Linköping University Medical Dissertation
No.1457

Development of body composition and its relationship with physical activity in healthy Swedish children

- A longitudinal study until 4.5 years of age including evaluation of
methods to assess physical activity and energy intake

Hanna Henriksson

Linköping University

Department of Clinical and Experimental Medicine
Faculty of Health Sciences
Linköping University, Sweden
Linköping, 2015

© Hanna Henriksson, 2015.

Cover drawing by Per Lagman.

Published articles (Papers I, III, IV) have been reprinted with permission from the respective copyright holder.

Permission to reproduce pictures of Pea Pod and the Bod Pod Pediatric option been obtained from COSMED. Permission to reproduce pictures of Actiheart has been obtained from Camntech Ltd.

Printed by Liu-Tryck, Linköping, Sweden, 2015.

ISBN 978-91-7519-093-8

ISSN 0345-0082

*To Pontus
and our beautiful children
Elias and Siri*

Drawing by Elias, 4 years

CONTENTS

1. ABSTRACT.....7

2. LIST OF PUBLICATIONS8

3. RELATED ARTICLES9

4. ABBREVIATIONS10

5. INTRODUCTION11

 5.1 Overweight and obesity in young children11

 5.2 Longitudinal body composition development11

 5.3 Assessment of body composition using air-displacement plethysmography in infancy and early childhood.....12

 5.4 Determinants of childhood obesity13

 5.5 The doubly labelled water method.....13

 5.5.1 Total energy expenditure13

 5.5.2 Physical activity level14

 5.5.3 Body composition.....14

 5.6 Assessment of physical activity and energy intake during early childhood14

 5.6.1 Physical activity14

 5.6.2 Energy intake15

6. SPECIFIC AIMS17

7. MATERIAL AND METHODS.....18

 7.1 Study design.....18

 7.1.1 Subjects (Papers I-IV).....18

 7.1.2 Study outline (Papers I-IV)20

 7.2 Methods.....23

 7.2.1 Body weight, length and height (Papers I-IV)23

 7.2.2 TEE and body composition using the doubly labelled water method (Papers I-IV)23

 7.2.3 SMR using indirect calorimetry (Papers I-III)23

 7.2.4 Reference estimates of energy expenditure in response to physical activity (Papers I-III).....24

 7.2.5 Body composition using the ADP technique (Papers I, II).....24

 7.2.6 FMI, FFMI and BMI (Paper II).....26

 7.2.7 Actiheart (Paper III)26

 7.2.8 Activity diary (Paper III)27

7.2.9 <i>Tool for Energy Balance in Children (TECH) (Paper IV)</i>	28
7.3 Ethics.....	29
7.4 Statistics	29
8. RESULTS	31
8.1 Longitudinal study (Papers I, II).....	31
8.1.1 <i>Longitudinal changes in body composition between 1 week and 4.5 years of age (Paper II)</i>	31
8.1.2 <i>Body composition in relation to PAL at 1.5 and 3 years of age (Papers I, II)</i>	36
8.2 Evaluation of Actiheart and the activity diary (Paper III)	43
8.2.1 <i>Actiheart</i>	43
8.2.2 <i>Activity diary</i>	45
8.4 Evaluation of TECH (Paper IV)	49
9. GENERAL DISCUSSION	50
9.1 Comments on the population and methods.....	50
9.1.1 <i>Study population</i>	50
9.1.2 <i>Methods to assess body composition and energy metabolism</i>	50
9.1.3 <i>Relationship between PAL and body composition</i>	51
9.1.4 <i>Choice of reference values</i>	51
9.2 Main findings: interpretation and implications.....	52
9.2.1 <i>Development of body composition during infancy and early childhood</i>	52
9.2.2 <i>Associations between PAL and body composition at 1.5 and 3 years of age</i>	53
9.2.3 <i>Assessment of physical activity and energy intake in young children</i>	53
9.2.4 <i>Assessment of energy metabolism and physical activity during growth</i>	56
10. CONCLUSIONS.....	57
11. ACKNOWLEDGEMENTS	58
12. SAMMANFATTNING PÅ SVENSKA	60
13. REFERENCES	62
14. PAPERS I-IV	

1. ABSTRACT

Childhood obesity according to the World Health Organization is one of the most serious public health challenges of the 21st century. The proportion of childhood obesity is high both globally and in Sweden. This is of great concern since obese children tend to stay obese in adulthood. In order to develop strategies to prevent early childhood obesity more knowledge is needed regarding factors explaining why children become overweight and obese. Preventive strategies require accurate and easy-to-use methods to assess physical activity in response to energy expenditure as well as energy intake in young children, but such methods are largely lacking or have shown limited accuracy. The aims of this thesis were: 1) to describe the longitudinal development of body composition from 1 week to 4.5 years of age; 2) to study relationships between measures of body composition and the physical activity level (PAL) at 1.5 and 3 years of age; 3) to evaluate if heart rate recording and movement registration using Actiheart can capture variations in total energy expenditure (TEE) and activity energy expenditure (AEE) at 1.5 and 3 years; 4) to evaluate the potential of a 7-day activity diary to assess PAL at 1.5 and 3 years of age; 5) to evaluate a new tool (TECH) using mobile phones for assessing energy intake at 3 years of age.

