} for prediction equations by Fjeld *et al.* (155), Kushner *et al.* (156) and Rush *et al.* (158). However, an analysis of the potential bias of FM assessed by DXA compared to the four-compartment model in 9- to 14-year-old children showed that DXA under-predicted FM in leaner subjects and over-predicted FM in more obese subjects (172). Thus, albeit being a valued technique for measuring body composition, DXA has its own limitations (159;173). Also inconsistency has been seen when using different DXA maschines or software (159).

An alternative reference method suitable for this age group could have been determination of total body water using deuterium dilution with subsequent application of age and gender specific hydration factors. However, this method also relies on the assumption of a constant hydration of FFM which varies according to growth, maturity, aging, ethnicity, disease, and obesity (145;149;153). Total body water measured by the deuterium dilution technique has also been shown to overestimate FM in children compared to the four-compartment model (172). Thus, for this age group we find that DXA is an appropriate non-invasive method with an extremely low radiation dose (159).

The rapid maturation of body tissues during childhood leads to alterations in the hydration level of FFM (153). Age-specific regression equations of FFM against RI resulted in lower prediction errors within a group of children and young adults aged 7 to 25 years (174). Children aged 7 to 9 years formed one group because they had similar RI-values. During this period the hydration of FFM changes with 0.5 % for boys and 0.2% for girls (153) while it changes about 2.3 - 2.9 % from age 7 years to adult hydration (75.3 - 75.9 % in 7-year-olds to 73.0 % in chemically mature adults). The predictive equation by Rush *et al.* (171) generated from DXA scans of 2-year-old children were applicable in the prediction of FFM in this group of 3-year-old children. This indicates that the equations are able to cover this age span despite age-dependent differences in the hydration level. We find it likely that the applicable age for the predictive equations generated in paper I is from 2 - 4 years of age. During this period the hydration level of FFM changes with approximately 1.1 % in boys and 0.5 % in girls (153). The generated equations can prove useful for population studies linking early risk factors to body composition and early onset of obesity. However, researchers should be aware that the relative error is greater when using the equations to calculate FM than when calculating FFM.

3.6 Main results and discussion - paper II

As described in the background section 1.2, early nutrition influence early growth, and early growth is highly related to later risk of overweight. A possible link between early growth, early feeding, and body composition at 3 years of age was examined in paper II. The focus here is on two main results: how early weight gain was related to body composition at 3 years – and how some of these relations were modified by duration of full breastfeeding.

We calculated FMI (FM (kg)/height (m)^n) and FFMI (FFM (kg)/height (m)^n) to achieve height independent indices of FM and FFM. The value of *n* was estimated from by log-log regressions of FM and FFM on height and it was found to be 2.4 for FMI and 1.9 for FFMI. However, $n = 2$ was in the confidence interval in both regressions and we chose this value for simplicity and to ease interpretation of results. Linear regression confirmed that FMI and FFMI generated as FM/height^2 and FFM/height^2 were no longer associated with height ($p > 0.5$). In the absence of well-established reference data for FM or FMI to identify cut-off values for overweight in young children, in some analyses FMI and FFMI was grouped according to gender-specific quartiles.

A simple display of the relation between birth weight, early growth and later body composition is shown in **Table 4**. Here the probability of being placed in the highest quartile of FMI and FFMI according to birth weight and early weight gain is shown. A birth weight above 4000 g increased the probability for being in the highest quartile of both FMI and FFMI at 3 years with approximately 50 % compared to children with birth weight from 3000 – 3500 g (Table 4). Other studies examining the relation between birth weight and later body composition support a stronger association with FFM than FM (23;24). Especially low birth weight could be speculated to program a smaller proportion of FFM later in life (23). A reason why we find birth weight equally related to FFM and FM could be that the children from the SKOT cohort were well nourished and only two were born with birth weight below 2500 grams. Furthermore, most of the studies reviewed by Wells *et al.* (24) have focussed on body composition in older children or adults. Interestingly, rapid weight gain from 0 - 5 months was not predictive of being in the highest FFMI quartile at 3 years in the SKOT cohort. Following the speculations by Wells *et al.* (24) (section 1.1.1) it is possible that the children in the SKOT cohort had reached their genetic potential for lean mass accumulation *in utero* whereby excessive energy intake in early infancy was rather stored as fat. Rapid weight gain above 0.67 z-scores from 0 – 5 months increased the probability of being in the highest quartile of FMI compared to the other groups with a change in WAZ from 0 - 5 months below 0.67 (Table 4). Other studies have also found rapid early weight gain associated with FM rather than FFM (6;27;31;32;61;175) while several studies from non-Western countries have found infant weight gain rather associated with later FFM (24).

Table 4: Prevalence of children in the 4th quartile of FMI and FFMI according to birth weight and change in WAZ from 0 - 5 months.

	n	n placed in 4th quartile of FMI (%)	P[1]	n placed in 4th quartile of FFMI (%)	P[1]
Birth weight (g)					
< 3000	23	5 (21.7)	0.167[2]	3 (13.0)	0.039[2]
3000 - 3499	92	16 (17.4)	0.007	16 (17.4)	0.007
3500 - 3999	83	23 (27.7)	0.189	25 (30.1)	0.297
≥ 4000	35	14 (40.0)	reference	14 (40.0)	reference
Δ WAZ, 0 - 5 months					
< -0.67,	49	7 (14.3)	0.001	10 (20.4)	0.472
-0.67- 0,	41	6 (14.6)	0.001	7 (17.1)	0.288
0- 0.67;	40	10 (25.0)	0.031	9 (22.5)	0.645
> 0.67	37	18 (48.7)	reference	10 (27.0)	reference

[1] Comparison of group difference against reference by Pearson X^2. [2] Comparison by Fischer's exact.
FMI, fat mass index (kg/m^2); FFMI, fat-free mass index (kg/m^2); Δ WAZ, change in weight-for-age SDS.

A more detailed analysis of the relation between change in WAZ during four time periods and body composition outcomes at 3 years of age is found in **Table 5.** The outcomes FM and FFM are not presented in paper II. The most important finding is that BWZ and change in WAZ from 0 - 5 months are both positively related to height, weight, BMI, FM, FMI, FFM and FFMI at 3 years of age. In addition to this, change in WAZ from 0 - 5 months was positively associated with sum of skinfolds ($p < 0.001$). Height, weight and FFM were also positively related to weight gain in other periods but FFMI as a measure of FFM uncorrelated to height was not related to weight gain after 5 months. No measures of adiposity (BMI, FM, FMI and skinfold thickness) were significantly associated to weight gain after 5 months. The associations persisted after control for parental height, weight or BMI, gestational weight gain, mother's educational level, household income, and smoking during pregnancy and the results indicate that birth weight and growth the first 5 months of life are strong predictors for body composition at 3 years of age.

It can be questioned if the fourth FMI quartile at 3 years is a good measure for later obesity risk. However, a high level of tracking of FM have been shown from 2 to 7 years (6) and 4 to 9 years (7) with higher increase among those who acquired a high fat percentage early (6;7). Therefore, we find it likely that FMI in 3-year-old children is a good predictor for later risk of obesity. A Danish representative survey of 5580 preschool children found a strong relation between overweight and obesity measured at 3 and 5 years of age (4). However, another proposed risk factor for later risk of obesity is an early adiposity rebound which can occur as early as 2 – 3 years (19). As described in section 1.1.3, some studies have shown lower BMI and greater height at 3 years in children with early adiposity rebound (39). With the 18 and 36 months measurements being within the age of very early adiposity rebound, a nadir in the BMI curve could easily be masked.

Table 5. Relation between weight gain during four time periods (Δ weight-for-age SDS) and body composition outcomes at 3 years of age expressed as fully adjusted regression coefficients.

	Outcomes at 3 years										
Adjusted model		Height (cm)	Weight (kg)	BMI (kg/m^2)		FM (kg)	FMI (kg/m^2)	FFM (kg)	FFMI (kg/m^2)		∑ SF (mm)
	n	β (SE)	β (SE)	β (SE)	n	β (SE)	β (SE)	β (SE)	β (SE)	n	β (SE)
BWZ	215	0.68 (0.21)[c]	0.52 (0.10)[c]	0.35 (0.08)[c]	215	0.23 (0.05)[c]	0.21 (0.05)[c]	0.32 (0.07)[c]	0.13 (0.05)[b]	206	0.24 (0.25)
Δ WAZ, 0-5 mo	152	0.70 (0.28)[a]	0.78 (0.10)[c]	0.64 (0.09)[c]	152	0.42 (0.05)[c]	0.42 (0.06)[c]	0.36 (0.09)[c]	0.20 (0.06)[c]	147	1.43 (0.26)[c]
Δ WAZ, 5-9 mo	152	1.26 (0.46)[b]	0.56 (0.21)[c]	0.10 (0.17)	152	0.07 (0.10)	-0.02 (0.11)	0.53 (0.15)[c]	0.10 (0.10)	147	-0.30 (0.45)
Δ WAZ, 9-18 mo	211	0.94 (0.46)[a]	0.19 (0.21)	-0.10 (0.16)	211	-0.12 (0.10)	-0.18 (0.10)	0.31 (0.13)[a]	0.07 (0.08)	202	-0.47 (0.43)
Δ WAZ, 18-36 mo	211	1.09 (0.44)[a]	0.62 (0.21)[b]	0.22 (0.18)	211	0.18 (0.11)	0.10 (0.12)	0.50 (0.14)[c]	0.10 (0.10)	202	0.04 (0.41)

[a] $p < 0.05$, [b] $p \leq 0.01$, [c] $p \leq 0.001$.

Each cell represents a multiple regression model with body composition at 3 years as dependent variables and left row as explanatory variables. The model is adjusted for gender, BWZ, income, mother's educational level, smoking during pregnancy and gestational weight gain. Height was additionally adjusted for parental height. Weight was additionally adjusted for parental weight. BMI, FFM, FFMI, FM, FMI and sum of skinfolds were all additional adjusted for parental BMI. BWZ, birth weight SDS; FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index; ∑ SF, sum of triceps and subscapular skinfolds; WAZ, weight-for-age SDS.

3.6.1 Duration of full breastfeeding as effect modifier for birth weight and early weight gain

While the above-mentioned results are in line with many other studies as described in the background section 1.1.1and 1.1.2, few other studies have focused on how the effect of early growth on later body composition can be modified by early nutrition (68-70).

In paper II, we found that duration of full breastfeeding considerably modified the effect of both BWZ and early weight gain from 0 – 5 months (**Table 6**). The effect of BWZ on FMI was eliminated by full breastfeeding for 6 months compared with less than 1 month (from β = 0.69 (0.39 – 0.98) to β = - 0.07 (-0.55 – 0.40), p = 0.002) (**Figure 11A**). No effect modification was seen for duration of full breastfeeding less than 6 months. Duration of full breastfeeding for 4 – 5 months compared with less than 1 month attenuated the positive effect of change in WAZ from 0 - 5 months on FMI by 47 % (from β = 0.81 (0.46 – 1.17) to β = 0.43 (0.05 – 0.81), p = 0.05) while 6 months of full breastfeeding eliminated the effect of WAZ (β = -0.15 (-0.76 – 0.45), p = 0.002) (**Figure 11B**). A borderline effect modification on WAZ change from 0 – 5 month by full breastfeeding for 1 – 3 months compared less than 1 month was also seen (45 % reduction, β = 0.45 (0.04 – 0.85), p = 0.075). This indicates that besides the direct effect breastfeeding has on early growth (58;123;176), longer duration of full breastfeeding influence the extent to which early rapid weight gain adversely affects FM development. A similar protective effect of full breastfeeding for at least 4 months among infants with early rapid weight gain with respect to body fat percentage at 2 and 5 years of age have been demonstrated in 249 children from the DONALD study (70). For normal growing infants, breastfeeding for more than 4 months had no effect on the percentage of body fat at 2 years (70). Likewise, a small retrospective cohort study showed that in the group of children with a weight gain above 8.15 kg within the first 2 years of life, fewer children had become overweight at 7 - 8 years of age if they had been exclusively breastfed for 6 months and were introduced to solids late (68). Preliminary data from the PROBIT study has also shown that the strong relationship between early rapid weight gain and later obesity was higher among children who had been exclusively formula fed from 1 months of age compared to other infants (69).

The grouping of duration of breastfeeding was critical for the effect modifications in paper II. A less detailed categorization of breastfeeding with less than 90 days of full breastfeeding as reference revealed a significant interaction with age of introduction to solids and birth weight. With introduction to solids at 3 - 4 months as reference group, age of introduction to solids at 6 months reduced the effect of birth weight by 55% (p = 0.028, data not shown). Duration of full breastfeeding became less powerful for weight gain 0 – 5 months (full breastfeeding for 5 - 6 months compared to less than 3 months tended to reduce the effect of weight gain by 50 %, p = 0.095). The effect modifications of early feeding are likely to depend on the alternatives to breastfeeding which differs from country to country and we encourage other studies to investigate similar effect modifications of infant feeding on early growth to extend the generalizability to populations with other characteristics.

Since the SKOT study is observational, we cannot say if the interactions are explained by physiology, genetic factors influencing growth or rather decisions made by the mother or advices given by health care workers. It is possible that infants who grow more slowly are satisfied by continued breastfeeding and late introduction to solids, while rapid growing infants have higher energy de-

mands and increased appetite (73). This could easily lead some mothers to supplement with formula or begin the complementary feeding period early. Early introduction to solids has been associated with both low and high birth weight (67;86). It is a possibility that mothers to infants with low birth weight or slow postnatal weight gain are encouraged by their health nurse or surroundings to begin introduction to solids earlier to achieve better weight gain. At the same time, the timing of complementary foods have been shown to be dependent on maternal perception of the appetite signals from the infant (90) a possible explanation for early introduction to solids in big babies. Residual confounding might also be present if mothers to large babies continue breastfeeding because they have heard it protects against later overweight.

It is a limitation that only 21 infants were fully breastfed at 6 months. The interaction between birth weight and full breastfeeding was not seen for full breastfeeding for less than 6 months, and thus the conclusion is based only on these 21 children. Neither duration of full or partial breastfeeding were directly associated with BMI, FMI or FFMI at 3 years. This could be due to a low share of overweight individuals in our cohort, to insufficient power, or that the differences in FM at 3 years are too small compared with later ages. Breastfeeding have been shown to have no effect on mean BMI (50;177), perhaps since BMI in the lower end of the BMI scale has been found to increase with duration of breastfeeding (177).

Table 6: Multiple regression model of the association between birth weight, weight gain from 0-5 months, infant feeding and FMI at 3 years of age (n = 152).

Risk factor	Estimate ± SE	P
Intercept	2.42 ± 0.64	< 0.001
BWZ	0.69 ± 0.15	< 0.001
Δ WAZ 0 - 5 months	0.81 ± 0.18	< 0.001
Sex (0, female; 1, male)	-0.59 ± 0.09	< 0.001
Full breastfeeding		
< 1 months	reference	
1 - 3 months	-0.03 ± 0.20	0.881
4 - 5 months	-0.07 ± 0.15	0.661
6 months	-0.29 ± 0.31	0.339
Age of introduction		
3 - 4 months	reference	
5 months	0.14 ± 0.12	0.227
6 months	0.44 ± 0.28	0.122
Maternal BMI	0.00 ± 0.02	0.850
Gestational weight gain	-0.01 ± 0.01	0.337
Maternal educational level		
No education above school level	reference	
1 - 2 years	0.53 ± 0.35	0.130
Further education < 3 years	0.45 ± 0.32	0.154
Further education 3 - 4 years	0.35 ± 0.30	0.240
Further education > 4 years	0.40 ± 0.31	0.200
Smoking during pregnancy (0, No; 1, Yes)	0.07 ± 0.29	0.796
Paternal BMI	0.00 ± 0.02	0.881
Household income	0.00 ± 0.01	0.878
BWZ x full breastfeeding		
< 1 months	reference	
1-3 months	-0.09 ± 0.22	0.670
4-5 months	-0.17 ± 0.17	0.329
6 months	-0.76 ± 0.24	0.002
Δ WAZ 0 - 5 months x full breastfeeding		
< 1 months	reference	
1-3 months	-0.37 ± 0.20	0.075
4-5 months	-0.38 ± 0.19	0.050
6 months	-0.97 ± 0.30	0.002

A multiple regression model with FMI at 3 years as the dependent variable and variables listed in the left row as explanatory variables. The intercept represents the mean value of FMI at 3 years in the reference groups. BWZ, birth weight SDS; FMI, fat mass index; WAZ, weight-for-age SDS.

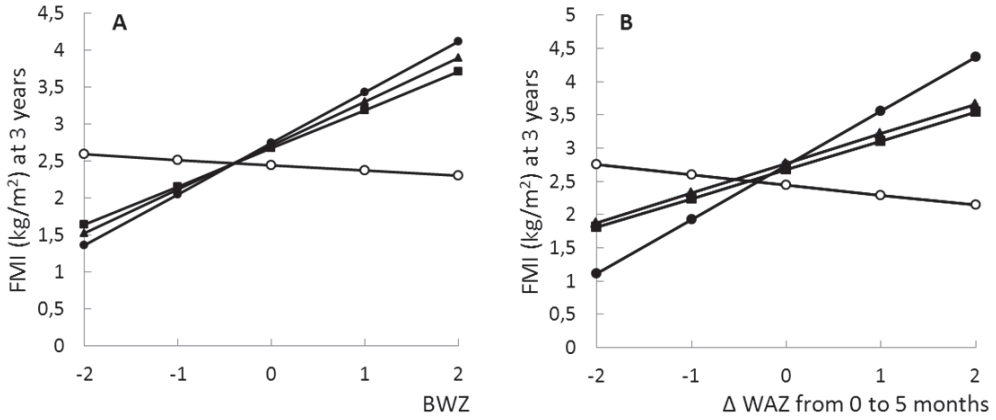

Figure 11. Predicted mean fat mass index (FMI, kg/m^2) at 3 years according to A, birth weight SDS (BWZ) and B, change in weight-for-age SDS (WAZ) from 0 - 5 months by subgroups of breastfeeding duration in 152 children in the SKOT cohort. Figure lines are drawn from the coefficients in question derived from the multiple regression model presented in Table 6. A, Illustrates the interaction between BWZ and duration of full breastfeeding. B, Illustrates the interaction between WAZ change from 0 – 5 months and duration of full breastfeeding. Duration of full breastfeeding < 1 months (●, n = 36), 1 - 3 months (▲, n = 39), 4 - 5 months (■, n = 137) and 6 month (○, n = 21).

Several studies have suggested an effect size of infant growth rate on later obesity risk of around 20 % (12-14). Results from the meta-analyses and systematic reviews of breastfeeding and risk of obesity estimated that breastfeeding resulted in a lower odds ratio of 7-22 % of becoming overweight (47;48) with a 4 % decrease in risk for each months of continued partial breastfeeding (49). These effect sizes are quite large when considering obesity as a multifactorial disease or condition. At the same time, the fact that only 1 in 5 of children with rapid weight gain from 0 – 24 months becomes overweight can be interpreted as possibility to intervene to overcome the effect of early rapid weight gain. Other factors related to a healthy lifestyle – a healthy diet with regular meals, limiting the ingestion of rapidly absorbed carbohydrates and simple sugars, exercise, less sedentary behaviour, are all plausible factors involved in reducing the risk of later obesity (83). The results from paper II suggest that prolonged breastfeeding can reduce the effect of very early rapid growth and prolonged exclusive breastfeeding could be particularly beneficial among rapid growers with regards to FM development. Of course it is not possible to say beforehand which infants will grow rapidly but with the many other beneficial effects of breastfeeding seen with breastfeeding in mind (44), normal growing infants achieve other benefits from being breastfed.