Healthy children were investigated at 1 and 12 weeks (n=44), at 1.5 (n=44), 3 (n=33) and 4.5 (n=26) years of age. Body composition was measured using air-displacement plethysmography at 1 and 12 weeks and at 4.5 years of age. At 1.5 and 3 years, body composition, TEE, PAL and AEE were assessed using the doubly labelled water method and indirect calorimetry. Heart rate and movements were recorded using Actiheart (four days) and physical activities were registered using the 7-day diary. Energy intake was assessed using TECH during one complete 24-hour period.

Average percentage of total body fat (TBF) and average fat mass index (FMI) were higher (+3 to +81 %), while fat-free mass index (FFMI) was slightly lower (-2 to -9 %), in children in the study from 12 weeks until 4.5 years of age when compared to corresponding reference values. A relationship between TBF% and PAL was found both at 1.5 and 3 years of age. At 3 years, but not at 1.5 years, this could be explained by a relationship between PAL and FFMI. Actiheart recordings explained a significant but small fraction (8%) of the variation in free-living TEE at 1.5 and 3 years, and in AEE (6 %) at 3 years, above that explained by body composition variables. At 1.5 and 3 years of age, PAL estimated by means of the activity diary using metabolic equivalent (MET) values by Ainsworth et al. was not significantly different from reference PAL, but the accuracy for individuals was low. Average energy intake assessed by TECH was not significantly different from TEE. However, the accuracy for individuals was poor.

The results of this thesis suggest that 1) The higher body fatness of the children in the study compared to the corresponding reference values may indicate the presence of a secular trend in body composition development characterized by a high body fatness. 2) Body fatness might counteract physical activity at 1.5 years of age when the capacity to perform physical activity is limited, but not at 3 years of age when such a capacity has been developed. 3) Actiheart recordings explained a significant but small fraction of the variation in TEE at 1.5 and 3 years, and in AEE at 3 years of age, above that explained by body composition variables. 4) The activity diary and TECH produced mean values in agreement with reference PAL and TEE, respectively, but the accuracy for individual children was low.

In conclusion, the results of this thesis suggest the presence of a secular trend in body composition development in healthy Swedish children, from infancy up to 4.5 years of age, which is characterized by a high body fatness. Methods to assess physical activity and energy intake at 1.5 and 3 years of age provided some promising results on a group level, although further research is needed to increase the accuracy of these methods in individual children.

2. LIST OF PUBLICATIONS

- I. Eriksson B*, **Henriksson H***, Löf M, Hannestad U, Forsum E. Body-composition development during early childhood and energy expenditure in response to physical activity in 1.5-y-old children. *Am J Clin Nutr* 2012; 96: 567-73.
* BE and HH contributed equally to this article.
- II. **Henriksson H**, Eriksson B, Forsum E, Flinck Carlsson E, Löf M. Development of body composition and its relationship with physical activity: A longitudinal study of Swedish children until 4.5 years of age. 2015. (Manuscript)
- III. **Henriksson H**, Forsum E, Löf M. Evaluation of Actiheart and a 7 d activity diary for estimating free-living total and activity energy expenditure using criterion methods in 1.5- and 3-year-old children. *Br J Nutr* 2014; 111(10): 1830-40.
- IV. **Henriksson H**, Bonn S, Bergström A, Bälter K, Bälter O, Delisle C, Forsum E, Löf M. A New Mobile Phone-Based Tool for Assessing Energy and Certain Food Intakes in Young Children: A Validation Study. *JMIR mHealth uHealth* 2015;3(2):e38.

3. RELATED ARTICLES

- I. Forsum E, Flinck Carlsson E, **Henriksson H**, Henriksson P, and Löf M, Total Body Fat Content versus BMI in 4-Year-Old Healthy Swedish Children, *Journal of Obesity*, vol. 2013, Article ID 206715, 4 pages, 2013. doi:10.1155/2013/206715.
- II. Forsum E, Flinck Carlsson E, **Henriksson H**, Henriksson P, and Löf M. BMI kan inte säkert identifiera 4-åringar med hög kroppsfehold. *Läkartidningen* 2013;110:CEXL.
- III. Löf M, **Henriksson H**, Forsum E. Evaluations of Actiheart, IDEEA® and RT3 monitors for estimating activity energy expenditure in free-living women. *J Nutr Sci* 2013 Sep 6;2:e31. doi: 10.1017/jns.2013.18.
- IV. Delisle C, Sandin S, Forsum E, **Henriksson H**, Trolle-Lagerros Y, Larsson C, Maddison R, Ortega F.B, Ruiz J.R, Silfvernagel K, Timpka T, Löf M. A web- and mobile phone-based intervention to prevent obesity in 4-year-olds (MINISTOP): a population-based randomized controlled trial. *BMC Public Health* 2015, 15, 95. Doi: 10.1186/s12889-015-1444-8. Published online 7th February 2015.