3.7 Main results and discussion - paper III

The results from paper III that will be emphasized here is the relation between total and free IGF-I at both 9 months and 3 years and anthropometry and body composition at 3 years. Moreover, the relation between IGF-I and diet will be discussed in relation to the literature.

There was a positive correlation between total and free IGF-I at 9 and 36 months (total IGF-I: rho = 0.49, $p < 0.001$; free IGF-I: rho = 0.39, $p < 0.001$) and values were considerably higher in girls (43% and 25%, respectively). Total IGF-I concentration at 9 months were positively associated with height, weight, BMI, FM and FFM at 36 months (**Table 7**). The only difference with free IGF-I at 9 months was that free IGF-I was not associated with BMI at 36 months. At 36 month, results for total and free IGF-I differed slightly from 9 months levels, especially by being positively associated with FFMI. Change in total and free IGF-I from 9 months to 3 years were positively related to FFM and FFMI while no association was found with FM (Table 7). This implies that the impact of IGF-I changes according to the timing of IGF-I increase with different effect on body composition if the rise in IGF-I occur after 9 months of age.

Children breastfed at 9 months had lower values at 9 months but reached the same IGF-I levels at 36 months as infants not breastfed at 9 months. Thus, the modulating effect of breastfeeding on IGF-I concentrations seen at 9 months (123) seemed to be transient. As we in this study found a positive correlation between total and free IGF-I at 9 and 36 months, it is possible that that the programming effect of the IGF-I axis (111;120) is not yet expressed at 36 months.

Opposite to what we had expected, intake of protein had limited influence on IGF-I concentrations at 36 months. No relation was seen for protein energy percentage (E %), meat and milk intake as otherwise found in the literature (106;107;112;113;115;126). It is possible that the variations in intake of milk (mean ± SD; 377 ± 141 ml) and meat intake (50 ± 23 g) in our cohort were too small to have an impact on IGF-I concentration but in a cross-sectional study of 2.5-year-old Danish children with significant correlations with animal protein, milk and IGF-I, intakes of meat and milk were close to the intake and variation seen in this study (112). For each increase in E % of fat and saturated fat IGF-I decreased by 1.6 % (-2.8 – -0.3 %, $p = 0.015$) and 3 % (-5.0 – -0.9 %, $p = 0.006$), respectively. Likewise, each increase in fat and saturated fat E % was associated with a 1.0 % (-1.9 – 0 %, $p = 0.046$) and 2.1 % (-3.6 – -0.5 %, $p = 0.009$) decrease in free IGF-I. It is possible that there is a link between this finding and the modulating effect of human milk on IGF-I since human milk also contains high amounts of fat and saturated fat (178). There is no evidence that fat intakes during infancy increase the risk of obesity in later life (179;180). A recent study even found intake of fat at 2 years negatively related to FM and subscapularis skinfold thickness at 20 years of age (78). These observations could suggest a role of fat and saturated fat in the regulation of IGF-I and a possible protective effect of high fat intake in early childhood on later obesity risk could be mediated through lower IGF-I levels. However, a possible relation could be complex since results from the DONALD study showed different effect of high fat intake in early childhood and change in adiposity from 2 - 5 years according to weight gain in infancy (70). A consistently high fat intake at 12 and 18 – 24 months reduced the physiologic decline in FM % from 2 – 5 years among children with rapid weight gain from 0 – 24 months of age, while a greater decrease in adiposity was seen among children without rapid weight gain if they had a high fat intake during the same period (70).

Thus, the relation between diet, IGF-I and risk of early development of obesity is very complex and needs to be investigated further.

There are some indications that IGF-I enhance subsequent linear growth and FFM rather than FM in infancy (106;118;123) and in prepubertal children (116;117;119). The present study showed no relation between IGF-I and FM % or FMI in this group of 3-years-old Danish children. This seems to question a role for IGF-I in the development of obesity. However, as discussed in section 1.3.3, the relation between early growth and later risk of obesity is not always simple since early accelerated growth including linear growth could be leading to accelerated maturity and early adiposity rebound (39;41). Our results indicate that IGF-I might have different effect on body composition according to the timing of the increase. It is possible that during early infancy when fat accumulation is high, high IGF-I concentrations will stimulate the differentiation of adipocytes, while high IGF-I concentrations during periods of length gain will rather stimulate longitudinal growth and protein synthesis. However, in disfavour of these speculations is the results from the meta-analysis by Gale *et al.* (59) showing lower FM in formula fed compared with breastfed from 3-6 months of age but higher FM at 12 months compared with breastfed infants. At the same time, FFM from 3-12 months was higher in the formula fed infants compared to breastfed infants. Since breastfeeding is associated with lower IGF-I concentrations (106;118;128), this finding seems to support a stimulating effect of IGF-I on lean mass and length gain in early infancy. As suggested by Ong *et al.* (118) it is possible that IGF-I stimulates the accumulation of lean mass and length gain in infancy and that insulin or another component stimulates FM. Findings from the 9 months examination in SKOT support a role for insulin in FM storage, since insulin concentration was positively related to weight gain from 5 - 9 months, waist circumference and borderline positively associated with subscapularis skinfolds (168). Further, there was a strong negative association between intensity of breastfeeding at 9 months and insulin concentration.

In favour of an association between IGF-I and later obesity risk is the positive correlation between overweight children and height until puberty (15;136;137) and that overweight children have normal or high IGF-I concentrations (139;140). Further, the findings that early adiposity rebound has been associated with lower BMI and greater height at 3 years in other studies (19;38;39) supports a potential role for IGF-I in the timing of the adiposity rebound through increased tempo of weight gain and linear growth. As mentioned in the discussion of paper II, two growth patterns are related to later adiposity risk, 1) early tracking of high BMI or FM from early childhood and 2) low BMI in early childhood followed by an early adiposity rebound (19). In paper II, we found that being in the highest quartile of FMI at 3 years was related to tracking of higher WAZ from early infancy onwards, rather that early adiposity rebound. On the basis of the results from paper III, we cannot rule out that early IGF-I levels can influence adiposity risk at later ages through accelerated growth leading to early adiposity rebound. Therefore it is highly relevant to continue the SKOT study with a follow up visit at 7 - 8 years of age. At this age most children have passed the BMI nadir and it should be possible to elucidate further on a role for IGF-I, early growth and the relation with later risk of overweight.

Table 7. Associations between IGF-I at 9 months and 3 years, anthropometry and body composition at 3 years of age[1].

		Model: 9 months[2]			Model: 3 years[2]			Model: Delta IGF-I[3]	
		IGF-I (ng/ml)	Free IGF-I[4]		IGF-I (ng/ml)	Free IGF-I[4]		IGF-I (ng/ml)	Free IGF-I[4]
Outcomes at 3 year	n	β (SE)	β (SE)	n	β (SE)	β (SE)	n	β (SE)	β (SE)
Height (cm)	231	0.054 (0.008)‡	39.37 (7.98)‡	229	0.035 (0.006)‡	28.45 (7.16) ‡	202	0.026 (0.007)†	23.55 (7.81)*
Weight (kg)	231	0.027 (0.004)‡	19.33 (3.96)‡	229	0.018 (0.003)‡	17.95 (2.99) ‡	202	0.013 (0.003)‡	14.46 (3.49)‡
BMI (kg/m^2)	231	0.011 (0.003)†	7.89 (3.11)[6]	229	0.008 (0.002) †	10.23 (2.55) †	202	0.005 (0.003)[6]	7.95 (2.81)*
∑ SF (mm)[5]	203	- 0.017 (0.011)	-12.25 (9.29)	212	-0.021 (0.005)†	-15.46 (6.02)[6]	185	-0.015 (0.006)[6]	-10.57 (6.23)
WC (cm)[5]	214	-0.003 (0.006)	-5.89 (5.44)	220	-0.004 (0.004)	0.29 (4.54)	193	-0.007 (0.005)	-0.62 (4.90)
FM %	207	0.025 (0.010)[6]	20.35 (10.80)	215	0.010 (0.007)	14.13 (8.22)	188	0.002 (0.010)	7.04 (9.78)
FM (kg)	207	0.008 (0.002)†	5.87 (2.06)*	215	0.005 (0.001) †	5.21 (1.55)†	188	0.003 (0.002)	3.65 (1.74)[6]
FFM (kg)	207	0.017 (0.003)‡	12.24 (2.71)‡	215	0.012 (0.002)‡	12.24 (1.94)‡	188	0.010 (0.002)‡	10.56 (2.16)‡
FMI (kg/m^2)	207	0.006 (0.002)[6]	4.14 (2.19)	215	0.003 (0.001)[6]	4.13 (1.68)[6]	188	0.001 (0.002)	2.67 (1.87)
FFMI (kg/m^2)	207	0.004 (0.002)[6]	2.26 (1.80)	215	0.004 (0.001)†	5.80 (1.34)‡	188	0.004 (0.001)*	5.40 (1.49)†

[1] p-values corrected for multiple comparisons (181). [2] Adjusted for gender and birth weight. [3] Delta IGF-I: Change in total and free IGF from 9 – 36 months. Adjusted for gender, birth weight and IGF-variable at 9 months. [4] Free IGF-I correspond to the IGF-I/IGFBP-3 molar ratio. [5] Further adjusted for weight at 3 years. [6] Significant without correction for multiple comparisons.

*, $p \leq 0.05$; †, $p \leq 0.01$; ‡, $p \leq 0.001$

Outcomes at 3 years as dependent variable, IGF-I variables as independent variables. FM %, percentage of body fat; FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index; ∑ SF, sum of subscapular and triceps skinfolds, WC, waist circumference.

3.8 Strengths and weaknesses

A strength of the SKOT study is the prospective study design with a large number of children having repeated standardized anthropometric measurements at 9, 18 and 36 months, blood samples taken at 9 months and 3 years, prospectively collected information on diet and breastfeeding, as well as detailed information on many possible covariates and confounders. The observational design of the SKOT study is useful to generate new ideas and research questions that can be further investigated through experimental approaches examining possible underlying mechanisms. The longitudinal design enables examination of how early exposures or blood values relates to outcomes later and hypotheses can be tested with adjustment for many of the known confounders. However, since traits are often correlated we cannot separate cause from effect and thus, causality of the findings cannot be determined. Although we have taken the known confounding factors into account in many of the statistical analyses, some confounders will be unknown and some we don't have information on. As discussed in section 1.2, 1.2.1 and 1.2.2 both physical and physiological factors are in play making interpretation of data complex.

The timing for the examinations was chosen from the interest of the role of the complementary diet on early growth and development. At 9 months, most children are well into the complementary feeding period and at the same time many are still partly breastfed. The period from 9 months to 3 years is special since this is a time where the child gradually changes from a predominantly milk-based diet to the family diet. Further, it is characterized by being a period with high growth velocities and brain development.

Information on breastfeeding and age of introduction to solids was collected retrospectively, since our first meeting with the parents was at the information meetings at around 8½ months. This might have induced some uncertainty of the exact ages for changes in the infant feeding practices. However, we believe the recall bias for these practices are still relatively low at 8½ - 9 months. The prospective collection of dietary information through dietary records reduced risk for recall bias regarding types and amounts of foods consumed. However, since 7-day pre-coded dietary records put quite a high work load on the parents and increase the awareness of what kind of food is given to the child, it is a risk that the method of registration has changed the kind of foods and drinks given to the child or reduced the complexity of the food intake during the registration period (169). However, all methods for registering food intake have strength and weaknesses. A validation study of the pre-coded dietary records against 7-day weighed food record and total energy expenditure measured by doubly labelled water in 9 and 36 months old children showed an overestimation of energy intake and most energy yielding nutrients but the estimates were found to be acceptable (169). Limitations of the information obtained from the children's clinical charts are discussed in section 3.2.

Estimates of body composition in paper II and III were made using the predictive equations for FFM and FM. This was chosen since it is a highly challenging task to obtain high quality DXA scans of 3-year-old children. Therefore, it was a great strength to be able to predict FFM and FM for a high number of children by using BIA and anthropometry.

4 Conclusions

This thesis and associated papers is based on the large prospective cohort study SKOT which have provided detailed prospective data on anthropometry, body composition, blood variables and diet.

In **paper I** we showed that it was possible to predict fat-free mass and fat mass with high precision by using bioelectrical impedance and anthropometry compared with DXA. The generated prediction equation provided a basis for paper II and III with information on body composition for more than double as many children as we had high quality DXA scans of. We anticipate that the prediction equation can be used in other studies with a similar study population aged 2 - 4 years.

The main conclusions to be drawn from the results presented in **paper II** are that birth weight as a measure of growth *in utero* and weight gain the first 5 months of life were highly related with anthropometry and body composition at 3 years of age. Our results showed that longer duration of full breastfeeding could reduce and even neutralize the effect of birth weight and early rapid growth on fat mass index at 3 years of age. This effect modification could be due to confounding but the relation is physiologically plausible. A few other studies have also reported effect modifications of infant feeding on the effect of rapid weight gain on later obesity and future studies should consider feeding mode when examining the association between rapid weight gain in infancy and obesity risk.

In **paper III**, we showed that total and free IGF-I at 3 years were positively associated with height, fat-free mass, and fat mass but not with adiposity in terms of body fat percentage and fat mass index. However, the higher tempo of growth could result in an earlier adiposity rebound followed by an earlier increase in BMI and thereby later risk of obesity. Breastfeeding had a strong modulating impact on IGF-I at the time of breastfeeding, but at 3 years of age children that were breastfed at 9 months had reached the same IGF-I level as children who were not breastfed at 9 months. Since there was a positive correlation between IGF-I at 9 and 36 month, it is possible that the suggested programming effect of early nutrition on the IGF-I axis is not yet expressed at 36 months of age. Milk and protein intake at 3 years of age did not influence IGF-I levels but there was a negative association with intake of fat and saturated fat expressed as energy percentages. The implications of this finding for the regulation of IGF-I and development of obesity need further exploration.

5 Perspectives for further research

It would be highly relevant to continue the SKOT study with a follow-up visit when the children have reached 7 - 8 years of age. At this age most children have passed the adiposity rebound and it should be possible to elucidate further on a role for IGF-I on accelerated growth and the timing of the adiposity rebound. It is possible that the relation between early growth and infant nutrition on the relation with later risk of overweight and obesity will be clearer. Hereby, a follow-up study of the SKOT cohort would enable further elaboration on the findings presented in paper II and III.

As mentioned the SKOT study has got a "sister study" called SKOT II where we follow a cohort of children born by obese mothers who have been involved in an intervention study during pregnancy to limit gestational weight gain at Hvidovre Hospital. The original SKOT cohort functions as a normal reference group to these children. Preliminary results have already shown that these children are larger at birth and at 9 months compared with the SKOT children. Differences in infant feeding are also seen with shorter duration of exclusive breastfeeding and earlier introduction to solids in the SKOT II cohort compared to the original SKOT cohort. It will be very interesting to investigate whether the impact of early weight gain on later adiposity is also modified by duration of full breastfeeding in this cohort. Further, it would be highly relevant to follow growth and development among children born by mothers who have been through gastric bypass to evaluate if this operation influences the health of the offspring. At Hvidovre Hospital, they now study these women during pregnancy and DXA scan their offspring at birth. Further, we are interested in creating a cohort of children with rapid weight gain the first 4 - 5 months of life. A close collaboration with the health nurses in Copenhagen and Frederiksberg to locate children with rapid growth could ease recruitment considerably. Such a cohort would enable further elaboration of the findings in paper II; especially it would be interesting and important with more information on a possible effect modification by full breastfeeding. Answers on what to advise families with large infants are requested but at the moment it is not possible to provide evidence based advice.

As already discussed in the thesis, early rapid growth is associated with an increased risk of overweight of approximately 20 %. Being aware of the many other risk factors involved in the obesity epidemic around the world, this effect size is substantial and a possible effect modification of breastfeeding on the relationship between rapid weight gain in infancy and obesity is highly relevant in this perspective. The long term effect of the modulating effect of breastfeeding on IGF-I levels in early infancy leading to later increase in IGF-I is unknown. More knowledge of the impact of high IGF-I concentrations in early childhood on later body composition is definitely needed. This will be relevant with regards to recommendations of protein and fat intake as well as cow's milk consumption in early childhood. Regarding the impact of age of introduction to solids on obesity risk, the evidence for 6 months as opposed to 4 months is weak and it could be interesting to go further into this issue with a qualified study design such as randomized controlled trials. However, my own personal belief of this matter is that the quality of the diet and the dietary pattern has much greater impact on body composition and later obesity risk than the exact age at which solids are introduced. Thus, I think more effort should be invested in examining the relation between the quality of the infant diet, body composition and later risk of obesity.

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7 Paper I – III

PAPER I

Prediction of fat-free body mass from bioelectrical impedance and anthropometry among 3-year-old children using DXA

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Abstract

For 3-year-old children suitable methods to estimate body composition are sparse. We aimed to develop predictive equations for estimating fat-free mass (FFM) from bioelectrical impedance (BIA) and anthropometry using dual-energy X-ray absorptiometry (DXA) as reference method using data from 99 healthy 3-year-old Danish children. Predictive equations were derived from two multiple linear regression models, a comprehensive model (height^2/resistance (RI), six anthropometric measurements) and a simple model (RI, height, weight). Their uncertainty was quantified by means of 10-fold cross-validation approach. Prediction error of FFM was 3.0 % for both equations (root mean square error: 360 and 356 g, respectively). The derived equations produced BIA-based prediction of FFM and FM near DXA scan results. We suggest that the predictive equations can be applied in similar population samples aged 2 - 4 years. The derived equations may prove useful for studies linking body composition to early risk factors and early onset of obesity.

Introduction

An association between body composition in early childhood and risk of overweight and non-communicable diseases later in life has been found in many studies (1;2). In particular, there is an increasing focus on factors that influence early proportions of fat-free mass (FFM) and fat mass (FM) (3;4). However, precise predictions of body composition in young children are difficult to obtain. The reference method used in this study, dual-energy X-ray absorptiometry (DXA), is a non-invasive technique that estimates bone mineral content, lean mass and FM with high reproducibility (5). However, in addition to high costs and being stationary, the DXA technique brings about practical challenges when used in young children e.g., the need for lying completely still for 5 minutes.

It is difficult to obtain reliable predictions of total FFM and total FM from simpler techniques (6). In young children weight-for-height standard deviation scores and body mass index (BMI) are frequently used as indirect estimates of total adiposity even though both are of limited use as measures of adiposity in early childhood (3;7;8). Anthropometric measurements like skin folds, waist- and arm circumference provide information on regional fat stores but become inaccurate when converted to full body FM and FFM in individuals (6;7). Bioelectrical impedance analysis (BIA) is a recognized method to estimate total body water (TBW) in epidemiologic studies (6;9), although some limitations have been identified (10;11). BIA estimates TBW by sending an electrical impulse through the body and measuring the resistance which depends on the amount and distribution of FM and FFM (9). The BIA method is quick, inexpensive, portable and easy to use in young children. In children, FFM can be calculated from TBW using age- and gender specific hydration factors (12). However, the hydration levels of FFM vary according to growth, maturity, ethnicity, disease, and obesity (6;9;12) and therefore require population and age-specific interpretation of the output. Only few predictive equations for TBW or FFM are available for children in early childhood (2 – 4 years) (13-17). None of these studies validates how accurate FM is estimated from the predicted FFM values as it is the underlying assumption that FM and FFM sum up to the total body weight of the child.

It has been recommended to begin prevention of obesity at the age of 2 - 4 years (2), and the age of the children in the present analysis (3 years) is relevant in the search for possible determinants for early body composition but also for studying the relationship between early body composition and adiposity later in life. Three-year-old children can be difficult to examine using DXA but BIA and anthropometry are measurements that are easily obtained. The aim of this study was to develop predictive equations for estimating FFM in 3-year-old children from BIA and anthropometry using DXA as reference measurement. We were mostly interested in establishing a comprehensive predictive equation for FFM that could explain as much variation as possible but we also considered a simpler version with BIA (resistance), height and weight only to increase the general applicability and suitability for clinical and epidemiological work.

Methods

Study design and participants

Data were from the observational cohort study SKOT (in Danish: Småbørns Kost Og Trivsel). Mailed invitations were sent to 2211 families randomly selected from the National Danish Civil Registry, and 330 Danish children were enrolled in the study and monitored at 9, 18 and 36 months of age (described in details elsewhere (23)). Inclusion criteria were singleton infants born ≥ 37 week of gestation, without diseases expected to affect growth or food intake. Eighteen children dropped out before the first examination and one child with late manifestation of a severe chronic disorder was excluded. All physiological measurements were made at the Department of Nutrition, Exercise and Sports, Copenhagen, Denmark.

A total of 263 (80%) completed the 36-months examination which was conducted from October 2009 to October 2010. As part of the 36-months examination all children were invited to a DXA scan. Except for three parents of Asian origin, all were Caucasian.

Anthropometrics

Weight at 36 months was measured without clothes to the nearest 0.1 kg using a yearly calibrated digital scale (Tanita WB-100MA, Tanita Corporation, 1-14-2, Maeno-chi, Itabashi-ku, Tokyo, Japan). Height was measured using a stationary digital height measurer (235 Heightronic Digital Stadiometer, Issaquah, WA, USA) to the nearest 0.01 cm. Waist circumference was measured on naked skin at the level of the umbilicus to the nearest mm with a nonflexible tape measure (Lasso-o; Child Growth Foundation, London, UK). This tape measure was also used to measure mid-upper-arm circumference at the mid-point between the olecranon process of the ulna and acromion process on the shoulder blade. The same mid-point point was used for measuring the triceps skinfold thickness while the subscapular skinfold was measured 2 cm below the inferior angle of the scapula (the lower left shoulder blade). Triceps and subscapularis skinfolds were measured using a Harpenden skinfold calliper (Chasmors Ltd, London, UK) and recorded to the nearest 0.1 mm. Except for weight, all measurements were performed in triplicates, and the mean value was used in subsequent analyses. Four well-trained observers conducted the examinations following standardized procedures. Age- and gender-specific BMI z-scores were obtained using WHO Anthro 2005 (24).

Bioelectrical Impedance Analysis

Whole body resistance, reactance and impedance were measured using a single frequency (50 kHz) tetrapolar bioelectrical impedance analyser Quantum III (RJL Systems, Michigan, USA) between right hand and right foot. The child had been fasting approximately 2 hours prior to the examination with no restrictions on physical activity. No request for bladder voiding was given before the BIA measurement. During measurement the child was lying relaxed on an examination couch in light clothing, without metal or persons touching the skin. The signal electrode (LMP3 Diagnostic Tab Electrodes, Kendall, Covidien, Mansfield, USA) on the foot were placed over the distal portion of the second metatarsal (the base of the second toe). The signal electrode on the hand was placed above the metacarpophalangeal joint of the middle finger and not wrapped around the middle finger

(proximal phalanx) as specified by the manufacturer, because the hands of the children were too small for this placement. The detecting electrode on the foot was placed at the anterior ankle on an imaginary line bisecting the medial malleolus and the detecting electrode on the hand was placed on an imaginary line bisecting the ulnar head as specified by the manufacturer. The procedure was performed twice with approximately 60 s between. Electrodes were not replaced. Mean values of resistance, reactance and impedance were used in the analyses. At the time the study was conducted the Quantum III software 'New pediatric' was only validated for individuals above 4 years of age. This has no influence of the physical measurements of impedance, resistance and reactance (RJL Systems, personal communication, 2010).

Dual-energy X-ray absorptiometry

DXA was used as the reference method for body composition measurements of FM, lean tissue mass, and bone mineral content. Whole body DXA scans were performed with a Lunar Prodigy Advance (GE Healthcare, Madison, WI, USA) using the software enCore, version 12.30. Radiation dose from each scan was maximum 0.0012 mSv according to the manufacturer. No fasting was required before the DXA measurement. The parents were requested to take the child to the toilet before the scan if the child needed to empty the bladder. The children were scanned lying supine in light clothing without metal and with no or dry nappy. It was a challenge to keep the children lying still for 5 minutes during the scanning process. The quality of the scans varied with several not being suitable for use. All scans were subsequently assessed by one person to ensure consistency. This person manually went through all scans to see if the body regions defined by the software were correct. The cut lines were adjusted if there were disagreement between the placement of the child and the software's definition of regions. DXA scans were divided into four categories ("perfect scans", "good scans with minor irregularities", "scans with several irregularities" and "useless scans") according to the quality of the scan. The procedure for selecting usable scans was described in detail by Jensen *et al.* (18).

Ethics

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 Committees on Biomedical Research Ethics for the Capital Region of Denmark (H-KF-2007-0003). Written consent was obtained from both parents or custody holders of all participating children.

Statistical analysis

FFM was calculated as the sum of lean tissue mass and bone mineral content measured by DXA and will be referred to as FFM_{DXA}. It is normal that the total body weight estimated by the DXA scanner (lean tissue mass + bone mineral content + FM) differs slightly from the measurement obtained using a digital weight. A deviation of 100-200 grams is more influential in children since their total body weight is lower and the relative error is therefore higher compared to adults. This means that a prediction of FFM (FFM_{pred}) can show a high degree of agreement with FFM_{DXA} but a discrepancy between the digital weight and the DXA weight will also be reflected in the calculated FM value (FM_{cal}) when subtracting FFM_{pred} from the digital weight. Consequently, the prediction

error had to be taken into account in order to calculate FM values equivalent to DXA FM values (FM_{DXA}). We generated an adjusted weight variable, $weight_{adj}$, obtained by simple linear regression of the DXA weight and the digital weight and used the slope estimate as a correction factor in the multiple regression analysis used for generating the predictive equations.

Characteristics of anthropometry, BIA and DXA were expressed as means ± SD or, if appropriate, median + interquartile range. Differences between genders were evaluated using two-sample *t*-test or, when appropriate, Wilcoxon rank test.

$Height(cm)^2/resistance(\Omega)$, subsequently referred to as the resistance index (RI), was chosen as the variable representing the BIA outcome since it has proven to be an accurate measure for prediction of TBW and FFM in linear regression models (9;15). FFM_{DXA} was considered the dependent variable. For the comprehensive model the predictor variables included in the multiple linear regression were: RI, height, $weight_{adj}$, sum of triceps and subscapularis skinfolds, waist circumference, mid-upper-arm circumference, and gender. Stepwise backward elimination resulted in a simplified model containing RI, height, $weight_{adj}$, sum of triceps and subscapularis skinfolds, and gender. The simple model included only RI, height, $weight_{adj}$, and gender as predictor variables. Age was not included in the analyses, since all children were scanned at 3.1 years (±0.1).

The prediction error of the obtained predictive equations was assessed through a 10-fold cross validation approach. Subjects were randomly placed in 10 groups, equally distributed by gender. Predictive equations were generated ten times, each time leaving out one group that subsequently served as a test data set for validation. The remaining 9 groups that were used to generate each predictive equation is called a training set. In total, we ended up with 10 training set and 10 test set. An average root mean square error (RMSE) was calculated from the predictions in all test sets and used to quantify the predictive performance of the two models for both FFM and FM. We used the adjusted R^2 to compare the proportion of the variability in data accounted for by the multiple regression models. Adjusted R^2 values for the predictive equations reported were obtained as the average of the adjusted R^2 values from the reduced regression models in the 10 training sets. To quantify the prediction errors in terms of percentages, we calculated prediction errors for FFM by dividing mean RMSE from the 10 test sets with mean FFM_{DXA} and multiplying by 100. The same procedure was applied for calculating prediction errors for FM. The level of agreement between DXA and the final predictive equations applied to the whole dataset was assessed through Bland-Altman approach. Potential bias between the predicted values and DXA (magnitude of bias) was quantified by means of the estimated slope parameter obtained from a linear regression model relating differences to mean-centered FM_{DXA}. A significant correlation indicates a bias in the prediction equation across the range of fatness.

Five published BIA-based prediction equations covering the age group of 2-3-year-old children (**Table 4**) were compared with FFM_{DXA} in this sample of children. The performance of each of these equations was assessed by means of Pearson's correlation coefficient and Bland-Altman approach. The difference between predicted and DXA values was evaluated using a paired *t*-test. The magnitude of bias of the predicted FFM was quantified as described above.

Data were analysed by STATA version 11.0. Statistical significance was set at $P < 0.05$.

Results

Anthropometric measurements (height, weight, sum of triceps and subscapularis skinfolds, waist circumference, and mid-upper-arm circumference) were available from 263 children; BIA data were obtained from 250 children; and 189 children completed the DXA scan. Only perfect scans and scans with minor irregularities (described in details in a previous paper (18)) were included in this analysis, giving a total of 101 scans. In total, we had complete data from 98 children for the full model and 99 children for the simple model (**Table 1**).

No differences were found between children who were and were not DXA scanned in weight, height and BMI at 9 months (n = 310) and 36 months (n = 263) (data not shown). Characteristics of the included population divided by gender are shown in Table 2.

Average subscapularis skinfolds, resistance, reactance, FFM_{DXA}, FM_{DXA} and bone mineral content were significantly different between boys and girls (Table 1). Boys had more muscle mass and bone mass compared to girls, while girls had thicker subscapularis skinfolds and higher FM. Triceps skinfolds tended to be thicker for girls (P = 0.06). BIA data showed that girls had higher resistance, reactance, and lower RI compared to the boys. No gender differences were seen in age, weight, height, BMI, mid-upper-arm circumference and waist circumference.

Weight estimated by DXA was slightly but significantly higher than the digital weight (mean difference 99 g (95 % CI: 27 – 171 g), P = 0.008) and an adjusted weight variable, $weight_{adj}$, was derived and used in the subsequent regression analyses. FM_{cal} was obtained from the following equations:

$$Weight_{adj} = 0.981\ digital\ weight + 0.374$$

$$FM_{cal} = Weight_{adj}\ x\ 1000 - FFM_{pred}$$

FM_{cal} and FFM_{pred} are both in g, while $weight_{adj}$ and digital weight are in kg.

The final full model included RI, height, $weight_{adj}$, sum of subscapular and triceps skinfolds and gender (**Table 2**) and explained 85 % of the variance in the training sets while the simple model including RI, height and $weight_{adj}$ explained 84 % of the variance.

Validation of the predictive equations

RMSE for the test sets and complete dataset are found in Table 2. Prediction errors (mean RMSE/mean FFM_{DXA} * 100%) in the full model were 2.9 % for girls and 3.1 % for boys, and 2.8 % for girls and 3.1 % for boys in the simple model. The mean difference between FFM_{pred} and FFM_{DXA} was -4 g in the full model (95% limits of agreement -816;808) and 2 g (- 852;856) in the simple model (**Figure 1 a & b**). Both models showed bias across the range of FM indicating that FFM was underestimated in leaner children and overestimated among children with higher FM_{DXA} (magnitude of bias: full model: β = 0.12 g (0.05), P = 0.026; simple model: β = 0.16 g (0.06), P = 0.004).

The values of RMSE for FM_{cal} were 264.3 g (girls 250.7 g; boys 277.3 g) in the full model and 303.4 g (girls 307.1 g; boys 299.9 g) in the simple model. Due to the lower FM-total body weight ratio prediction errors for FM_{cal} were 10.5 % (girls 9.1 %; boys 12.1 %) in the full model and 12.0 % (girls 11.2 %; boys: 13.0 %) in the simple model. Analysis of the level of agreement showed that the mean difference between FM_{cal} and FM_{DXA} was 6 g based on the full model (95%

limits of agreement -623;636) and 0 g (- 724;725) in the simple model (**Figure 2 a & b**). The magnitude of bias for FM_{cal} (FM_{cal} - FM_{DXA}) was depended on FM indicating an overestimated of FM among the leaner children and underestimated among children with higher FM_{DXA} (full model: β = -0.14 g (0.04), P = 0.001; simple model: β = -0.18 g (0.05), P < 0.001).

Comparison of FFM predicted from published equations and FFM_{DXA}

The agreement between FFM_{DXA} and FFM_{pred} based on previously published predictive equations is shown in **Table 3**. The newest predictive equation by Rush *et al.* (17), which is also using DXA as the reference method, showed the best accordance with FFM_{DXA} with a mean over-estimation of 0.21 kg (95% CI: 0.13 – 0.30 kg) and mean underestimation of FM of 0.31 kg (-0.39 – -0.24 kg). The other equations were based on other reference methods to predict TBW and underestimated FFM by 0.87 – 2.68 kg. In most of the predictive equations the magnitude of bias was not independent of FM indicating that FFM was underestimated in leaner subjects and overestimated in fatter subjects. For all predictive equations considered differences between predicted values and DXA values were significant (Table 3).

Discussion

In the present study, predictive equations for FFM have been generated and validated using data from a large sample of 99 healthy Caucasian children aged 3 years. This is one of few predictive equations covering the age group of 3-year-old children and the first equation to show how accurate FM can be calculated based on the predicted FFM. Only a small gain in explained variance was obtained by including sum of subscapularis and triceps skinfolds in the predictive equations. We recommend using the simple predictive equation since the difference between the two models was shown to be negligible and the use of skinfold measurement, which can be highly dependent on the examiner, is avoided. The relatively wide 95 % limits of agreement indicate that despite high agreement between predicted and DXA values on population level, there is some predictive uncertainty at the individual level. In this age group, BIA and anthropometry have practical advantages compared to DXA and other sophisticated techniques as the measurements are easily obtained.

We found only five other published equations that included children aged 2-3 years (13-17). Rush *et al.* (17) predicted FFM with DXA as reference method, while the other four equations predicted TBW with different methods as reference. Our study indicates that the Rush *et al.* equation provides good predictions also for FFM in the group of 3-year-old Danish children considered in the present study. The Rush *et al.* prediction equation was generated in a group of 77 2-year-old children from New Zealand with mixed ethnicity born to mothers treated for gestational diabetes (17). The other validated prediction equations over-estimated FM compared to DXA and this finding is in line with an earlier study showing that TBW determined by deuterium dilution led to higher estimation of FM than DXA and the four-compartment model in children (19). The magnitude of bias of FFM_{pred} increased with increasing FM_{DXA} for prediction equations by Fjeld *et al.* (14), Kushner *et al.*(15) and Rush *et al.* (17). For Rush *et al.* the magnitude bias was of the same size as the magnitude of bias in our predictive equation derived from the simple model. The magnitude of bias shows a discrepancy between the predictive equations in question and DXA. However, an analysis of the potential bias of FM assessed by DXA compared to the four-compartment model in 9 to 14-year-old children showed that DXA underestimated FM in leaner subjects and overestimated FM in more obese subjects (19). Thus, albeit being a valued technique for measuring body composition, DXA has its own limitations (20). An alternative reference method suitable for this age group could have been determination of TBW using deuterium dilution with subsequent application of age and gender specific hydration factors. However, this method have other limitations and has also been shown to overestimate FM in children compared to the four-compartment model (19). Besides different measurement errors by the different techniques used as reference methods in the evaluated equations, the discrepancies among the equations can be explained by large age spans, varying numbers of participants, and differences in population characteristics and settings that may influence the relative proportion of TBW and hydration level of FFM (6;9;12). The equations by Fjeld *et al*. (14) and Kushner *et al.* (15) were developed on children from Peru, while the equation by Masuda & Komiya (16) was developed on children from Japan.

It is challenging to get high quality DXA scans of 3-year-old children since they often find it difficult to lie still for 5 minutes. A considerable strength of our study is that we excluded the 47 %

of the DEXA scans with low quality and still retained a large number of high-quality DXA scans for use as a reference. We see it as a great strength that the generated equations are made to predict FFM directly without requiring age and gender dependent determination of hydration factors to account for different hydration level in FFM. Cross validation was used for calculating RMSE, ensuring that the uncertainty of the prediction model when applied to new data (data not used for fitting the model) was more appropriately accounted for than would be the case if we reported the RMSE derived directly from the fitted values. However, the reported cross validation-based RMSE may still be slightly too optimistic as it is based on the same data as the prediction model. Therefore, the reported RMSE may serve as a lower bound on the uncertainty to expect when using the prediction model for new data.

The generated predictive equations are derived from a group of 3-year-old children who were homogeneous in age (3 years ± 1 mo) and ethnicity. This setting should increase accuracy, also in case the predictive equations are applied in other populations that are similar to the SKOT cohort. However, in terms of generalizability this is a limitation as our equation might be less accurate for studies with a focus on obese children or very undernourished children. Only few of the SKOT children whose data were used forming the predictive equations were overweight or obese (7.8 % overweight and none obese according to the IOTF cut-off values (21) with 17.1 % having BMI z-scores above 1, 2 % above 2, and none exceeding 3 z-scores according to the WHO growth standards (22)). None of the children had BMI z-scores below minus 2. Limitations of the BIA method is especially the responsiveness to variations in the hydration state seen with age, size, ethnicity, temperature, clinical conditions, fasting state, bladder voiding and exercise (9;11) but also positioning of the body and electrode placements affect impedance measures (9). Therefore, caution should be taken before the predictive equation is applied in study settings where the children differ considerably in age, size or ethnicity or in studies with sick children if the disease is likely to affect the hydration level of FFM.

A possible source of error in this study was the BIA electrodes being placed less than 5 cm apart on the hand due to the small size of the hand. There is a risk that this placement has increased resistance, leading to a systematic underestimation of FFM (9). However, this seemed not to be the case, since FFM estimated by the BIA software did not differ from FFM_{DXA} for the boys and was significantly overestimated in girls. We used the BIA instrument from RJL systems in this study. It is a risk that different BIA machines measure resistance slightly different (9;11). However, RJL models are some of the most frequently used BIA instruments in epidemiologic studies (11).

It is of interest which age span the predictive equations can be applied to. We speculate that the applicable age range for the generated predictive equations is 2 - 4 years of age where the hydration level of FFM only changes with approximately 1.1 % in boys and 0.5 % in girls (12). This age span has been found to be a critical period for excessive weight gain and risk of overweight in adolescence (2).