4. ABBREVIATIONS

ADP	Air-displacement plethysmography
AEE	Activity energy expenditure
BMI	Body mass index
BMR	Basal metabolic rate
Bpm	Beats per minute
CO ₂	Carbon dioxide
Cpm	Counts per minute
DIT	Dietary induced thermogenesis
DLW	Doubly labelled water
FFM	Fat-free mass
FMI	Fat mass index
FFMI	Fat-free mass index
² H	Deuterium
MET	Metabolic equivalent
mHR	Mean heart rate (beats per minute) provided by Actiheart
mAC	Mean activity count (counts per minute) provided by Actiheart
O ₂	Oxygen
¹⁸ O	Oxygen-18
PAL	Physical activity level
PAL _{SMR}	Physical activity level obtained as TEE divided by SMR
PAL _{Ainsworth}	PAL estimated by the activity diary using MET values published by Ainsworth et al.
PAL _{Adolph}	PAL estimated by the activity diary using MET values published by Adolph et al.
PAL _{Torun}	PAL estimated by the activity diary using MET values published by Torun.
SD	Standard deviation
SMR	Sleeping metabolic rate
TBF	Total body fat
TBW	Total body water
TECH	Tool for Energy Balance in Children
TEE	Total energy expenditure

5. INTRODUCTION

5.1 Overweight and obesity in young children

In the early 2000s, when the studies in this thesis were planned, the obesity epidemic had come into focus. Of special concern were the alarming reports that the prevalence of overweight and obesity had also increased in young children. These reports also included Sweden, where approximately 20 % of the 4-year-old children were reported to be overweight or obese ^(1;2). This is distressing, since overweight and obesity established early in life tend to persist into adulthood ⁽³⁾ where they are associated with increased risk for diseases such as diabetes, cardiovascular disease and some forms of cancer ⁽⁴⁾. A considerable amount of knowledge has been obtained in this area since the early 2000s both in terms of observational and interventional studies. Nevertheless, a recent compilation of global data has shown that obesity prevalence has risen substantially in adults and children in the past three decades and it is expected to increase further in developing countries ⁽⁵⁾. In addition, although the increase in obesity prevalence has levelled off in developed countries ⁽⁵⁾, the prevalence is still high and no substantial decrease has occurred in any developed country. Fortunately, the prevalence of overweight and obesity in Sweden is lower than in southern Europe ⁽⁶⁾ and recent reports indicate that it is no longer increasing in Swedish children at 4 ⁽⁷⁾, 7-9 ⁽⁸⁾ and at 12 years of age ⁽⁹⁾. However, the prevalence of overweight is still almost twice that of 20 years ago ⁽⁸⁾, and there is a socioeconomic gradient with higher proportions of overweight and obesity among socially disadvantaged groups ⁽¹⁰⁾. Clearly, more research is needed to understand how overweight and obesity are established. Such knowledge is important in order to prevent these conditions at a young age.

5.2 Longitudinal body composition development

Overweight and obesity are commonly defined in terms of the body mass index (BMI) of a subject, and this definition is widely used and well-accepted in adults. In children, age- and sex-specific cut-offs for overweight and obesity have been established by Cole et al. in 2-18-year-old children ⁽¹¹⁾. However, as pointed out by Wells ⁽¹²⁾, BMI is a global proxy of nutritional status and cannot differentiate between the components of body weight, i.e. the total body fat (TBF) and the fat-free mass (FFM). Indeed, we have previously shown in 4-year-old children that BMI explained only about 15 % of the variation in TBF% ⁽¹³⁾, which is much lower than the corresponding figure (50–70 %) in adults ⁽¹⁴⁾. It is also relevant to note

that TBF% has limitations, since it reflects not only the proportion of TBF but also the proportion of FFM in the body ⁽¹²⁾. A commonly used alternative is to divide BMI into two measures, the fat mass index (FMI; TBF/height²) and the fat-free mass index (FFMI; FFM/height²) ⁽¹²⁾.

In order to develop strategies for prevention of childhood obesity, knowledge is needed regarding longitudinal body composition development, i.e. changes in the amounts of TBF and FFM from birth throughout early childhood in healthy children. This may offer a possibility to identify, early in life, children at risk for obesity. However, few longitudinal data of this kind are available.

5.3 Assessment of body composition using air-displacement plethysmography in infancy and early childhood

Previously, assessment of body composition in infants and small children was difficult due to a lack of simple, yet valid, body composition methodology suitable for these age groups. It has become easier to investigate this area since the air-displacement plethysmography (ADP) technique (5) became applicable in 2004 in infants with up to 8 kg body weight using Pea Pod ^(15; 16), and in children between 2-6 years of age (8-25 kg) in 2012 using the Bod Pod Pediatric option ^(17; 18). With ADP, body volume of infants and children can be measured in an accurate, safe and non-invasive manner.