In conclusion, the derived predictive equations enable BIA-based prediction of FFM and FM close to DXA scan results in a preschool population. The equations are particularly relevant for use among healthy Caucasian children aged 2 to 4. The predicted FFM proved useful at calculating FM although researchers should be aware that the relative error is greater when using the equations to

calculate FM than when calculating FFM. The generated equations can prove useful for population studies linking early risk factors to body composition and early onset of obesity.

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Author contribution statement

KFM and CM designed the study. KFM obtained the funding. KTE and LBC managed the data collection at the 3-year examinations. KTE analysed the data and prepared the first draft of the manuscript. SMJ and CR supervised the quality standards of the statistical analyses. All authors contributed to interpretation of results and commented on drafts and approved the final version of the manuscript.

Additional information

Competing financial interest: The authors have no conflicts of interest to disclose.

Figure 1: The Bland-Altman plots show the difference between predicted values of fat-free mass (FFM) and FFM measured by DXA plotted against their mean result for girls (○) and boys (●) in **a**, full model and **b**, simple model. The predictive equations for the full and simple model are presented in Table 3.

Figure 2: The Bland-Altman plots show the difference between calculated values of fat mass (FM) and FM measured by DXA plotted against their mean result for girls (○) and boys (●) in **a**, full model and **b**, simple model.

Table 1. Characteristics of the population - anthropometry, BIA and DXA

	n	Girls	n	Boys	*P*-value
Age (months)	49	36.2 [35.7;36.9]	50	36.1 [35.5;37.6]	0.92
Wt (kg)	49	14.26 (1.30)	50	14.61 (1.46)	0.21
Wt_{adj} (kg)	49	14.36 (1.28)	50	14.71 (1.43)	0.21
Ht (cm)	49	95.0 (3.0)	50	96.0 (3.5)	0.13
BMI (kg/m^2)	49	15.8 (1.3)	50	15.8 (1.1)	0.77
BMI z-score	49	0.24 (0.92)	50	0.15 (0.82)	0.58
Wc (cm)	49	50.3 (2.4)	50	50.1 (2.9)	0.67
SFt (mm)	48	9.5 [8.2;10.5]	50	8.8 [7.6;9.9]	0.06
SFs (mm)	49	6.6 [5.9;7.9]	50	6.0 [5.4;6.9]	0.01
MUAC (cm)	49	16.6 (1.1)	50	16.4 (1.1)	0.45
R (Ω)	49	765.0 (65.9)	50	734.5 (64.4)	0.02
Xc (Ω)	49	58.3 [56.4:62.1]	50	55.4 [52.5;57.4]	<0.001
RI ($cm^2/Ω$)	49	11.9 (1.3)	50	12.7 (1.5)	0.006
BMC (g)	49	428.9 (49.7)	50	452.6 (52.0)	0.02
FFM_{DXA} (g)	49	11,600 (852)	50	12,427 (1,138)	<0.001
FM_{DXA} (g)	49	2743 (771)	50	2299 (662)	0.003

Data presented as mean (SD) or median (25;75 percentile). Tested for statistical significance by two-sample *t*-test or Wilcoxon rank test.

BMC; Bone Mineral Content; DXA, dual-energy X-ray absorptiometry; FFM, Fat-free mass; FM, Fat mass; Ht, height; MUAC, mid-upper-arm circumference; R, resistance; RI, Resistance Index; SFt, skinfold thickness triceps; SFs, skinfold thickness subscapularis; Wc, waist circumference; Wt, digital weight; Wt_{adj}, weight adjusted to agree with DXA weight; Xc, reactance. BMI calculated as Wt/Ht^2. Xc and R measured by bioelectrical impedance. FFM_{DXA} calculated as lean tissue mass + BMC. FM_{DXA}, BMC and lean tissue mass was measured by DXA. RI calculated as Ht^2/R.

Table 2. Predictive equations for FFM (g) developed by 10-fold cross validation based on 3-year-old Danish children

Equation	Predictor variables[a]										
	n	RI	Wt	Ht	Sex[b]	Sum SF	Constant	Adj R^2	RMSE	RMSE[c]	PE	PE[c]
Full	98	297.3	354.3	43.5	331.7	- 64.7	-62.7	0.85	360.4	321.1	3.0	2.7
Simple	99	327.2	223.8	76.8	417.6	-	-2784.4	0.84	355.8	333.3	3.0	2.8

[a] Results presented as regression coefficients.

[b] Female = 0, male = 1.

[c] Based on the complete dataset.

Adj R^2, adjusted R^2; FFM, Fat-free Mass (g); Ht, height (cm); PE, Prediction error; RI, Resistance Index (cm^2/Ω); RMSE, Root mean square error (g); Sum SF, Sum of skinfold thickness triceps and subscapularis (mm); Wt, digital weight (kg). Adj R^2 is the weighted mean of the adj. R^2 based on the 10 training sets. RMSE is the mean of the individual RSME in the test sets. PE was calculated by dividing mean RMSE from the test sets with mean FFM_{DXA} and multiply by 100%.

Table 3. Comparison of DXA FFM and FFM predicted by other published BIA-based equations in this group of 3-year-old children.

Equation	FFM_{pred} (kg)	Rho[a]	Bias[b]	LOA (kg)	Magnitude of bias[c] β (SE)	FM_{cal} (kg)	Rho[a]	Bias[b]	LOA (kg)
Fjeld *et al.*[d]	11.07	0.87	-0.94*	-2.03;0.14	0.48 (0.05)*	3.36	0.82	0.85*	-0.06;1.75
Kushner *et al.*[d]	10.64	0.88	-1.38*	-2.53;-0.22	0.20 (0.08)†	3.80	0.75	1.23*	0.18;2.37
Bedogni *et al.*[d]	9.34	0.88	-2.68*	-3.95;-1.41	0.12 (0.09)	5.09	0.73	2.58*	1.33;3.83
Masuda & Komiya[d]	11.15	0.88	-0.87*	-1.91;0.18	0.10 (0.07)	3.29	0.83	0.77*	-0.14;1.68
Rush *et al.*	12.23	0.91	0.21*	-0.65;1.08	0.16 (0.06)†	2.21	0.87	-0.31*	-1.06;0.43

[a] Pearson's correlation coefficient.

[b] Predicted – DXA value. Tested for significance by paired *t*-test that bias = 0.

[c] Linear regression model relating differences (FFM_{pred} - FFM_{DXA}) to mean-centered FM_{DXA}.

[d] Predicted TBW was converted to FFM by the assumption than the age standardized hydration levels of FFM in 3-year-old children is 77.9 % for girls and 77.5 % for boys (12).

* $P \leq 0.001$; † $P \leq 0.01$.

FFM, Fat-free mass; FM, Fat mass; LOA, Limits of agreement by Blandt Altman.

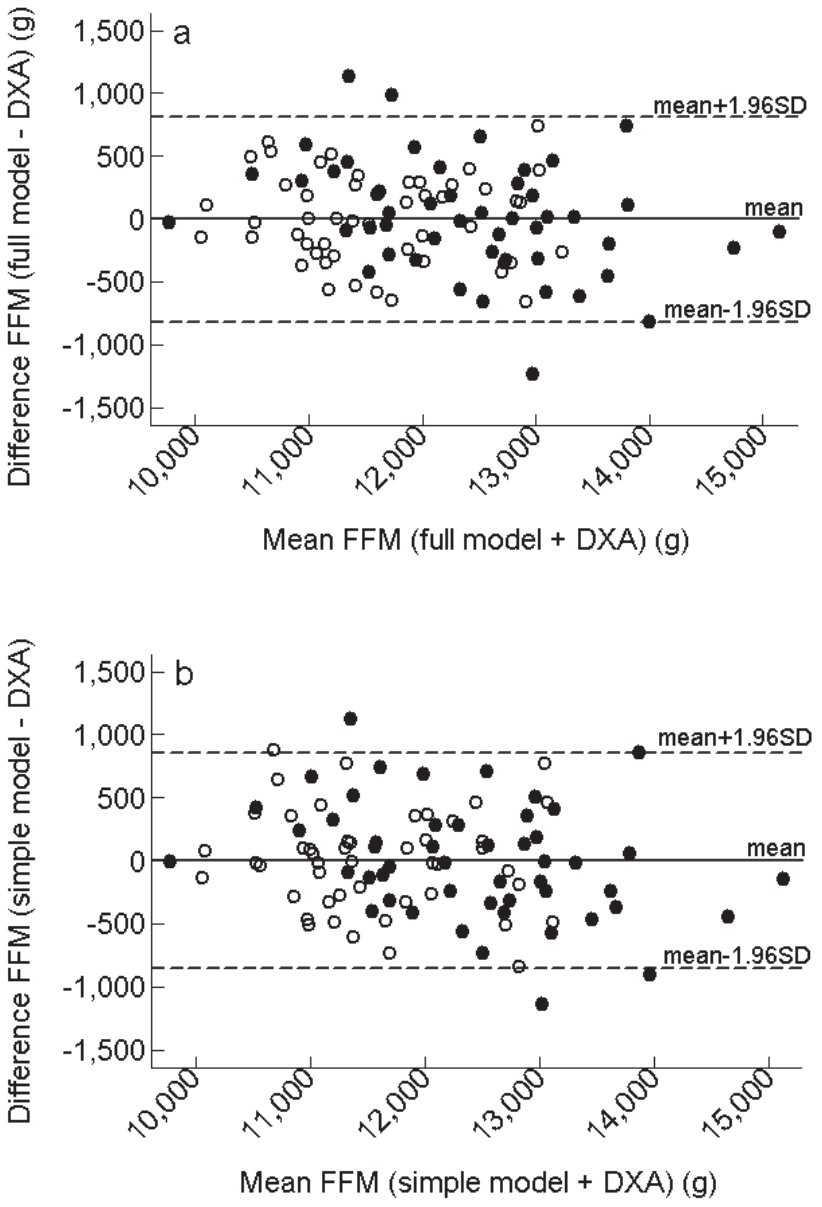
Table 4. Published BIA-based equations relevant for 3-year-old children.

Source	Reference method	BIA instrument	Equation for FFM	n	Age (years)	Country
Fjeld *et al.*[a]	^{18}O dilution	RJL	(0.18 Ht^2/R + 0.39 Wt + 0.76) / HF	30	0.3 – 2.5	Peru
Kushner *et al.*[a]	^{18}O dilution	RJL-101	(0.593 Ht^2/R + 0.065 Wt + 0.04) / HF	29	Preschool	Peru
Bedogni *et al.*[a]	D_2O dilution	Dietosystem Human IM scan	(0.716 Ht^2/I -1.504) / HF	23	3 – 19	Italy
Masuda & Komiya[a]	D_2O dilution	TP-95 K	(0.149 Ht^2/R + 0.244 Wt + 0.460 age + 0.501 sex + 1.628) / HF	46	3 – 6	Japan
Rush *et al.*	DXA	BIM4	0.367 Ht^2/R + 0.188 Wt + 0.077 Ht + 0.273 sex - 2.490	77	2	New Zealand

[a] Original prediction of TBW - converted to FFM by dividing with the age standardized hydration levels of FFM (12).

BIA, bioimpedance analysis; D_2O, deuterium dilution; DXA, Dual-Energy X-ray Analysis; FFM, fat free mass; HF, hydration factor; Ht, height (cm); I, impedance (Ω); ^{18}O dilution, Oxygen-18 dilution; R, resistance (Ω); Wt, weight (kg).

Figure 1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Difference FFM (full model - DXA) (g)
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Mean FFM (full model + DXA) (g)
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Difference FFM (simple model - DXA)
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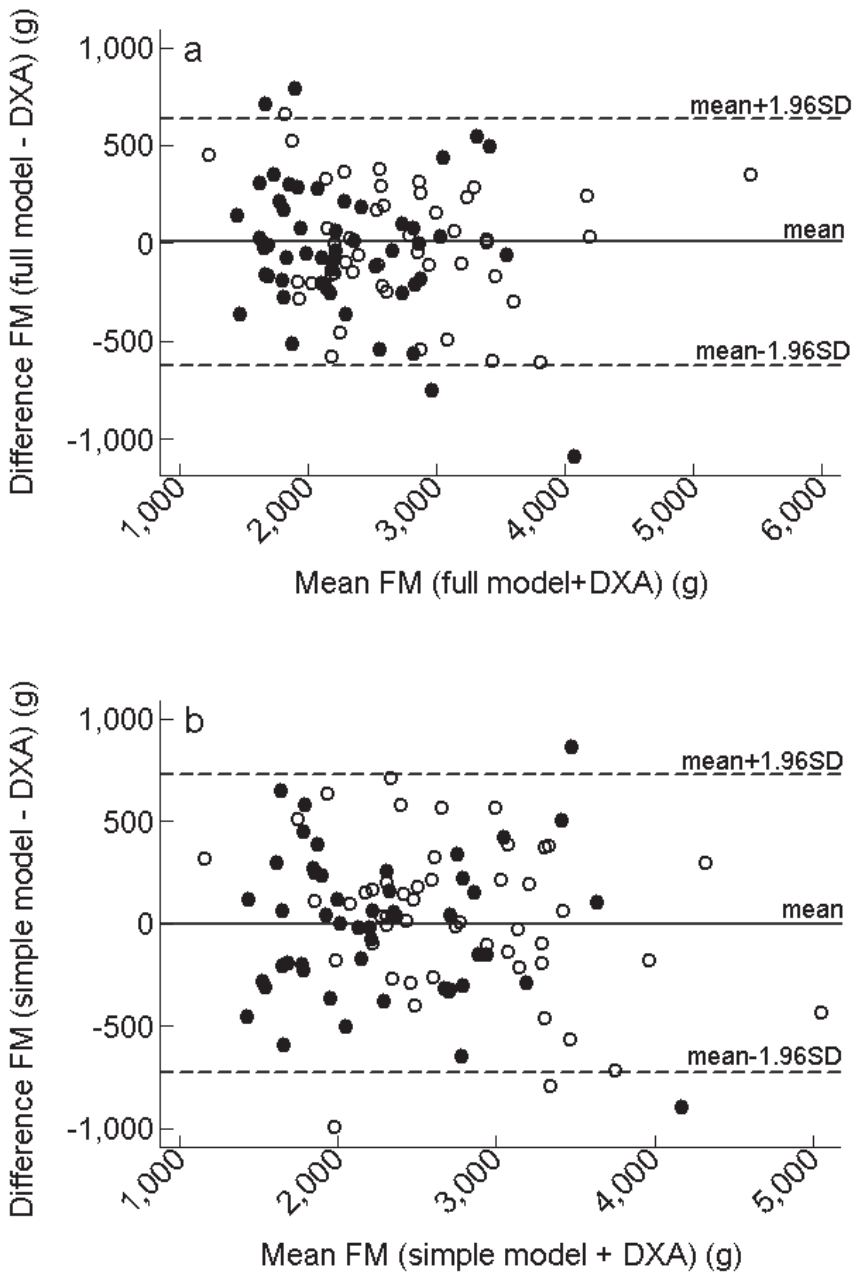
Mean FFM (simple model + DXA) (g)

Figure 2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Mean FM (full model+DXA) (g)
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Difference FM (simple model - DXA) (g)
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Mean FM (simple model + DXA) (g)

PAPER II

Early growth patterns and infant feeding: Impact on body composition at 3 years of age.

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Running head: Early growth, diet and later body composition

Keywords: Early growth patterns, infant feeding, body composition, fat mass index, childhood.

Abbreviations:

BAZ: BMI-for-age z-score

BIA: Bioelectrical impedance analysis

BWZ: Birth weight z-score

DXA: Dual-energy X-ray absorption

FFM: Fat-free mass
FFMI: Fat-free mass index
FM: Fat mass

FMI: Fat mass index
HAZ: height/length-for-age z-score
WAZ: weight-for-age z-score
WFH: weight-for-height/length z-score.

Clinical trial registry: The study is not registered at clinical trials. It was initiated in 2007 where clinical registration of prospective studies was not common practice.

Abstract:

Background: Early weight gain is positively associated with later obesity, but the effect of weight gain during specific periods and the impact of infant feeding practice are debated.

Objective: The objective was to examine the impact of weight gain in periods of early childhood on body composition at 3 years and whether infant feeding modified the relationship between early growth and body composition at 3 years.

Design: We studied 233 children from the prospective cohort study, SKOT. Birth weight z-score (BWZ) and change in weight-for-age z-scores (WAZ) from 0-5, 5-9, 9-18 and 18-36 months were analysed for relations with body composition (anthropometry and bioelectrical impedance) at 3 years by multivariate regression analysis.

Results: BWZ and change in WAZ from 0-5 months were positively associated with BMI, fat mass index (FMI) and fat-free mass index (FFMI) at 3 years. Full breastfeeding for 4-5 months compared with less than 1 month attenuated the effect of early growth on FMI by 47 % ($p = 0.05$) while full breastfeeding for 6 month eliminated the effect of early growth ($p = 0.002$). Full breastfeeding for 6 months compared with less than 1 months also eliminated the positive relation between BWZ and FMI ($p = 0.002$). No effect modification of was seen for FFMI.

Conclusions: High birth weight and rapid growth from 0-5 months were associated with increased FMI and FFMI at 3 years. Longer duration of full breastfeeding reduced the effect of birth weight and early weight gain on fat mass considerably.

Introduction

Many studies have shown a highly significant association between weight gain during the first months or years of life and later adiposity (1;2). Rapid growth during the first 4 – 9 months of life have been identified as sensitive period predicting fat mass (FM) at later ages (3-5) and later risk of obesity (6-8) but other studies have proposed excessive weight gain from 2 – 6 years (5;9) or no specific periods from birth to 15 years (10).

Nutrition in infancy is thought to be particularly influential since growth patterns and changes in body composition in early infancy are closely linked with feeding patterns (3;11-13). Breastfed infants show slower weight gains the first year of life (11;12) and have lower FM at 12 months compared with formula fed infants (13). Three meta-analyses have shown that breastfeeding is associated with a small but consistently reduced risk of obesity determined by BMI (14-16) while breastfeeding do not seem to reduce mean BMI (17). Less consistency is found in studies of how infant feeding is linked with FM and fat-free mass (FFM) at later ages. Three studies have found an inverse relationship with duration of breastfeeding and FM at 4 years (18), 9 – 10 years (19) and 16 years (20), one have found an inverse effect in 9 year old girls but not boys (21) while three studies have not found differences at 2 years (22), 5 years (23) and 18 years (24). In favour of the theory of early overnutrition leading to increased risk for obesity at later ages, two randomised controlled trials demonstrated increased growth rates and higher FM at 5 – 8 years of age in the group of children born small for gestational age who had received nutrient-enriched formula versus control formula until 6 – 9 months of age (3). Fewer studies have focussed on the relation between the timing of introduction to solids and later risk of obesity and findings are inconsistent (25;26) Early introduction to solids have been found to be associated with higher energy intake (27;28) and earlier introduction to unhealthy foods (29). Breastfeeding, formula feeding and complementary feeding practices are three highly related components and it can be difficult to distinguish which component is responsible for certain effects. Therefore, both breastfeeding and information on complementary feeding should be included in analyses of early diet and body composition in later childhood (18;23).

Although large variations in body composition exists within a given BMI (30) most studies of both early growth and early feeding have used BMI or BMI standard deviation scores as indirect measures of adiposity at later age stages (1;2;14-17). In this study we supplement anthropometric measurements and BMI with fat mass index (FMI) and fat-free mass index (FFMI) estimated from bioelectrical impedance.

In order to elucidate the mechanisms behind rapid growth and to improve prevention strategies of childhood overweight, it is important to identify the most sensitive age window and modifiable factors that influence growth in this period. The main aim of this study was to make a detailed investigation of how weight gain in four periods from birth to 3 years of age influenced body composition at 3 years of age. Our secondary aim was to examine the impact of breastfeeding and age of introduction to solids on FMI and FFMI.

Subjects and Methods

Study design and participants

Data are from the SKOT cohort, a prospective observational cohort study that has monitored healthy Danish children at 9, 18 and 36 months. The study has previously been described in detail (31). In brief, inclusion criteria were singleton term infants (gestational age: 37 – 42 weeks) without diseases expected to affect growth or food intake. Recruitment took place from April 2007 to May 2008, when 2211 families randomly selected from the National Danish Civil Registry were invited to participate. Out of these, 330 families accepted and were enrolled in the study. The 36 months' examinations took place from October 2009 to October 2010.

Anthropometry

Birth weight and weight at 5 months was obtained from health records kept by parents. All other anthropometric measurements, weight, height, and skin folds, were obtained from the physical examinations at the Department of Nutrition, Exercise and Sports, Copenhagen, Denmark at age 9 (±2 weeks), 18 (± 1 months) and 36 months (± 3 months). The procedure for the anthropometric measurements at 9 and 18 months has been described previously (32). At 36 months naked body weight was measured to the nearest 0.1 kg on a yearly calibrated digital scale (Tanita WB-100MA, Tanita Corporation, 1-14-2, Maeno-chi, Itabashi-ku, Tokyo, Japan) and height was measured by a stationary digital height measurer (235 Heightronic Digital Stadiometer, Issaquah, WA, USA) to the nearest 0.01 cm. Triceps and subscapular skinfolds were measured by a Harpenden skinfold calliper (Chasmors Ltd, London, UK) and recorded to the nearest 0.1 mm. Except for weight, all measurements were performed in triplicates, and average was used in analysis. Four well-trained observers conducted the examinations following standardized procedures. Weight and height/length were entered into the software WHO Anthro 2005 (33) to achieve gender-specific z-scores. The number of overweight and obese children at 3 years was determined according to the cut-off values by IOTF (34) and according to WHO growth standards (35).

Body composition assessment at 3 years

Body composition at 36 months was measured by bioelectrical impedance analysis (BIA) (described in details by Ejlerskov *et al.* (36)). Whole body resistance, reactance and impedance was measured with the child in a supine position by a single frequency (50 kHz) tetra polar BIA (Quantum III, RJL Systems, Michigan, USA). On the right foot, the signal electrode (LMP3 Diagnostic Tab Electrodes, Kendall, Covidien, Mansfield, USA) was placed over the distal portion of the second metatarsal (the base of the second toe) and the detecting

electrode was placed at the anterior ankle on an imaginary line bisecting the medial malleolus. On the right hand, the signal electrode was placed above the metacarpophalangeal joint of the middle finger. The detecting electrode on the right hand was placed on an imaginary line bisecting the ulnar head as specified by the manufacturer. The measurement was performed twice consecutively, and the mean values of resistance, reactance and impedance were used. We have previously generated a prediction equation for FFM in this cohort of 3-year old children using BIA, height and weight with dual-energy x-ray analysis (DXA) as reference method (36). In short, high quality DXA scans were achieved in a sub-group of the SKOT children (n = 101) and the prediction equation was generated by linear regression models using a 10-fold cross validation approach with BIA, weight, height and gender as independent variables (36). FFM and FM were calculated using the following equations:

$$FFM = 327.2\ RI + 223.8\ weight + 76.8\ height + 417.6\ sex - 2784.4$$

$$FM = (weight * 0.981 + 0.374)*1000 - FFM$$

FFM and FM are in grams, RI is the resistance index (height $(cm)^2$/resistance (Ω)) and weight is digital weight computed in kg, height is in cm and sex was recorded as male = 1 and female = 0.

Feeding patterns and other information

At the 9 months examination the parents filled out questionnaires about household income, age, height and weight of parents, gestational age, gestational weight gain and smoking during pregnancy. At the 36 months examination we measured height and weight of the accompanying parent for more exact data (same measuring equipment as for the child). Data on feeding patterns including duration of full and partial breastfeeding as well as age of introduction to complementary foods were obtained by interviews at each examination. Full breastfeeding was defined as receiving only breast milk, water and vitamins but allowed exceptional bottle feeding e.g. if the child have been babysit for a single night (this changed the length of exclusive breastfeeding for 15 infants). Age of introduction to solids was registered as the earliest age in months at which the infant first received one of 19 food categories. At all three examinations, parents filled out follow-up questionnaires with updated information regarding educational status from which a variable of the mother's educational level at 3 years was generated.

Ethics

The parents of all participants received verbal and written information about the study and written consent was obtained from all. The study was approved by The Committees on Biomedical Research Ethics for the Capital Region of Denmark (H-KF-2007-0003).

Statistical analysis

The dataset only includes children with information on FM and FFM. Missing values were considered missing at random and available-cases analyses were carried out.

To account for natural variation in FM and FFM due to body size, we calculated FMI (FM (kg)/height $(m)^2$) and FFMI (FFM (kg)/height $(m)^2$) (37). Linear regression confirmed that FMI and FFMI were no longer associated with height ($p > 0.5$). In the absence of well-established reference data for FM or FMI to identify cut-off values for overweight in young children, we grouped FMI and FFMI according to gender-specific quartiles.

Differences between weight-for-age z-scores (WAZ), height/length-for-age z-scores (HAZ), weight-for-height/length z-scores (WFH) and BMI-for-age z-scores (BAZ) in the study sample and the WHO standard were evaluated by means of one-sample *t*-tests. Differences in feeding patterns between genders were assessed either using *t*-tests or, if not normally distributed, Wilcoxon rank tests. Anthropometry at 3 years, parental characteristics and infant feeding practice were evaluated according to FMI and FFMI quartiles using means ± SD and median + interquartile range (IQR) (if not normally distributed). Test for trends across quartiles of FMI and FFMI were based on linear regression.

Associations between weight gain in four age intervals (0 - 5 months, 5 - 9 months, 9 - 18 months and 18 - 36 months) and body composition at 3 years of age were analysed using multiple linear regression. Body composition outcomes were regressed on change in WAZ in each of the four age intervals in both a simple model only adjusting for gender and BWZ and in a fully adjusted model controlling for gender, BWZ, household income, educational level of the mother, smoking during pregnancy, gestational weight gain, and parental BMI or height or weight according to outcome of interest. Educational level for the mother was grouped in five categories: "no education above school level", "trainee or vocational education", "short academic education < 3 years", "academic education 3 - 4 years" and "long academic education > 4 years". Data on household income were collected in categories from 1 "less than 200.000 DKK" to 14 "more than 800.000 DKK", with an interval of 50.000 DKK (≈ 8700 US$) and used as a quantitative variable in the analyses. Since only few mothers reported smoking in pregnancy, this variable was included as a dichotomous variable, yes/no. Model assumptions were evaluated using residual and normal probability plots. Robust standard errors were employed in case substantial departures were found.

For each quartile of FMI, the development of mean WAZ across the four time points was visualised by scatter plots. The same was done for each quartile of FFMI. Differences between quartiles in WAZ over time were evaluated using linear mixed models controlling for gender, income, educational level of the mother, smoking during pregnancy, gestational weight gain, and parental BMI, and including subject-specific random effects.

To see the predictive value of the associations between birth weight and early growth on FMI and FFMI, we grouped birth weight in four categories: "< 3000 g" (n = 23), "3000 – 3499 g" (n = 92), "3500 - 3999 g" (n = 83) and "≥ 4000g" (n = 35). Only two children were born with a weight below 2500 g. Change in WAZ from 0 - 5 months was also divided into four categories: "< - 0.67", "-0.67 - 0", "0 - 0.67" and "> 0.67". Change in WAZ exceeding

0.67 represents upward centile crossing at standard growth curves and this threshold is found clinically relevant (38). Differences in the proportion of children in the highest quartile of FMI and FFMI across the categories were tested by X^2-tests.

Associations between FMI or FFMI and duration of full and partial breastfeeding and age of introduction to solids were assessed by multiple regression in simple models only adjusted for gender and in fully adjusted models controlling for BWZ, WAZ change from 0 - 5 months, household income, educational level of the mother, smoking during pregnancy, gestational weight gain, and parental BMI. We included combined effects corresponding to BWZ-modified effect of full breastfeeding, BWZ-modified effects of age of introduction to solids, effect modification of full breastfeeding by WAZ change from 0 - 5 months, effect modification of age of introduction to solids by WAZ change from 0 - 5 months in a fully adjusted regression model with FMI or FFMI as outcome variables. For this analysis full breastfeeding was categorized as"< 1 months" (< 31 days, n = 36), "1 - 3 months" (31 – 120 days, n = 39), "4 - 5 months" (121 – 180 days, n = 137) and "6 months" (>180 days, n = 21). Age of introduction were grouped as "3 – 4 months" (n = 138), "5 months" (n = 63) and "6 months" (n = 32). Backwards stepwise elimination was used for removing non-significant combined effects one by one (by means of likelihood-ratio tests). The same procedure was applied to a similar model with partial breastfeeding ("< 4 months" (< 121 days; n = 30), "4 – 5 months" (121 - 182 days, n = 30), "6 - 8 months" (183 – 273 days; n = 60), "9 – 11 months" (274 – 364 days, n = 56) and "≥ 12 months" (> 365 days, n = 54) instead of full breastfeeding in the interaction terms.

Data were analysed by STATA version 11.0 (StataCorp LP, Texas, USA). The significance level was set at $\alpha = 0.05$.

Results

Out of the 330 children initially recruited for SKOT, 263 (79.7%) completed the 36 month examination. FFM and FM were calculated for the 233 children with complete data of BIA, height and weight. Data for weight and length at 5 months were missing from 66 children. There were no difference in weight and length at 9 months between children with and without 5 months values.

Sample characteristics

Mean WAZ of the SKOT children were above average compared with WHO growth standards at all examinations (all $p < 0.001$) (**Table 1**). HAZ at birth and 5 months were measured by midwifes and practitioners and substantially higher than the WHO standards most likely because of inaccurate measures of length in the primary health care sector. At 3 years 19 children were overweight (8.2%) and none obese according to the IOTF criteria. Forty six children (19.7 %) were at risk of overweight (BAZ above 1 SD), 4 children (1.7 %) were overweight with BAZ above 2 SD and none were obese using the WHO growth standards. One hundred fifty-eight children (67.8 %) were fully breastfed 4 months or more, while 99 children (42.5 %) were still partially breastfed at 9 months. Five children (2 %) were introduced to solids earlier than 4 months, 133 (57 %) at 4 months, 63 (27 %) at 5 months and 32 children (14 %) at 6 months. Feeding patterns did not differ between boys and girls (all $p > 0.3$, data not shown) but infants that were no longer fully breastfed at 4 months were introduced to solids earlier than infants fully breastfed at 4 months ($p < 0.001$) (data not shown). Significant positive trends across FMI and FFMI quartiles at 3 years of age was seen for FM, FFM, BMI and skin fold thickness at 3 years (**Table 2 and 3**). No trends across FMI or FFMI quartiles were seen for full breastfeeding, partial breastfeeding, breastfeeding at 9 months, intake of formula at 9 months, and age of introduction to solids, but a trend across FMI quartiles were seen for the number of children fully breastfed at 4 months (Table 2). In the higher quartiles fewer children were breastfed at 4 months ($p = 0.028$). Maternal age, height, gestational weight gain, smoking during pregnancy, parental educational level, paternal height and BMI, and family income were not related to FMI quartiles (all, $p > 0.13$, data not shown). There was a trend for higher maternal BMI in the higher FMI quartiles ($p = 0.066$). Positive trends across FFMI quartiles were seen for maternal BMI ($p = 0.019$), paternal BMI ($p = 0.016$) while negatives trends were seen for maternal education ($p = 0.023$) and paternal education ($p = 0.049$). The other parental characteristics and family income were not related to FFMI quartile (all, $p > 0.18$, data not shown).

Effect of early weight gain on body composition

BWZ and change in WAZ from 0 - 5 months were positively related to height, weight, BMI, FMI and FFMI at 3 years of age in both the simple and adjusted model (**Table 4**). In addition

to this, change in WAZ from 0 - 5 months was positively associated with sum of skin folds ($p < 0.001$).

The different growth patterns over time for WAZ according to FMI quartiles are shown in **Figure 1A.** A LR test of fully adjusted mixed model analysis showed significant interaction between FMI quartile and time ($p < 0.001$) with the most evident difference between growth patterns from 0 - 5 months. Differences between quartiles assessed by mixed model analyses controlling for educational level of the mother, smoking during pregnancy, gestational weight gain and parental BMI showed that mean WAZ in the highest FMI quartile was significantly higher than the other FMI quartiles at 5 months ($p < 0.002$) and 9 months ($p < 0.05$). At 18 months only WAZ in the first and second FMI quartile were significantly lower than the highest ($p < 0.002$). At 36 months, the third FMI quartile were only borderline lower than the fourth FMI quartile ($p = 0.08$) but the others remained lower ($p < 0.001$). Growth patterns over time did not differ between FFMI quartiles (LR test of interaction between examination age and FFMI, $p = 0.17$) but the fourth quartile were significantly higher than all other quartiles ($p < 0.001$) (**Figure 1B)**.

FMI and FFMI according to birth weight & early weight gain

The probability of being placed in the highest quartile of FMI and FFMI according to birth weight and early weight gain is shown in **Table 5.** We categorised birth weight into four categories and analysed the share of children from each category that were placed in the highest quartile of FMI and FFMI at 3 years. Same approach was done for change in WAZ from 0 - 5 months. More children with a birth weight above 4000 grams were placed in the highest quartile of FMI compared with children with birth weight from 3000 - 3500 g (40 compared with 17 %, $p < 0.007$). For the group of children undergoing rapid growth the first 5 months of life (change in WAZ > 0.67), 49 % were placed in the highest quartile of FMI at 3 years. This was more than children with weight gain not exceeding 0.67 WAZ from 0 – 5 months (all groups, $p < 0.031$). A significantly larger proportion of the children with birth weight above 4000 g compared with 3000 - 3500 g and less than 3000 g was in the highest FFMI quartile at 3 years (40 compared with 17 and 13 %, respectively, both $p < 0.04$). WAZ change from 0 - 5 months did not affect the probability for being in the highest FFMI quartile at 3 years.

Impact of early feeding practices

Full breastfeeding, partial breastfeeding, age of introduction to solids or age of introduction to cow's milk were not related to FMI or FFMI at 3 years in both unadjusted and adjusted regression analyses (data not shown). However, when testing for possible interactions between early feeding practices and early growth, we found two effect modifications of duration of full breastfeeding (**Table 6**). The effect of BWZ on FMI was eliminated by full

breastfeeding for 6 months compared with less than 1 month (from β = 0.69 (0.39 – 0.98) to β = - 0.07 (-0.55 – 0.40), p = 0.002) (**Figure 2A**). Duration of full breastfeeding for 4 – 5 months compared with less than 1 month attenuated the positive effect of change in WAZ from 0 - 5 months on FMI by 47 % (from β = 0.81 (0.46 – 1.17) to β = 0.43 (0.05 – 0.81), p = 0.05) while 6 months of full breastfeeding eliminated the effect of WAZ (β = -0.15 (-0.76 – 0.45), p = 0.002) (**Figure 2B**). A borderline effect modification on WAZ change from 0 – 5 month by full breastfeeding for 1 – 3 months compared with less than 1 month was also seen (45 % reduction, β = 0.45 (0.04 – 0.85), p = 0.075). The effect of age of introduction to solids on FMI did not interact with duration of full breastfeeding (p > 0.25), and no interactions between gender and age of introduction to solids or duration of full breastfeeding were seen (p > 0.23).

Neither age of introduction to solids or duration of partial breastfeeding modified the effect of BWZ or change in WAZ from 0 - 5 months on FMI (p > 0.3). No interactions were found between BWZ, WAZ change from 0 – 5 months and early feeding practice on FFMI (p > 0.6).

Discussion

In this prospective cohort, we found that high birth weight and rapid growth the first 5 months of life independently affected body composition and measures of adiposity at 3 years, while there were no effects of weight gain during the following periods. Full breastfeeding for 4 - 6 months compared with less than 1 month considerably attenuated the effect of birth weight and weight gain from 0 - 5 months on FMI at 3 years. Our results indicate that longer duration of full breastfeeding, which is a modifiable factor, can attenuate the effect of birth weight and early growth on FM at 3 years.

Our results, that birth weight and growth the first 5 months of life are strong predictors for body composition at 3 years of age persisted after control for parental height, weight or BMI, gestational weight gain, mother's educational level, and smoking during pregnancy. Weight gain in later periods from 5 to 36 months did not relate significantly to measures of adiposity or FFMI at 3 years. A birth weight above 4000 g increased the probability for being in the highest quartile of FMI and FFMI at 3 years with approximately 50 % compared to children with birth weight from 3000 – 3500 g. Rapid weight gain above 0.67 z-scores from 0 – 5 months were strongly related to FMI but not FFMI. Other studies have also found rapid early weight gain associated with FM rather than FFM (3-5;39-41) while studies of the effect of birth weight on later body composition support a stronger association with FFM than FM (42;43). Especially low birth weight could be speculated to program a smaller proportion of FFM later in life (42). One reason that we find birth weight equally related to FFM and FM could be the low share of children with birth weight below 2500 g in this cohort.

We find it likely that FMI in 3-year-old children is a good predictor for later risk of obesity. A high level of tracking of FM have been shown from 2 to 7 years (40) and 4 to 9 years (44) with higher increase among those who acquired a high fat percentage early (40;44). The association between early rapid growth and later risk of obesity is well documented (1;2). Several studies have suggested an effect size of infant growth rate on later obesity risk of around 20 % (6;7;45). With the perspective that obesity is a multifactorial disease, we find this effect size substantial.

Lenght measures at 0 and 5 months resulted in very high HAZ scores, most likely because of a tendency to overestimate length measurements in the primary health care sector as demonstrated by others (46). The WAZ values were above the WHO standards at all ages. Disregarding WFH and BAZ at birth and 5 months, WFH and BAZ values were also above WHO standards at 9, 18 and 36 months. Part of the explanation is that the cohort includes infants that have only been breastfed for a shorter period. We have previously shown that WAZ and BAZ of children from the SKOT cohort, who were breastfed at 9 months were closer to the median of the WHO growth standards at 9 and 18 months and lower compared with children that were no longer breastfed at 9 months (32). However, duration of full or partial breastfeeding was not directly associated with BMI, FMI or FFMI at 3 years.