Both Pea Pod and the Bod Pod Pediatric Option consist of a scale and a chamber in which the subject's volume is measured using ADP. This technique is based on relationships between pressure and volume as formulated by Boyle and Poisson ^(15; 18). Once the measurement has been conducted, estimates of weight and body volume are used to calculate the density of the subject. This density can be used to calculate TBF% assuming a fat mass density of 0.9007 g/ml and an appropriate density of FFM ⁽¹⁹⁾. The use of ADP to estimate body composition has been proven valid against a four-component model both in infants ⁽¹⁶⁾ and in 2-6-year-old children ⁽¹⁷⁾. The introduction of Pea Pod and the Bod Pod Pediatric Option offers new possibilities to study longitudinal development of body composition in children.

5.4 Determinants of childhood obesity

A positive energy balance will lead to fat retention and ultimately to the development of overweight and obesity. Although the mechanisms behind the increase in childhood obesity are not fully understood, it is likely that low levels of energy expenditure in response to physical activity⁽²⁰⁾, as well as high energy intake are of importance. For example, energy intake has been positively related to BMI in children^{e.g. (21; 22)} and the association between levels of physical activity and body fatness has been investigated in numerous studies in children^{e.g. (23; 24)} and several have reported an inverse relationship between the physical activity level (PAL) and body fatness. Such a relationship was reported for children as early as at 9 and 14 months of age⁽²⁵⁾, and the authors suggested that a high body fatness counteracts physical activity and consequently may lead to a positive energy balance with subsequent accumulation of body fat. However, it is not known whether such a mechanism may be present also in 1.5- and 3-year- old children.

5.5 The doubly labelled water method

The doubly labelled water (DLW) method is a widely used reference for assessment of total energy expenditure (TEE), PAL and activity energy expenditure (AEE) in human subjects during free-living conditions. The isotope dilution technique inherent in the DLW method also provides a possibility to estimate body composition. The DLW method is non-invasive, involves no health hazards, and can therefore be used in adults, children and infants.

5.5.1 Total energy expenditure

When using the DLW method an oral dose of the stable isotopes deuterium (^2H) and oxygen-18 (^{18}O) is given to the subject. Urine samples are collected before dosing and during approximately one to two weeks after dosing. Isotope enrichments of dose and urine samples are assessed using isotope-ratio massspectrometry. Briefly, the method is based on the following assumptions: ^2H mixes with body water, while ^{18}O mixes both with body water and carbon dioxide (CO_2). Consequently, ^2H is lost from the body as water, while ^{18}O is lost both as water and as CO_2 . The difference between the disappearance rates of ^2H and ^{18}O is therefore a measure of the CO_2 production rate. This rate can be converted to energy expenditure using the Weir equation⁽²⁶⁾ and an appropriate food quotient (generally 0.85 for mixed diets⁽²⁷⁾). It is well documented that the DLW method is able to provide accurate estimates of CO_2 production in humans⁽²⁸⁾.

5.5.2 Physical activity level

Combining estimates obtained using the DLW method with estimates of the resting energy expenditure gives a measure of the energy expended in response to physical activity during free-living conditions. For adults, the energy expenditure during resting conditions is measured when the subject is awake, and it is referred to as the basal metabolic rate (BMR). When BMR is measured, the subject should be resting, fasting, not experiencing stress, and the measurement should be conducted in a thermo-neutral environment ⁽²⁹⁾. In young children such measurements are difficult and are therefore generally carried out when the child is asleep (the so-called sleeping metabolic rate, SMR). Energy expended in response to physical activity can be calculated as 1) PAL i.e. TEE divided by BMR or SMR; 2) AEE i.e. TEE minus BMR or SMR.

5.5.3 Body composition

Body composition can be assessed using either of the isotopes ^2H or ^{18}O to estimate total body water (TBW). If the TBW content and the hydration factor (the proportion of water in FFM) are known, it is possible to calculate FFM (TBW divided by the hydration factor). TBF is then calculated by subtracting FFM from body weight.

5.6 Assessment of physical activity and energy intake during early childhood

In order to investigate underlying factors responsible for why young children become overweight and obese, as well as to evaluate the efficacy of treatment or preventive obesity intervention programs, further research is required. Such research requires accurate and easy-to-use methods to assess energy expenditure in response to physical activity as well as energy intake in young children. However, such methods are largely lacking or have shown limited accuracy ⁽³⁰⁾.

5.6.1 Physical activity

When developing methods for assessing energy expenditure in response to physical activity it is desirable that such methods are validated against criterion methods, i.e. measurements of TEE using the DLW method in combination with BMR/SMR obtained using indirect calorimetry. Conducting such methods in young children is demanding and represents a challenge. Therefore only a few attempts to develop such methods have been made in

preschool children ^(31; 32; 33; 34; 35) and no attempts have been reported for children aged 3 years or younger.

During the last decades, different kinds of activity monitors have been developed for assessment of physical activity in human subjects; for example accelerometers and heart rate recorders. Activity monitors provide objective assessment and also have the advantage of being non-intrusive and simple to use. The Actiheart combines a uniaxial accelerometer with a heart rate recorder ⁽³⁶⁾. Studies show that Actiheart may provide valid estimates of AEE in adults ⁽³⁷⁾ and young men ⁽³⁸⁾, and of TEE in children ^(35; 39). However, the potential of the Actiheart device to capture variations in free-living AEE or TEE has not been studied in children aged 3 years or younger.

Available methods to assess physical activity include activity questionnaires or diaries, tools which are cheap and relatively easy to apply. However, they rely on self-reporting and in many cases also on so-called metabolic equivalent (MET) values, which represent the intensity level of various activities. A MET value is calculated as the ratio between energy expenditure when performing a certain activity and BMR. Such values are useful for groups but tends to be inaccurate for individuals ⁽⁴⁰⁾. Nevertheless, there are many situations where a method based on self-reports and MET values is the only feasible option. For young children, self-reporting is not possible, but parents and other caretakers may record the child's physical activity pattern. Bratteby et al. developed a 7-day activity diary ⁽⁴¹⁾ which was able to provide valid estimates of PAL in a group of adolescents ⁽⁴¹⁾. Compilations of MET values have been published for adults ^(42; 43; 44) and youths ⁽⁴⁵⁾, but no corresponding compilation for younger children is available. However, Torun suggested a procedure to derive MET values for children from 1 to 15 years of age ⁽⁴⁶⁾ and Adolph et al. ⁽⁴⁷⁾ proposed MET values, intended for children aged 3 to 5 years, for seven activities. Bratteby's diary has not been applied in young children and it is not known how different sets of MET values ^(42; 46; 47) influence the accuracy of energy expenditure estimates in free-living children.

5.6.2 Energy intake

Traditional dietary assessment methods such as food records, dietary recalls, weighed food records and food frequency questionnaires have limited accuracy and, when applied in children, involve excessive effort for caretakers ⁽³⁰⁾. For example, the burden for parents of having to weigh or write down all consumed food items may result in changed eating

behaviours or misreporting. Although many dietary assessment methods may produce valid estimates for groups, application of the DLW method shows that self-reported energy intakes suffer from substantial systematic errors in both adults ⁽⁴⁸⁾ and children ⁽³⁰⁾.

Mobile phones offer possibilities for methodological advancements in this area for several reasons. For example, nowadays people carry mobile phones almost everywhere. In Sweden, 95% of the population have a mobile phone and 53% have a smartphone ⁽⁴⁹⁾. Mobile phones also enable instant reports of food intake and contain a digital camera which can be used for pre- and post-meal photographing of meals and food items. Indeed, photographing using digital cameras has shown potential for assessing dietary intake both in adults ^(50; 51; 52; 53; 54) and children ^(55; 56; 57). We have developed a new tool for assessing food and energy intakes using mobile phones in young children, the “Tool for Energy Balance in Children” (TECH). However, TECH needs validation before being used in further studies. A validation study, based on the DLW method, may reveal the potential of TECH to accurately assess energy intake of groups and individual children. TECH was developed in 2010-2011 which was at the same time as we planned the follow-up measurements at 3 years of age of the children in an ongoing study ⁽⁵⁸⁾. Since this study included DLW assessments, it provided a good possibility to conduct a first evaluation of TECH. To avoid that the addition of TECH would affect the parents’ participation in the original study by adding too much burden on the parents, this first evaluation only included one day of TECH recordings. This was considered sufficient for a pilot study.

6. SPECIFIC AIMS

This thesis investigates the following specific aims in healthy Swedish children:

- To describe the longitudinal development of different measures of body composition (i.e. TBF%, BMI, FMI and FFMI) from 1 week to 4.5 years of age, (Paper II).
- To study relationships between different measures of body composition (i.e. TBF%, BMI, FMI and FFMI) and PAL at 1.5 and 3 years of age, (Papers I and II).
- To investigate if heart rate recordings and movement registrations, using Actiheart, can capture variations in free living TEE and AEE at 1.5 and 3 years of age, (Paper III).
- To assess the potential of a 7-day activity diary to assess PAL using three different sets of MET values at 1.5 and 3 years of age, (Paper III).
- To evaluate, in a pilot study, the potential of a new tool (TECH) using mobile phones for assessing energy intake at 3 years of age, (Paper IV).