Early feeding patterns as effect modifiers

The effect of birth weight on FMI at 3 years was eliminated if the child had been fully breastfed for 6 months. No effect modification was seen for duration of full breastfeeding less than 6 months. Duration of full breastfeeding 4 – 6 months had a considerable modifying effect on the impact of early growth on FMI at 3 years. This indicates that besides the direct effect breastfeeding has on early growth (11;12;32), longer duration of full breastfeeding directly influence the extent to which early rapid weight gain adversely affects FM development. A similar protective effect of full breastfeeding for at least 4 months among infants with early rapid weight gain with respect to body fat percentage at 2 and 5 years have been demonstrated in 249 children from the DONALD study (47). Preliminary data from the PROBIT study has also shown that the strong relationship between early rapid weight gain and obesity at 6 – 7 years of age was higher among children who had been exclusively formula fed from 1 months of age compared to other infants (Manasseh *et al.*, 2010, abstr). Since the present study is observational, we cannot say if the interactions are explained by physiology or rather decisions made by the mother or advices given by health care workers. The duration of full breastfeeding and age of introduction to solids are by nature interdependent. Infants in the SKOT cohort still partially breastfed at 9 months had been introduced to complementary foods later than non-breastfed infants a 9 months (48). Moreover, at 9 months non-breastfed infants in this cohort had higher intakes of protein in the complementary diet compared to partially breastfed infants (48) also found to modify the effect of early growth on FM development (47). The fact that we did not see a direct effect of early feeding on FMI could be due to insufficient power, a low share of overweight children in our cohort or that the differences in FM at 3 years is too small compared with later ages.

The effects of birth weight and early growth on FFMI were not affected by infant nutrition. Thus, we speculate that factors in pregnancy, the child's level of physical activity and the dietary quality beyond infancy have greater impact on FFMI.

Strengths and limitations

In this study FM and FFM was predicted from predictive equations generated from a large number of DXA scans in the same cohort. This was done to be able to include more children in the analyses as we had acceptable DXA scans from only 101 children while we had bioelectrical impedance from 233 children (36). Compared with DXA the predictions are connected with a prediction error of 2.8 % (333 g) for FFM and 11.3 % (284 g) for FM (36). A strength of the study is the prospective and detailed information obtained on growth and dietary data. Unfortunately, information on age of introduction to solids was crude (in months) and did not allow for more detailed grouping of the age of introduction. It is a limitation that only 21 infants were fully breastfed at 6 months. Thus, the interaction between birth weight and duration of full breastfeeding on FMI was only based on these 21 infants since the effect was not modified by full breastfeeding for less than 6 months. The families in the SKOT study are characterized by long educations and high incomes known to influence

choice of breastfeeding, age of introduction to solids and the complementary diet (19) possibly reducing extremes in infant feeding practices. In contrast to other studies (28;29), most children in the SKOT study were introduced to solids at 4 months of age or later. Despite this relative homogeneity, infant feeding practices were found to modify the effect of early growth. The effect modifications of early feeding are likely to depend on the alternatives to breastfeeding which differs from country to country and we encourage other studies to investigate similar effect modifications of infant feeding on early growth to extend the generalizability to populations with other characteristics.

Conclusion

In conclusion, birth weight and weight gain the first 5 months of life had a strong influence on later body composition both in terms of FM and FFM. None of the other periods from 5 months to 3 years showed significant relations with FM and FFM at 3 years. Moreover, we demonstrated that full breastfeeding for 4 - 6 months attenuated the impact of early growth on FMI at 3 years while full breastfeeding for 6 months eliminated the impact of high birth weight on FMI. This complex relation suggests that breastfeeding has an additional protective effect besides the direct effect on early growth. Infant feeding did not modify the effect of early growth on FFMI. Our results suggest that early feeding can be important to modify the effect of high birth weight and excessive weight in early infancy and support current recommendations of duration of full breastfeeding.

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Table 1. Anthropometric characteristics in the SKOT cohort according to WHO z-scores.

	Mean	Range [min - max]	P-value
At birth (n = 233)[1]			
WAZ	0.48	-1.98 – 2.71	< 0.001
HAZ	1.39	-3.84 – 4.29	< 0.001
WFH	-0.95	-4.71 – 2.54	< 0.001
BAZ	-0.35	-3.99 – 2.63	< 0.001
At 5 months (n = 167)[2]			
WAZ	0.32	-2.00 – 3.29	< 0.001
HAZ	1.21	-1.12 – 4-21	< 0.001
WFH	-0.40	-2.57 – 2.89	< 0.001
BAZ	-0.48	-2.62 – 2.88	< 0.001
At 9 months (n = 233)			
WAZ	0.40	-1.62 – 3.00	< 0.001
HAZ	0.25	-1.94 – 2.84	< 0.001
WFH	0.43	-1.52 – 4.21	< 0.001
BAZ	0.35	-1.75 – 4.37	< 0.001
At 18 months (n = 229)			
WAZ	0.41	-2.02 – 2.58	< 0.001
HAZ[3]	0.11	-2.91 – 2.53	0.066
WFH[3]	0.49	- 1.86 – 3.00	< 0.001
BAZ[3]	0.49	-1.69 – 3.14	< 0.001
At 36 months (n = 233)			
WAZ	0.17	-2.06 – 2.04	< 0.001
HAZ	-0.05	-2.09 – 2.36	0.34
WFH	0.28	-1.76 – 2.36	< 0.001
BAZ	0.27	-1.78 – 2.44	< 0.001

[1] Measured by midwifes at the hospital.

[2] Measured by general medical practitioner

[3] Missing values for 3 children.

Data presented as mean [minimum - maximum]. Differences between WAZ, HAZ, WFH and BAZ in the study sample and the WHO standards were evaluated by means of one-sample *t*-tests. BAZ, BMI-for-age z-score; HAZ, height/length-for-age z-score; WAZ, weight-for-age z-score; WFH, weight-for-height/length z-score.

Table 2. Characteristics of the SKOT children according to FMI quartile at 3 years of age

		n	Overall	3-year FMI quartile				P for trend
				1 (n = 58)	2 (n = 58)	3 (n = 59)	4 (n = 58)	
Anthropometric characteristics								
FM (kg)[1]	Girls	122	2.71 (0.74)	1.82 (0.34)	2.44 (0.23)	3.00 (0.21)	3.61 (0.43)	<0.001
	Boys	111	2.29 (0.65)	1.56 (0.38)	2.03 (0.16)	2.41 (0.20)	3.13 (0.38)	<0.001
FFM (kg)[1]	Girls	122	11.65 (0.90)	11.26 (0.93)	11.33 (0.76)	11.97 (0.76)	12.05 (0.88)	<0.001
	Boys	111	12.67 (1.01)	12.46 (1.06)	12.41 (1.06)	12.67 (0.78)	13.14 (0.99)	0.008
BMI (kg/m^2)		233	15.8 [15.1;16.6]	14.8 [14.3;15.3]	15.4 [15.1;15.8]	16.0 [15.7;16.4]	17.4 [16.9;17.9]	< 0.001
Skin folds triceps (mm)		224	9.3 [8.1;10.4]	8.2 [7.6;9.3]	8.8 [8.0;10.3]	9.4 [8.3;10.4]	10.4 [9.4;11.8]	< 0.001
Skin folds subscapular (mm)		230	6.3 [5.5;7.2]	5.8 [5.2;6.4]	6.1 [5.4;6.7]	6.3 [5.7;7.2]	7.7 [6.3;8.7]	< 0.001
Infant feeding								
Days of exclusive BF		233	126 [91;152]	125 [122;152]	135 [117;166]	122 [61;143]	129 [61;152]	0.20
Fully BF at 4 months, % (n)		233	67.8 (158)	77.2 (44)	74.6 (44)	56.9 (33)	62.7 (37)	0.028
Days of any BF		230	274 [183;349]	304 [212;357]	284 [183;365]	227 [152;318]	265 [196;365]	0.98
Partial BF at 9 months, % (n)		233	42.5 (99)	50.9 (29)	42.4 (25)	34.5 (20)	42.4 (25)	0.31
Formula at 9 months (g/d)		233	169 [0;410]	93 [0;334]	169 [7;401]	269 [11;449]	138 [0;447]	0.15
Age of introduction to solids (mo)		233	4.0 [4.0;5.0]	4.0 [4.0;5.0]	4.0 [4.0;5.0]	4.0 [4.0;5.0]	4.0 [4.0;5.0]	0.95

Data presented as mean (SD) or median [IQR] if not normal distributed. Trends across FMI quartiles were assessed by linear regression models adjusted for gender.

[1]Not adjusted for gender

BF, Breastfed; FFM, fat-free mass; FM, fat mass; FMI, fat mass index (kg/m^2); mo, months.

Table 3. Characteristics of the SKOT children according to FFMI quartile at 3 years of age

		n	Overall	3-year FFMI quartile				P for trend
				1 (n = 57)	2 (n = 59)	3 (n = 59)	4 (n = 58)	
Anthropometric characteristics								
FM (kg)[1]	Girls	122	2.71 (0.74)	2.37 (0.59)	2.46 (0.62)	2.78 (0.65)	3.24 (0.76)	< 0.001
	Boys	111	2.29 (0.65)	1.99 (0.48)	2.28 (0.51)	2.19 (0.78)	2.69 (0.59)	< 0.001
FFM (kg)[1]	Girls	122	11.65 (0.90)	11.22 (0.85)	11.31 (0.82)	11.77 (0.80)	12.30 (0.73)	< 0.001
	Boys	111	12.67 (1.01)	12.03 (0.89)	12.49 (0.83)	12.79 (0.90)	13.38 (0.95)	< 0.001
BMI (kg/m^2)		233	15.8 [15.1;16.6]	14.8 [14.4;15.2]	15.6 [15.2;16.0]	15.9 [15.5;16.6]	17.3 [16.7;17.9]	< 0.001
Skin folds triceps (mm)		224	9.3 [8.1;10.4]	8.6 [7.7;9.9]	9.0 [8.0;10.2]	9.4 [8.3;10.7]	9.7 [8.8;11.1]	< 0.001
Skin folds subscapular (mm)		230	6.3 [5.5;7.2]	5.9 [5.2;6.7]	6.3 [5.4;6.9]	6.4 [5.7;7.4]	6.9 [5.9;8.2]	< 0.001
Infant feeding								
Days of exclusive BF		233	126 [91;152]	132 [105;152]	136 [115;152]	122 [44;152]	122 [75;152]	0.24
Fully BF at 4 months, % (n)		233	67.8 (158)	70.7 (41)	74.1 (43)	62.7 (37)	63.8 (37)	0.26
Days of any BF		230	274 [183;349]	274 [183;335]	318 [197;365]	244 [173;335]	274 [183;365]	0.99
Partial BF at 9 months, % (n)		233	42.5 (99)	48.3 (28)	48.3 (28)	33.9 (20)	39.7 (23)	0.19
Formula at 9 months (g/d)		233	169 [0;410]	253 [14;429]	89 [0;316]	231 [0;432]	146 [0;422]	0.83
Age of introduction to solids (mo)		233	4.0 [4.0;5.0]	4.0 [4.0;5.0]	4.0 [4.0;5.0]	4.0 [4.0;5.0]	4.0 [4.0;5.0]	0.52

Data presented as mean (SD) or median [IQR] if not normal distributed. Trends across FFMI quartiles were assessed by linear regression models adjusted for gender. [1]Not adjusted for gender

BF, Breastfed; FFM, fat-free mass; FM, fat mass; FFMI, fat-free mass index (kg/m^2); mo, months.

Table 4. Relation between weight gain during four time periods (Δ weight-for-age z-score) and body composition outcomes at 3 years of age expressed as regression coefficients.

	Outcomes at 3 years										
		Height (cm)		Weight (kg)		BMI (kg/m^2)		FMI (kg/m^2)	FFMI (kg/m^2)		∑ SF (mm)
	n	β (SE)	n	β (SE)	n	β (SE)	n	β (SE)	β (SE)	n	β (SE)
Simple model											
BWZ	233	0.80 (0.23)[c]	233	0.59 (0.10)[c]	233	0.38 (0.08)[c]	233	0.21 (0.05)[c]	0.15 (0.04)[c]	223	0.32 (0.23)
Δ WAZ, 0-5 mo	167	0.83 (0.30)[b]	167	0.85 (0.11)[c]	167	0.66 (0.09)[c]	167	0.42 (0.06)[c]	0.22 (0.05)[c]	161	1.33 (0.22)[c]
Δ WAZ, 5-9 mo	167	1.34 (0.38)[c]	167	0.68 (0.19)[c]	167	0.29 (0.15)	167	0.12 (0.10)	0.15 (0.09)	161	0.09 (0.35)
Δ WAZ, 9-18 mo	229	0.72 (0.42)	229	0.12 (0.21)	229	-0.10 (0.16)	229	-0.18 (0.10)	0.08 (0.08)	219	-0.46 (0.37)
Δ WAZ, 18-36 mo	229	1.66 (0.47)[c]	229	0.72 (0.21)[c]	229	0.24 (0.18)	229	0.14 (0.11)	0.08 (0.09)	219	0.10 (0.38)
Adjusted model											
BWZ	217	0.68 (0.21)[c]	215	0.52 (0.10)[c]	215	0.35 (0.08)[c]	215	0.21 (0.05)[c]	0.13 (0.05)[b]	206	0.24 (0.25)
Δ WAZ, 0-5 mo	153	0.70 (0.28)[a]	155	0.78 (0.10)[c]	152	0.64 (0.09)[c]	152	0.42 (0.06)[c]	0.20 (0.06)[c]	147	1.43 (0.26)[c]
Δ WAZ, 5-9 mo	153	1.26 (0.46)[b]	155	0.56 (0.21)[b]	152	0.10 (0.17)	152	-0.02 (0.11)	0.10 (0.10)	147	-0.30 (0.45)
Δ WAZ, 9-18 mo	213	0.94 (0.46)[a]	211	0.19 (0.21)	211	-0.10 (0.16)	211	-0.17 (0.10)	0.07 (0.08)	202	-0.47 (0.43)
Δ WAZ, 18-36 mo	213	1.09 (0.44)[a]	211	0.62 (0.21)[b]	211	0.22 (0.18)	211	0.10 (0.12)	0.10 (0.10)	202	0.04 (0.41)

[a] $p < 0.05$.

[b] $p \leq 0.01$.

[c] $p \leq 0.001$.

Each cell represents a multiple regression model with body composition at 3 years as dependent variables and left row as explanatory variables. The simple model adjusted for gender and BWZ. The adjusted model adjusted for gender, BWZ, income, mother's educational

level, smoking during pregnancy and gestational weight gain. Height was additionally adjusted for parental height. Weight was additionally adjusted for parental weight. BMI, FMI, FFMI and sum of skinfolds were all additional adjusted for parental BMI. BWZ, birth weight z-score; FMI, fat mass index; FFMI, fat-free mass index; mo, months; ∑ SF, sum of triceps and subscapular skin folds; WAZ, weight-for-age z-score.

Table 5: Prevalence of children in the 4th quartile of FMI and FFMI according to birth weight and WAZ change from 0 - 5 months.

	n	n placed in 4th quartile of FMI (%)	P[1]	n placed in 4th quartile of FFMI (%)	P[1]
Birth weight (g)					
< 3000	23	5 (21.7)	0.167[2]	3 (13.0)	0.039[2]
3000 - 3499	92	16 (17.4)	0.007	16 (17.4)	0.007
3500 - 3999	83	23 (27.7)	0.189	25 (30.1)	0.297
≥ 4000	35	14 (40.0)	reference	14 (40.0)	reference
Δ WAZ, 0-5 months					
< -0.67	49	7 (14.3)	0.001	10 (20.4)	0.472
-0.67- 0	41	6 (14.6)	0.001	7 (17.1)	0.288
0 - 0.67	40	10 (25.0)	0.031	9 (22.5)	0.645
> 0.67	37	18 (48.7)	reference	10 (27.0)	reference

[1] Comparison of group difference against reference by Pearson X^2.

[2] Comparison by Fischer's exact.

FMI, fat mass index (kg/m^2); FFMI, fat-free mass index (kg/m^2); Δ WAZ, change in weight-for-age z-score.

Table 6: Multiple regression model of the association between birth weight, weight gain from 0-5 months, infant feeding and FMI at 3 years of age (n = 152).

Risk factor	Estimate ± SE	P
Intercept	2.42 ± 0.64	< 0.001
BWZ	0.69 ± 0.15	< 0.001
Δ WAZ 0-5 months	0.81 ± 0.18	< 0.001
Sex (0, female; 1, male)	-0.59 ± 0.09	< 0.001
Full breastfeeding		
< 1 months	reference	
1-3 months	-0.03 ± 0.20	0.881
4-5 months	-0.07 ± 0.15	0.661
6 months	-0.29 ± 0.31	0.339
Age of introduction		
3-4 months	reference	
5 months	0.14 ± 0.12	0.227
6 months	0.44 ± 0.28	0.122
Maternal BMI	0.00 ± 0.02	0.850
Gestational weight gain	-0.01 ± 0.01	0.337
Maternal educational level		
No education above school level	reference	
1-2 years	0.53 ± 0.35	0.130
Further education < 3 years	0.45 ± 0.32	0.154
Further education 3-4 years	0.35 ± 0.30	0.240
Further education > 4 years	0.40 ± 0.31	0.200
Smoking during pregnancy (0, No; 1, Yes)	0.07 ± 0.29	0.796
Paternal BMI	0.00 ± 0.02	0.881
Household income	0.00 ± 0.01	0.878
BWZ x full breastfeeding		
< 1 months	reference	
1-3 months	-0.09 ± 0.22	0.670
4-5 months	-0.17 ± 0.17	0.329
6 months	-0.76 ± 0.24	0.002
Δ WAZ 0-5 months x full breastfeeding		
< 1 months	reference	
1-3 months	-0.37 ± 0.20	0.075
4-5 months	-0.38 ± 0.19	0.050
6 months	-0.97 ± 0.30	0.002

A multiple regression model with FMI at 3 years as the dependent variable and variables listed in the left row as explanatory variables. The intercept represents the mean value of FMI

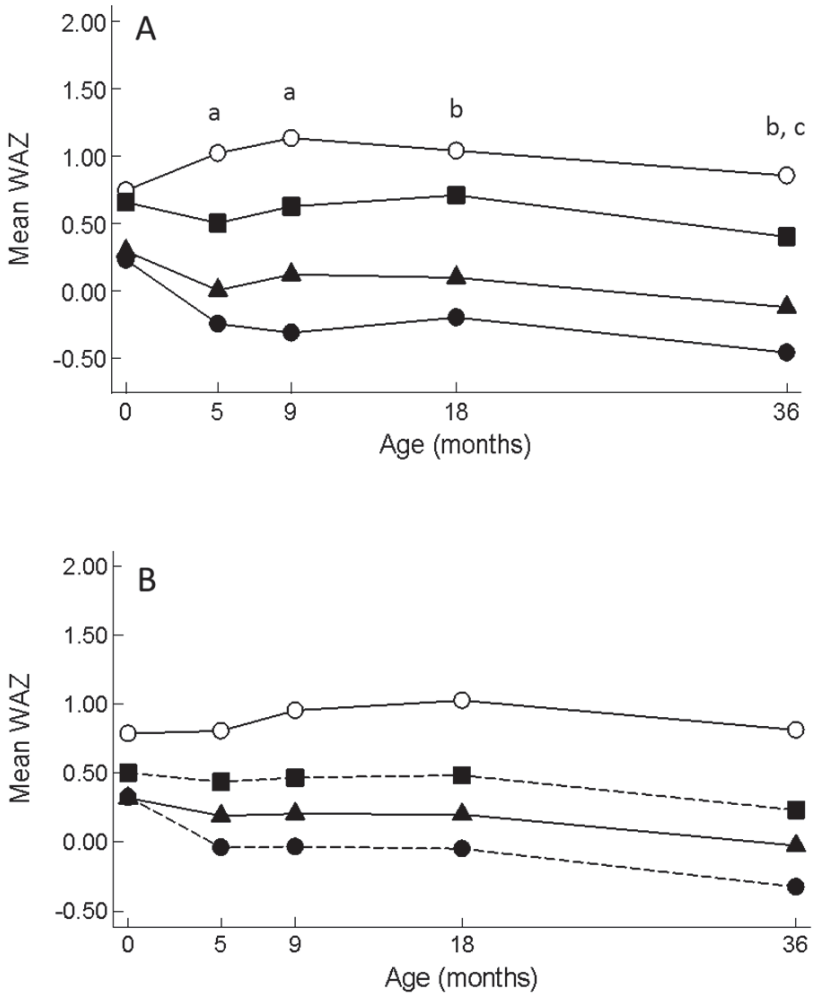
at 3 years in the reference groups. BWZ, birth weight z-score; FMI, fat mass index; WAZ, weight-for-age z-score.