7. MATERIAL AND METHODS

7.1 Study design

7.1.1 Subjects (*Papers I-IV*)

A total of 798 women, pregnant in approximately gestational week 24 and living in the city of Linköping or its surroundings, were contacted by mail during 2007 and 2008 and asked to participate in a study on the body composition of their infants. Addresses for the women were obtained from the maternity clinic in Linköping. The participating parent couples lived in an area with a well-educated middle income population. Inclusion criteria were singleton birth and a healthy infant born after at least 37 weeks of gestation. One hundred and seventy-seven parent couples consented to participate but 69 left the study for various reasons (premature birth, sick infant, withdrawn consent and participation in only the first measurement). Thus, body composition using ADP ⁽⁵⁹⁾ was successfully measured both at 1 and 12 weeks of age in 108 children. All of these 108 parent couples were asked to participate with their child in a follow-up study investigating body composition, energy metabolism and physical activity at 1.5 years of age and 45 couples agreed to do so. One child was excluded due to poor health and hence 44 children were included in the study. These 44 couples were asked to repeat this study when their children were 3 years old, then including also a dietary assessment, and 33 couples accepted. At 1.5 and 3 years of age the DLW method was used to measure body composition since, at that time, the isotope dilution technique inherent in this method was the only possibility for accurate assessment of body composition of children at this age. The parents of all 108 children were asked to participate in another follow-up when their child was 4.5 years old. At this age body composition was assessed using ADP ⁽¹³⁾, since this technique was then available for children weighing 8-25 kg ⁽¹⁷⁾. Body composition was successfully assessed in 76 children at 4.5 years of age. For this thesis, data from all five measurements were used, from 1 week to 4.5 years of age, and 26 children participated at all ages. The number of children in the different papers and their characteristics (age, weight, height, BMI) are given in **Table 1**. Fifty-seven percent of the participating children went to day-care at 1.5 years of age, and at 3 years all children went to day-care.

Table 1. Characteristics (age, weight, height, BMI, weight-for-age and height-for-age) of children in the different papers.

	Papers I, II		Papers I, II		Papers I, II, III		Papers II, III*, IV†		Paper II	
	1 week (n=44)	12 weeks (n=44)	1.5 years (n=44)	3 years (n=33)	4.5 years (n=26)					
Age (weeks)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	1.0	0.3	12.1	0.6	1.53	0.04	3.00	0.04	4.41	0.04
Body weight (kg)										
boys‡	3.8	0.5	6.3	0.5	12.1	1.1	15.5	1.7	19.2	2.9
girls‡	3.7	0.5	6.0	0.7	11.7	1.4	15.2	1.4	18.2	1.8
all	3.7	0.5	6.2	0.6	11.9	1.2	15.4	1.6	18.8	2.5
Height (m)										
boys	0.52	0.02	0.62	0.02	0.83	0.02	0.97	0.03	1.08	0.04
girls	0.52	0.02	0.61	0.02	0.83	0.03	0.96	0.03	1.08	0.03
all	0.52	0.02	0.61	0.02	0.83	0.03	0.96	0.03	1.08	0.04
Weight-for-age z score§										
boys	0.47	1.07	0.27	0.74	-0.14	0.92	-0.06	0.99	0.26	1.34
girls	0.54	1.00	0.43	1.04	0.10	1.13	0.06	0.81	-0.01	0.76
all	0.50	1.03	0.34	0.89	-0.02	1.02	-0.02	0.92	0.16	1.14
Height-for-age z score§										
boys	0.26	1.00	0.21	0.65	0.02	0.82	-0.08	0.92	0.00	0.87
girls	0.49	1.02	0.26	0.88	0.45	1.13	-0.16	0.90	0.11	0.78
all	0.37	1.00	0.23	0.77	0.22	0.99	-0.10	0.90	0.04	0.82
BMI (kg/m²)¶										
boys	13.7	1.2	16.5	1.6	17.5	1.5	16.6	4.4	16.4	1.77
girls	13.8	1.0	16.2	1.4	16.9	1.0	16.7	1.1	15.7	0.84
all	13.7	1.1	16.3	1.5	17.3	1.3	16.6	1.3	16.1	1.50

BMI, body mass index.

* In Paper III data on 31 of these 33 children are reported. One boy and one girl were excluded due to incomplete Actiheart recordings.

† In paper IV data on 30 of these 33 children are reported. Two boys and one girl were excluded due to incomplete recordings of dietary intake.

‡ At 1 and 12 weeks as well as at 1.5 years of age 23 of the children were boys and 21 were girls. At 3 years of age 22 were boys and 11 were girls and at 4.5 years of age 16 of the children were boys and 10 were girls.

§ Calculated using reference data by Albertsson-Wikland et al. ⁽⁶⁰⁾.

|| At the age of 3 years 6 of 33 (18%) of the children were classified as overweight and none were classified as obese. At the age of 4.5 years 3 of 26 (12%) of the children were classified as overweight and 1 (4%) as obese ⁽¹¹⁾.

7.1.2 Study outline (Papers I-IV)

An overview of the study outline is provided in **Figure 1**. In addition, a description of the aims, design, methods and data analyses in the four included papers are shown in **Table 2**.

1 and 12 weeks of age: Measurement sessions were scheduled at approximately 1 (1.0 ± 0.3) and 12 (12.1 ± 0.6) weeks of age ⁽⁶¹⁾. First the infant's length was measured. Thereafter, weight and body composition of the infant were measured using Pea Pod ⁽¹⁵⁾.