Legend for figures

Figure 1: Change in WAZ (weight-for-age z-scores) from 0- 36 months according to A, fat mass index (FMI) and B, fat-free mass index (FFMI) quartiles at 3 years of age. 1st quartile (●), 2nd quartile (▲), 3rd quartile (■), 4th quartile (○). Differences between quartiles were assessed by mixed model analyses controlled for educational level of the mother, smoking during pregnancy, gestational weight gain, household income and parental BMI. [a] 4th quartile (reference) higher than all other quartiles ($p < 0.05$). [b] 4th quartile higher than 1st and 2nd quartiles ($p < 0.002$). [c] 4th quartile borderline higher than 3rd quartile ($p < 0.08$).

Figure 2: Predicted mean fat mass index (FMI, kg/m^2) at 3 years according to A, birth weight z-score (BWZ) and B, change in weight-for-age z-scores (WAZ) from 0 - 5 months by subgroups of breastfeeding duration in 152 children in the SKOT cohort. Figure lines are drawn from the coefficients in question derived from the multiple regression model presented in Table 6. A, Illustrates the interaction between BWZ and duration of full breastfeeding. B, Illustrates the interaction between WAZ change from 0 – 5 months and duration of full breastfeeding. Duration of full breastfeeding < 1 months (●, n = 36), 1 - 3 months (▲, n = 39), 4 - 5 months (■, n = 137) and 6 month (○, n = 21).

Figure 1.


A
2.00
1.50
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Mean WAZ
a
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36
Age (months)
B
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-0.50
Mean WAZ
0
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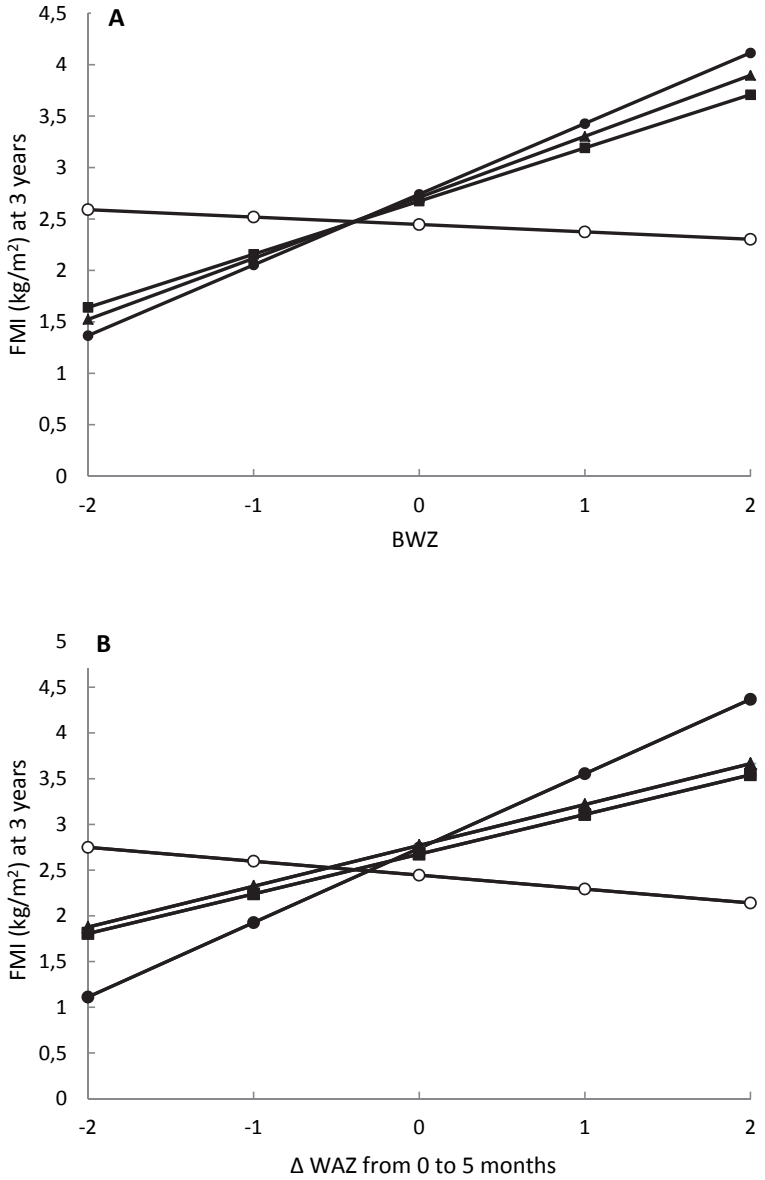
18
36
Age (months)

Figure 2.


A
4,5
4
3,5
3
2,5
2
1,5
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FMI (kg/m2) at 3 years
-2
-1
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1
2
BWZ
B
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
FMI (kg/m2) at 3 years
-2
-1
0
1
2
Δ WAZ from 0 to 5 months

PAPER III

IGF-I at 9 and 36 months of age - relations with body composition and diet at 3 years - the SKOT cohort

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Running title:
IGF-I, diet and body composition at 3 years

ABSTRACT

Objective: High infancy levels of insulin-like growth factor-I (IGF-I) have been associated with increased linear growth and fat-free mass (FFM) but also with risk of obesity. This paper examines how IGF-I at 9 and 36 months relates to diet and body composition.
Design: Healthy term infants were examined at 9 and 36 months with anthropometry, bioelectrical impedance (36 months), 7-day food records and blood analysis of IGF-I and IGFBP-3 by chemiluminescence.
Results: Total and free IGF-I at 36 months (n=229) were positively correlated with 9 months values and values were considerably higher in girls (43% and 25%, respectively). Children breastfed at 9 months had lower values at 9 months but reached the same IGF-I levels at 36 months as infants not breastfed at 9 months. IGF-I values at 36 months were positively associated with height, weight, BMI, FFM and FFM index. Although there were also a positive association with fat mass (FM) there was no association with FM index and a negative association with skin fold thickness. A change in IGF-I from 9 – 36 months was positively related to FFM and FFM index but not BMI, FM and FM index. No associations were seen between IGF-I variables and current intake of milk, meat or protein energy percentage, but both fat and saturated fat energy percentage were negatively associated with IGF-I variables.
Conclusion: IGF-I concentrations were positively associated with growth but not with adiposity. However, the higher tempo of growth could influence age at adiposity rebound and thereby later risk of obesity. Milk and protein intake at 36 months did not influence IGF-I but there was a negative association with intake of fat and saturated fat. The implications of this finding for development of obesity need further exploration.

Keywords: IGF-I, IGFBP-3, breastfeeding, diet, body composition, growth, early childhood.

INTRODUCTION

Insulin-like growth factor-I (IGF-I) enhance bone growth, muscle mass and in some studies adipogenic activity [1-3]. High IGF-I concentrations are associated with rapid growth in infancy and early childhood [4-6] which is a recognised predictor for later obesity [7;8]. However, the role of IGF-I in the development of obesity is complex as there are some indications that IGF-I enhance linear growth and fat-free mass (FFM) in infancy rather than fat mass (FM) [6] . New data suggest that IGF-I levels decline from about 2 months to about 6 - 8 months and thereafter increase again, hereby following the inverse pattern of change in BMI in the same period [1]. One of the proposed mechanisms for a role of IGF-I in the development of later obesity is that high levels of IGF-I in early childhood lead to accelerated maturation or tempo of growth and induce early adiposity rebound which is associated with elevated risk of obesity [9].

IGF-I concentration is mainly regulated by growth hormone but is also highly responsive to nutrition during the first 1 - 2 years [2]. Especially intake of cow's milk and protein intake seems to stimulate IGF-I [1;10;11]. However, the immediate positive relation between IGF-I and cow's milk intake appears to be modified later in childhood. In one of our cohorts we found a negative correlation between IGF-I levels at 9 months and 17 years indicating a long-term programming effects on the IGF-I axis [11]. Early diet has been hypothesised to have a programming effect through a resetting of the pituitary control of growth hormone on IGF-I levels as a response to high IGF-I in childhood [11]. Breastfeeding is associated with lower IGF-I concentrations in infancy [5;6;12] and earlier finding from the SKOT cohort showed a negative dose-response relationship between intensity of breastfeeding at 9 months and IGF-I concentrations at 9 months [4]. There are some indications that the relationship between breastfeeding and IGF-I reverse later in childhood with higher IGF-I concentrations among children who had been breastfed as infants [13;14]. However, new results from the PROBIT study showed no effect of a breastfeeding intervention prolonging duration of breastfeeding on IGF-I concentrations at 11.5 years, and there were no difference in IGF-I concentrations at 11.5 years between infants exclusively breastfed for 3 to less than 6 months compared with 6 months or more [15]. The longer duration of breastfeeding had no effect on overweight and obesity in this population.

Differences in the protein content of breast milk and formula milk is likely to be a main factor responsible for differences in IGF-I concentrations in infancy. The large randomised multicentre trial, CHOP, that compared infant formula with high or low protein content showed approximately 40 % higher IGF-I levels at 6 months in the high protein group compared to the low protein group while breastfed infants had approximately 60 % lower IGF-I compared to those getting the formula with low protein [5]. Also beyond infancy IGF-I concentrations have been found to be influenced by diet; especially protein and cow's milk [16-18]. Furthermore, several observational studies have shown that high protein intake in early childhood is related to early adiposity rebound and higher risk of obesity [19-22].

Modulation of the IGF-I axis and how it relates to growth and body composition during the first years of life is therefore complex and still not fully understood. In this study we aimed to investigate the relation between IGF-I at two time points in early childhood (9 and 36 months) and

relate this to body composition at 36 months of age. Further, we wanted to examine the influence of current diet on total and free IGF-I concentrations at 3 years. The associations between IGF-I at 9 months, early growth, complementary feeding and breastfeeding practices in this cohort have been reported by Madsen *et al.* [4]. We hypothesised that IGF-I concentrations at 9 and 36 months would be positively related to height, FFM and FM at 36 months and that history of breastfeeding and current diet would be important predictors of IGF-I concentrations at 36 months.

MATERIALS AND METHODS

Study design

Analyses include data from the SKOT study, which has previously been described in details elsewhere [4;23-25]. In brief, SKOT was a prospective observational cohort study monitoring young children from 9 - 36 months of age. During the study, children were examined three times at age 9 months ± 2 weeks, 18 months ± 1 months and 36 months ± 3 months, respectively. The study was conducted at Department of Nutrition, Exercise and Sports, Frederiksberg, Denmark.

The study protocol was approved by The Committee on Biomedical Research Ethics for the Capital Region of Denmark (H-KF-2007-0003).

Body composition

Information on weight and length at birth was obtained from birth charts, while all remaining measurements were conducted at the examinations (described in detailed elsewhere [4;23-25]). All measurements (except weight) were performed in triplicates, with the mean value used in analysis.

We have previously generated an equation for predicting FFM in 3-year-old children using whole body resistance obtained from bioelectrical impedance, height and weight [25]. The bioelectrical impedance measurement was performed twice consecutively with the child in a supine position by a single frequency (50 kHz) tetra polar bioelectrical impedance analyser (Quantum III, RJL Systems, Michigan, USA). Mean values of resistance were used in the equation. The reference method for the predictive equation was dual-energy X-ray analysis (DXA), which was made in a sub-group of the SKOT children (n = 99) as previously described [25].

Blood variables

For the IGF-I and IGF binding protein-3 (IGFBP-3) analyses at both 9 and 36 months, a venous blood sample (6 ml) was taken in a test tube containing lithium-heparin. EMLA patches (AstraZeneca AB, Södertälje, Sweden) provided local anaesthesia of the skin. Children were fasted approximately 2 hours before blood sampling, and content and time of last meal before fasting were recorded and later analysed using Dankost (Dankost version 3000, Dankost Ltd, Copenhagen, Denmark). Plasma IGF-I and IGFBP-3 concentrations were determined by an automated, enzyme-labelled chemiluminescent immunometric assay performed on IMMULITE 1000 (Siemens Medical Solutions Diagnostics, Los Angeles, United States). The intra-assay variations for IGF-I and IGFBP-3 were 2.1% and 3.1%, respectively, while inter-assay variations were 7.2% and 4.3%, respectively. The IMMULITE system had a detection level of IGF-I at 25 ng/ml, and 20 children had a value below this level at 9 months and one child had a value below this level at 36 months. These IGF-I values were recoded with a value of 12.0 ng/ml.

Diet

At all three examinations, children's diet were recorded for at least 4 consecutive days by parents and caregivers in a validated pre-coded dietary questionnaire, as previously described [26]. Nutritional intake was calculated by the GIES software (Version 1.000 d, The National Food Institute, Søborg, Denmark). Information on type of infant feeding and duration of full and partial breastfeeding was obtained from the 9, 18 and 36 months examinations. Intensity of breastfeeding at 9 months defined as the number of meals per day was recorded as "not breastfed", "0 - 2 times per day", "3 - 5 times per day" and "more than 6 times day" as in Madsen *et al.* [4]. Breastfeeding was categorized as full, if the child only had received breast milk, water and vitamins but allowed exceptional bottle feeding e.g. if the child have been babysit for a single night (this changed the duration of exclusive breastfeeding for 15 infants).

Calculations

Free IGF-I was calculated as a ratio of IGF-I to IGFBP-3 using the following equivalents for conversion [27]: 1 ng/ml IGF-I = 0.133 nM IGF-I and 1 µg/ml IGFBP-3 = 33 nM IGFBP-3. Weight, length/height and BMI were converted into age- and gender-specific standard deviation scores (SDS) by the software program WHO Anthro 2005 [28]. The share of overweight and obese children in the cohort was determined according to the IOTF BMI cut-off values [29] and the WHO growth standards [30]. FFM, FM and FM % were calculated using the following equations:

$FFM = 327.2\ RI + 223.8\ Weight + 76.8\ Height + 417.6\ sex - 2784.4$

$FM = (0.981\ Weight + 0.374) \times 1000 - FFM$

$FM\ \% = FM / (FM + FFM) \times 100$

FFM and FM are in grams, RI is the resistance index (height $(cm)^2$/resistance (Ω)), Weight is the digital weight in kg, Height is in cm, and sex was recorded as male = 1 and female = 0. To analyse an effect of IGF-I on FM and FFM fully adjusted for height, we calculated fat mass index, FMI (FM (kg)/height $(m)^2$) and fat-free mass index, FFMI (FFM (kg)/height $(m)^2$) [31]. Regression analyses of these indices against height confirmed that neither FFMI nor FMI were longer associated with height ($p > 0.5$).

Statistics

Gender differences in anthropometry, diet, and IGF variables at 36 months were assessed using two-sample t-tests and, where appropriate, Wilcoxon rank sum tests. Likewise, comparison of children with and without IGF-I measurements was based on two-sample *t*-tests or Wilcoxon rank sum tests. For children who were and were not breastfed at 9 months, differences in changes in weight-for-age SDS (weight SDS) from 0 - 9 and 9 - 36 months were compared by means of two-sample *t*-tests, while differences in change in IGF-I from 9 - 36 months according to the breastfeeding status at 9 months were assessed by Wilcoxon rank sum tests. Test for trend in the change of IGF-I from 9 - 36 months according to degree of breastfeeding at 9 months was based on linear regression for logarithm-transformed changes. The associations between IGF variables at 36 months and duration of full and partial breastfeeding were investigated by multiple linear

regressions including adjustment for gender. Where appropriate IGF variables were logarithm transformed to meet the regression model assumptions. The analyses of current diet and IGF variables included adjustment for gender. Absolute intakes were additionally adjusted for weight at 36 months.

The relationships between total and free IGF-I at 9 months and body composition at 36 months were analysed by multiple regression models that were adjusted for both gender and birth weight, since it was anticipated that birth weight was correlated to both IGF-I at 9 months and body composition at 36 months. Additionally, the regression models for sum of triceps and subscapularis skinfolds and waist circumference included adjustment for weight at 36 months to assess if a possible association was explained by the current weight. The regression models for change in total and free IGF-I from 9 - 36 months and body composition at 36 months were adjusted for gender, birth weight, and total or free IGF-I at 9 months. All models were repeated with gender as possible effect modifier. For all regression analyses, model assumptions were checked using residual and normal probability plots. Robust standard errors were used in case substantial discrepancies were found. To control the overall risk of a false positive finding across all outcomes considered the corresponding p-values were adjusted. Data were analysed by STATA version 11.0 (StataCorp LP, Texas, USA) except for the adjustment of p-values, which was based on the method by Pipper *et al.* [32] and was performed in the open-source statistical programming environment R [33]. The significance level was set at $\alpha = 0.05$.

RESULTS

Subjects

Of the 263 children (79.7%) who completed the examination at 36 months, 229 (115 girls) had information on IGF-I concentrations and were included in the analyses. Two hundred thirty nine children had IGF-I measured at 9 months, and 202 children had IGF-I measured at both 9 and 36 months. At 36 months, 8.6 % of the children in this analysis (n = 20) were overweight and none were obese determined by the IOTF BMI cut-off values while 22.7 % were at risk for overweight (BMI-for-age SDS between 1 - 2, n = 52), 1.7 % were overweight (BMI SDS between 2 - 3, n = 4) and none were obese (BMI SDS above 3, n = 0) according to the WHO growth standards.

IGF-variables at 9 months and 3 years

Children with IGF-I measured at 36 months did not differ from children without IGF-I measurement at 36 months with regards to IGF-I concentrations at 9 months ($p = 0.26$), nor did they differ in BMI or FMI at 36 months (both $p > 0.85$) (data not shown). Girls had considerable higher values of IGF-I, IGFBP-3 and free IGF-I than boys (all, $p < 0.001$) (Table 1). Total and free IGF-I concentrations at age 9 and 36 months were positively correlated ($p < 0.001$) (**Figure 1**, A & B).