1.5 and 3 years of age: The parents collected two urine samples at home and brought their child to the measurement session which was started by giving each child a dose of stable isotopes in order to calculate body composition and TEE during the two following weeks ⁽⁵⁸⁾. The children consumed the isotopes mixed with fruit juice. Body weight and length/height were recorded. Indirect calorimetry was used to measure SMR. At 1.5 years of age SMR was measured during a mid-morning nap shortly after dosing. At 3 years of age SMR was measured in the evening on the day of dosing. Parents were instructed to collect urine samples at 1, 5, 10, and 14 days after dosing and to note the time of sampling. Urine samples were obtained by means of baby urine collector bags (B. Braun Medical), cotton balls in the diaper (using a syringe to recover the urine) or a pot. The activities of the children were recorded by parents and caretakers using an activity diary for 7 days following the day of dosing. Also, bodily movements and the heart rate of the children were recorded by means of the Actiheart during the two-week period. Actiheart was worn during the daytime for two days during the first week after the day of dosing, and for two days during the second week. The parents were asked to apply Actiheart on their child one weekday and one weekend day each week and to choose days with usual activity patterns. At 3 years of age, during the 14 days when TEE was measured, the children's intake of foods and drinks was assessed using TECH ⁽⁶²⁾ during one complete 24-hour period.

4.5 years of age: Body composition was assessed by means of the ADP technique using the Bod Pod Pediatric Option as previously described ^(13; 63). Body weight and height were recorded.

Age:	1 and 12 weeks	1.5 years	3 years	4.5 years
Number of children:	44	44	33 (of 44)	26 (of 44)
Methods:	Body composition <ul style="list-style-type: none"> - Air-displacement plethysmography by means of Pea Pod Weight and height	Body composition <ul style="list-style-type: none"> - The doubly labelled water method Total energy expenditure (TEE) <ul style="list-style-type: none"> - The doubly labelled water method Sleeping metabolic rate (SMR) <ul style="list-style-type: none"> - Indirect calorimetry Physical activity <ul style="list-style-type: none"> - Reference physical activity level (PAL, i.e. TEE/SMR) - Actiheart (movement and heart rate recordings) - Activity diary Weight and height	Body composition <ul style="list-style-type: none"> - The doubly labelled water method Total energy expenditure (TEE) <ul style="list-style-type: none"> - The doubly labelled water method Sleeping metabolic rate (SMR) <ul style="list-style-type: none"> - Indirect calorimetry Physical activity <ul style="list-style-type: none"> - Reference physical activity level (PAL, i.e. TEE/SMR) - Actiheart (movement and heart rate recordings) - Activity diary Weight and height	Body composition <ul style="list-style-type: none"> - Air-displacement plethysmography by means of the Bod Pod Pediatric Option Weight and height

Figure 1. Description of the study outline from 1 week until 4.5 years of age

Table 2. Description of papers included in the thesis

	Paper I	Paper II	Paper III	Paper IV
Aim	i) To study the development of body composition from 1 week to 1.5 years of age and relate this development to PAL at 1.5 years of age. ii) To study relationships between body fatness and PAL at 1.5 years of age.	i) To describe the longitudinal development of different measures of body composition from 1 week to 4.5 years of age. ii) To study relationships between different measures of body composition and PAL at 1.5 and 3 years of age.	To evaluate the potential for Actiheart and a 7-day activity diary for estimating total -and activity energy expenditure in 1.5 and 3-year-old children.	To evaluate, in a pilot study, a new tool (TECH) using mobile phones for assessing intakes of energy and certain foods in 3-year-old children.
Design	Longitudinal Cross-sectional	Longitudinal Cross-sectional	Cross-sectional	Cross-sectional
Participants	44 children at 1 and 12 weeks and at 1.5 years of age.	i) 26 children participating in all five measurements from 1 week to 4.5 years. ii) 44 children at 1.5 years and 33 at 3 years of age.	44 children at 1.5 years and 31 children at 3 years of age.	30 children at 3 years of age.
Methods/variables	Body composition and energy metabolism including energy expended in response to physical activity.	Body composition and energy metabolism including energy expended in response to physical activity.	Energy metabolism including energy expended in response to physical activity. Actiheart: accelerometer counts, heart rate. Activity diary: minutes spent in different activities.	Energy metabolism. Web-based food frequency questionnaire. TECH: energy and food intake by means of pictures and questions using mobile phones.
Analysis	Descriptive line plots. Linear regression and correlation analyses. Student's T-test.	Descriptive line plots. Correlation analyses. Linear and multiple regression analyses.	Student's T-test. Multiple regression analyses. Bland-Altman plot comparison.	Wilcoxon matched pairs test. Bland-Altman plot comparison. Spearman rank order correlation.

7.2 Methods

7.2.1 Body weight, length and height (Papers I-IV)

At 1 and 12 weeks as well as at 4.5 years of age, body weight was measured without clothes using the electronic scale accompanying the Pea Pod (COSMED USA, Inc., Concord, CA, USA) and the Bod Pod Pediatric Option (COSMED USA, Inc., Concord, CA, USA), respectively. At 1.5 and 3 years, body weight was recorded without clothes using an electronic scale (KCC 150; Mettler-Toledo). Length/height was measured to the nearest 0.5 centimetre using a length board (1 and 12 weeks and 1.5 years of age), or a wall stadiometer (3 and 4.5 years).