Anthropometrics and growth

At 36 months, all anthropometric variables differed between genders, except for waist circumference, triceps skinfold thickness and BMI (Table 1). Boys were heavier, taller and had more FFM, while girls had thicker subscapularis skin folds and more FM. The differences in body composition were still significant when expressing FM and FFM against height (FMI and FFMI, both $p < 0.001$). No differences in early growth were seen between genders ($p > 0.52$). Infants breastfed at 9 months had gained less weight from 0 - 9 months than infants not breastfed at 9 months (change in weight SDS: mean: -0.30 (SD: 0.93) versus 0.15 (1.03), $p = 0.0002$), but no differences in weight gain were observed between these two groups from 9 - 36 months (weight SDS change: -0.17 (0.52) versus -0.22 (0.60), $p = 0.47$).

Associations between diet and IGF-I at 3 years of age

Duration of exclusive and partial breastfeeding were not associated with total or free IGF-I concentrations at 36 months and there was no relation between breastfeeding status at 9 months and total IGF-I concentrations at 36 months (data not shown). However, infants who had been breastfed at 9 months had higher increase in total IGF-I between 9 and 36 months compared with infants that were not breastfed at 9 months (median: 28.3 ng/ml [25^{th}; 75^{th} percentile: 12.9; 49.3] versus 19.6 ng/ml [2.2; 35.6], $p = 0.007$) with a positive trend toward greater increase in IGF-I from 9 – 36 months with higher intensity of breastfeeding at 9 months ($p = 0.046$).

At 36 months, boys had higher energy intake than girls ($p = 0.006$) but not when computed as energy intake per kg body weight ($p = 0.26$) (Table 1). Boys also had higher intakes of protein expressed in both grams per day and energy percentage (E %), and meat and meat products (all $p < 0.044$). An increase in energy intake with 1 MJ per day increased total IGF-I concentrations with mean 7.8 % (95 % CI: 1.2 – 14.7 %, $p = 0.02$) and each extra gram of protein per day was associated with a 0.7 % increase in total IGF-I (0.03 – 1.2 %, $p = 0.039$). However, these associations did not persist when adjusting for body weight (both, $p > 0.0.25$). For each increase in fat and saturated fat E %, total IGF-I was reduced by 1.6 % (95% CI: -2.8 – -0.3 %, $p = 0.015$) and 3.0 % (-5.0 – -0.9 %, $p = 0.006$), respectively. Likewise, each increase in fat and saturated fat E % were associated with a 1.0 % (-1.9 – 0 %, $p = 0.046$) and 2.1 % (-3.6 – -0.5 %, $p = 0.009$) decrease in free IGF-I. No association was found for protein E %, meat or milk intake. Similar analysis with 18 months diet variables showed no associations with total or free IGF at 36 months (data not shown).

Associations between IGF-I and body composition at 3 years of age

Total IGF-I concentration at 9 months were positively associated with height, weight, BMI, FM and FFM at 3 years (**Table 2**). The same associations were seen for free IGF-I at 9 months except that BMI was not associated with free IGF-I. At 36 months, total and free IGF-I concentrations were positively associated with the same anthropometric parameters as at 9 months (height, weight, BMI, FM, FFM) and also with FFMI and negatively associated with the sum of skinfolds when adjusting for weight at 36 months. A change in IGF-I from 9 - 36 months was positively associated with height, weight, FFM and FFMI irrespective of the IGF-I concentration at 9 months (all $p < 0.05$). Change in free IGF-I from 9 - 36 months differed from total IGF-I by being positively associated with BMI ($p < 0.05$). Gender did not modify the effect of IGF-I on body composition (data not shown).

DISCUSSION

Total and free IGF-I at 9 and 36 months were markers for growth in terms of linear growth, FM and FFM. Since IGF-I was not related to FMI or FM % there was no clear role of IGF-I in the development of adiposity in this group of healthy Danish 3-year-old children. While IGF-I levels at 9 and 36 months were both strongly associated with FFM and FM at 36 months, increase in IGF-I from 9 – 36 months was positively associated to FFM and FFMI, but not FM. This implies that the impact of IGF-I on body composition, and especially FM, changes according to the timing of IGF-I increase. It is possible that it is too early to see an effect of accelerated growth and maturation on FM at 36 months of age when BMI and FM % is still decreasing. Although a high degree of tracking of FM has been demonstrated from 2 - 7 years [34] and 4 - 9 years [35], other studies have shown lower BMI in early childhood among children with early adiposity rebound and thereby a higher risk of obesity later in childhood [36;37].

IGF-I and diet

Duration of full or partial breastfeeding as well as intensity of breastfeeding at 9 months were not related with IGF-I concentrations at 36 months. However, infants who had been breastfed at 9 months had a higher increase in IGF-I from 9 - 36 months than infants who were not breastfed at 9 months, hereby reaching the same level as the children who terminated breastfeeding earlier. Thus, the modulating effect of breastfeeding on IGF-I concentrations seen at 9 months [4] seemed to be transient. As we in this study found a positive correlation between total and free IGF-I at 9 and 36 months, it is possible that that the programming effect of the IGF-I axis [13;14] is not yet expressed at 36 months.

Opposite to what we had expected, intake of protein had limited influence on IGF-I concentrations at 36 months. It is possible that the variations in intake of milk (mean ± SD; 377 ml ± 141) and meat intake (50 ± 23 g) in our cohort were too small to have an impact on IGF-I concentration. On the other hand, in a cross-sectional study of 2.5-year-old Danish children we found significant correlations with milk and protein with intakes of meat and milk close to the intake and variation seen in this study [16]. In an intervention study with 8-year-old children, we found an effect of skimmed milk intake on IGF-I levels but they had a very high milk intake (1.5 litres per day during one week) [27].

Interestingly, intake of fat and saturated fat expressed as energy percentages was negatively associated with both total and free IGF-I. It is possible that there is a link between this finding and the modulating effect of human milk on IGF-I, as human milk also contains high amounts of fat and saturated [38]. Also, it is noteworthy that in the CHOP randomised controlled trial with high and low protein formula, the fat content in the formula given at 6 months when IGF-I was measured was 22 % higher in the low protein formula compared with the high protein formula [5]. Thus, the difference in fat content between the two interventions might also play a role in the effects of the two formulas on IGF-I levels. There is no evidence that fat intakes during infancy increase the risk of obesity in later life [39;40]. A recent study even found intake of fat at 2 years negatively related to FM and

skinfold thickness at 20 years of age [41]. These observations could suggest a role of fat and saturated fat in the regulation of IGF-I and we speculate that a possible protective effect of high fat intake in early childhood on later obesity risk could also to some degree be mediated through lower IGF-I levels. However, in the DONALD study a high fat intake in the second year of life was associated with less decrease in body fat from 2 – 5 years in children with rapid weight gain from 0 – 24 months while a higher decrease in body fat in the same period was seen among children without rapid weight gain [42]. Thus, the relation between diet, IGF-I and risk of early development of obesity is very complex and needs to be investigated further.

IGF-I, adiposity and body composition

As expected total and free IGF-I at both 9 and 36 months were positively related to most measures of anthropometry and body composition at 36 months. The positive associations between IGF-I and height, weight, FM and FFM was almost the same at 9 and 36 months while an increase in IGF-I from 9 - 36 months was associated with FFM and FFMI rather than FM. This could indicate differences in the effect of IGF-I on body composition according to age and timing of IGF-I increase.

In relation to body composition it is interesting that girls, who have significantly more body fat than boys, had considerably higher levels of IGF-I at both 9 and 36 months, as found in other studies of IGF-I in childhood [6;43;44]. However, the associations between IGF-I and body composition were not modified by sex.

We have previously shown that high IGF-I concentrations at 9 months was positively related to weight, length and BMI gain from 0 – 9 months, as well as weight and BMI SDS at 9 months [4]. However, subsequent change in BMI SDS was negatively associated with 9-months IGF-I due to higher gain in length. These findings are in line with Socha *et al.* [5] who found IGF-I at 6 months highly positively correlated with weight-for-length SDS from 0 – 6 months but not with subsequent change in weight-for-length SDS from 6 – 12 months. Likewise, Ong *et al.* [6] found that while IGF-I at 3 months was positively related to early weight gain (0 – 3 months) it was unrelated to weight gain from 3 – 12 months, positively related to length gain and thus negatively related to BMI change in the same period. A recent systematic review and meta-analysis [45] showed lower FM in formula fed infants from 3-6 months of age but higher FM at 12 months compared with breastfed. At the same time, FFM from 3-12 months was higher in the formula fed infants compared to breastfed infants. This finding could support a stimulating effect of IGF-I on lean mass and length gain during this period and could question a role for IGF-I in the development of obesity. On the other hand, overweight children tend to be taller than normal weight children until puberty [46-48] and have higher or normal IGF-I levels [49;50]. Early adiposity rebound has been associated with low BMI at 3 years of age [36] and found to be better correlated with height at 3 years compared to BMI [51]. Thus, it could be speculated that increased tempo of weight gain and linear growth potentially induced by IGF-I is related to early adiposity rebound and later adiposity [51;52]. The present study showed no relation between IGF-I and FM% or FMI in this group of 3-years-old Danish children. However, we cannot rule out that early IGF-I levels can influence adiposity risk at later ages.

Strenght and limitations

A strength of our study is that we have measured IGF-I twice during early life. This enable us to distinguish between the impact of very early growth from 0 – 9 months which is a growth period highly influenced by infant nutrition, and growth from 9 – 36 months where there is a shift in the regulation of growth toward growth hormone control as well as a shift in the diet towards family foods. It is also a strength that the study have detailed prospective data on anthropometry, body composition and diet on a large group of children.

FM and FFM were predicted from bioelectrical impedance, height and weight using a predictive equation generated from DXA scans in the same cohort. Although bioelectrical impedance has some limitations compared to DXA [25], the use of a predictive equation validated in the same group of children enabled us to include more children in the analyses (215 versus 93 with DXA measurements). The predictions induced an error of 2.8 % (333 g) for FFM compared with DXA values while calculation of FM induced prediction errors of 11.3 % (284 g) compared with DXA FM due to the lower FM-total body weight ratio [25]. We had a low share of overweight children in our cohort. It is possible that an effect of IGF-I on FM may not be as evident in this cohort as in other studies with a higher proportion of overweight children.

Conclusion

In this group of healthy 3-year-old children we found no clear associations between IGF-I levels and early development of overweight and obesity. However, it is possible that IGF-I is related to later risk of obesity through increased tempo of weight gain and linear growth leading to early adiposity rebound. We had expected that intake of protein and milk was positively associated with IGF-I values but this was not the case in this study. However, we found quite strong negative associations between IGF-I levels and current intake of fat and saturated fat expressed as energy percentages. To our knowledge this is the first time this has been shown. The implications of this finding need further explorations.

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AUTHOR CONTRIBUTION STATEMENT

KTE, ALA and DP analysed data and wrote the first draft of the manuscript. CR supervised the quality standards of the statistical analyses and performed the correction for multiple testing in R. All authors have been involved in the interpretation of results and commented on drafts. All authors have approved the final version of the manuscript. The authors have no conflicts of interest to disclose.

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TABLES

Table 1. Anthropometry, diet and IGF-variables at 3 years according to gender

	N	Girls	n	Boys	p-value
Anthropometry at 3 years					
Weight (kg)	115	14.31 (1.52)	114	14.96 (1.55)	0.001
Height (cm)	115	94.9 (3.2)	114	96.6 (3.5)	<0.001
BMI (kg/m^2)	115	15.9 (1.3)	114	16.0 (1.1)	0.39
Waist circumference	112	50.1 (2.8)	108	50.4 (2.8)	0.47
Triceps skin folds	110	9.4 [8.3;10.7]	105	9.2 [8.0;10.2]	0.07
Subscapularis skin folds	112	6.6 [5.9;8.2]	107	5.9 [5.4;6.7]	<0.001
FM %	113	18.5 (3.8)	102	15.2 (3.4)	<0.001
FM (kg)	113	2.69 (0.75)	102	2.30 (0.66)	<0.001
FFM (kg)	113	11.66 (0.90)	102	12.68 (1.03)	<0.001
FMI (kg/m^2)	113	2.99 (0.82)	102	2.47 (0.67)	<0.001
FFMI (kg/m^2)	113	12.97 (0.59)	102	13.62 (0.64)	<0.001
Diet variables 3 years					
Energy intake (MJ/d)	107	4.95 (0.90)	103	5.30 (0.94)	0.006
Energy (kJ/kg/d)	107	348 (67)	103	359 (70)	0.26
Fat g/d	107	46.5 [38.7;54.5]	103	49.1 [41.0;56.3]	0.16
Fat (E%)	107	35.4 (4.6)	103	34.6 (4.6)	0.20
Saturated fat (E%)	107	15.0 (2.7)	103	14.5 (2.7)	0.20
Protein (E%)	107	14.3 (1.7)	103	14.7 (1.6)	0.044
Protein (g/d)	107	41.5 (8.6)	103	45.9 (8.8)	<0.001
Meat intake (g/d)	107	45 (32;60)	103	53 (39;71)	0.018
Milk intake (g/d)	107	343 [270;454]	103	388 [294;470]	0.06
IGF-I variables at 3 years					
IGF-I (ng/ml)	115	85.3 [61.0;108.0]	114	59.8 [44.2;81.9]	<0.001
IGFPB-3 (µg/ml)	115	3.3 [2.8;3.8]	114	2.9 [2.6;3.4]	<0.001
IGF-I/IGFBP-3 molar ratio	115	0.10 [0.08;0.13]	114	0.08 [0.07;0.11]	<0.001

Data shown as mean (SD) or median [25th;75th percentile]. Compared by unpaired *t*-test or Wilcoxon rank sum (Mann-Whitney). FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat-mass index; FM %; fat-mass percentage; MJ, mega-Joule; kJ, kilo-Joule. E%, energy-percentage. IGF-I, insulin-growth-factor I; IGFBP-3, IGF binding protein-3.

Table 2. Associations between IGF-I, anthropometry and body composition at 3 years of age[1].

		Model: 9 months[2]			Model: 3 year[2]			Model: Delta[3]	
		IGF-I (ng/ml)	Free IGF-I[4]		IGF-I (ng/ml)	Free IGF-I[4]		IGF-I (ng/ml)	Free IGF-I[4]
Outcomes at 3 year	n	β (SE)	β (SE)	n	β (SE)	β (SE)	n	β (SE)	β (SE)
Height (cm)	231	0.054 (0.008)‡	39.37 (7.98)‡	229	0.035 (0.006)‡	28.45 (7.16)‡	202	0.026 (0.007)†	23.55 (7.81)*
Weight (kg)	231	0.027 (0.004)‡	19.33 (3.96)‡	229	0.018 (0.003)‡	17.95 (2.99)‡	202	0.013 (0.003)‡	14.46 (3.49)‡
BMI (kg/m^2)	231	0.011 (0.003)†	7.89 (3.11)[6]	229	0.008 (0.002)†	10.23 (2.55)†	202	0.005 (0.003)[6]	7.95 (2.81)*
∑ SF (mm)	203	- 0.017 (0.011)[5]	-12.25 (9.29)[5]	212	-0.021 (0.005)[5]†	-15.46 (6.02)[5,6]	185	-0.015 (0.006)[5,6]	-10.57 (6.23)[5]
WC (cm)	214	-0.003 (0.006)[5]	-5.89 (5.44)[5]	220	-0.004 (0.004)[5]	0.29 (4.54)[5]	193	-0.007 (0.005)[5]	-0.62 (4.90)[5]
FM %	207	0.026 (0.010)[6]	20.53 (10.81)	215	0.010 (0.007)	14.13 (8.22)	188	0.002 (0.010)	7.28 (9.79)
FM (kg)	207	0.008 (0.002)†	5.90 (2.06)*	215	0.005 (0.001)†	5.21 (1.55)†	188	0.003 (0.002)	3.69 (1.74)[6]
FFM (kg)	207	0.017 (0.003)‡	12.21 (2.70)‡	215	0.012 (0.002)‡	12.24 (1.94)‡	188	0.010 (0.002)‡	10.52 (2.16)‡
FMI (kg/m^2)	207	0.006 (0.002)[6]	4.17 (2.19)	215	0.003 (0.001)[6]	4.13 (1.68)[6]	188	0.001 (0.002)	2.71 (1.87)
FFMI (kg/m^2)	207	0.004 (0.002)[6]	2.23 (1.80)	215	0.004 (0.001)†	5.80 (1.34)‡	188	0.004 (0.001)*	5.36 (1.49)†

Outcomes at 3 years as dependent variable and IGF-I variables as independent variables. FFM, fat-free mass; FFMI, fat-free mass index; FM %, percentage of body fat; FM, fat mass; FMI, fat mass index; ∑ SF, sum of subscapular and triceps skin folds, WC, waist circumference.

[1] p-values corrected for multiple comparisons [32].

[2] Adjusted for gender and birth weight.

[3] Delta IGF-I: Change in total and free IGF from 9 – 36 months. Adjusted for gender, birth weight and IGF-variable at 9 months.

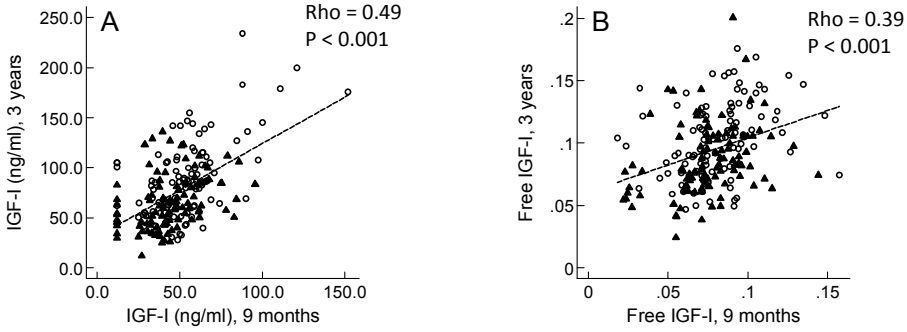
[4] Free IGF-I correspond to the IGF-I/IGFBP-3 molar ratio.

[5] Further adjusted for weight at 3 years.
[6] Significant without correction for multiple comparisons.
*, $p \leq 0.05$; †, $p \leq 0.01$; ‡, $p \leq 0.001$

FIGURE LEGEND

Figure 1. Scatter plots of A, IGF-I concentration at 9 months versus 3 years (boys: rho = 0.45; girls: rho = 0.49, both $p < 0.001$) and B, free IGF-I at 9 months versus 3 years (boys: rho = 0.38; girls: rho = 0.41, both $p < 0.001$). Girls (○), boys (▲).

Figure 1


A
Rho = 0.49
P < 0.001
IGF-I (ng/ml), 3 years
IGF-I (ng/ml), 9 months
250.0
200.0
150.0
100.0
50.0
0.0
0.0
50.0
100.0
150.0
B
Rho = 0.39
P < 0.001
Free IGF-I, 3 years
Free IGF-I, 9 months
.2
.15
.1
.05
0
0
.05
.1
.15

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KATRINE TSCHENTSCHER EJLERSKOV

Body composition at 3-years of age
The influence of early growth, infant feeding and IGF-I

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