7.2.2 TEE and body composition using the doubly labelled water method (Papers I-IV)

Each child consumed an accurate amount of the stable isotopes ^2H and ^{18}O (0.14 g $^2\text{H}_2\text{O}$ and 0.35 g H_2^{18}O per kg body weight) at 1.5 and 3 years of age, as described in Papers I-IV. Urine samples were stored in glass vials with an internal aluminium-lined screw cap sealing at $+4^\circ\text{C}$ until sample collection was completed, after which they were stored at -20°C until analysed. An isotope ratio mass spectrometer fitted with a $\text{CO}_2/\text{H}_2/\text{H}_2\text{O}$ equilibrium device (Deltaplus XL, Thermoquest, Bremen, Germany) was used to analyse ^2H and ^{18}O enrichments of dose and urine samples ^(64; 65). ^2H dilution space (N_D) and ^{18}O dilution space (N_O) were calculated using zero time enrichments obtained from the exponential isotope disappearance curves that provided estimates for the elimination rates for ^2H and ^{18}O , respectively. CO_2 -production was calculated according to Davies et al. ⁽⁶⁶⁾ assuming that 25 % of total water losses were fractionated. CO_2 -production was used to calculate TEE using the Weir formula ⁽²⁶⁾ assuming a food quotient of 0.85 ⁽²⁷⁾. TBW was the average of $N_D/1.041$ and $N_O/1.007$ ⁽⁶⁷⁾. N_D/N_O was 1.024 ± 0.013 and 1.028 ± 0.009 at 1.5 and 3 years of age, respectively. FFM was calculated as $\text{TBW}/0.784$ and $\text{TBW}/0.777$, at 1.5 and 3 years of age, respectively ⁽⁶⁸⁾. TBF was obtained by subtracting FFM from body weight. Analytic precision (in ppm) was 0.22 for ^2H and 0.03 for ^{18}O . Coefficients of variation when samples from one adult subject were analysed nine times were: 1.2 % for TEE, 0.3% for total body water, and 0.3% or less for k_D and k_O . These values are all well within the recommended criteria ⁽⁶⁷⁾.

7.2.3 SMR using indirect calorimetry (Papers I-III)

SMR was measured during sleep using a ventilated hood system (Deltatrac Metabolic Monitor; Datex Instrumentarium Corporation) at 1.5 and 3 years of age, as described in

Papers I and III. During this measurement session O₂ uptake and CO₂ production were recorded. When the recordings were stable, which occurred after approximately 10 min, the following 12–16 min were used to calculate SMR using the Weir equation ⁽²⁶⁾.

Child being measured using indirect calorimetry

7.2.4 Reference estimates of energy expenditure in response to physical activity (Papers I-III)

Reference PAL was calculated as TEE/SMR (PAL_{SMR}). AEE was calculated as TEE minus SMR.

7.2.5 Body composition using the ADP technique (Papers I, II)

At 1 and 12 weeks of age, body volume and weight of the infants were measured using Pea Pod ⁽⁶³⁾. Body weight was divided by body volume to obtain body density. Body composition was calculated using the two-component model based on a fat mass density of 0.9007 g/ml ⁽¹⁹⁾ and densities of FFM by Fomon et al. ⁽⁶⁸⁾ using the Pea Pod software 3.0.1 ⁽⁶¹⁾. At 4.5 years of age body volume and weight were measured using the Bod Pod Pediatric Option ⁽¹⁷⁾ with software 5.2.0 ⁽¹³⁾. Body density was converted to TBF% using a fat mass density of 0.9007 g/ml ⁽¹⁹⁾ and densities of FFM presented by Lohman ⁽⁶⁹⁾.

Pea Pod

The Bod Pod Pediatric Option

7.2.6 FMI, FFMI and BMI (Paper II)

At 1 and 12 weeks as well as 1.5, 3 and 4.5 years, FMI, FFMI and BMI were calculated as TBF (kg), FFM (kg) and body weight (kg), respectively, divided by height² (m). Reference BMI, FMI and FFMI were calculated using data by Fomon et al. ⁽⁶⁸⁾.

7.2.7 Actiheart (Paper III)

The Actiheart (Camntech Ltd, Cambridge, United Kingdom) (<http://www.camntech.com>) contains a uniaxial accelerometer which measures bodily movements in counts per minute (cpm) and a pulse monitor which measures heart rate in beats per minute (bpm). The device has two electrodes, connected by a lead, which are attached to the chest by two electrocardiography pads (2660-3, 3M Svenska AB, Sollentuna, Sweden). The Actiheart software Version 4.0.11 (Camntech Ltd, Cambridge, United Kingdom) was used to initiate, transfer and analyse the recorded information. For each child, mean heart rate (mHR) in bpm was calculated as the sum of the recorded heart rates (in bpm) divided by the number of recorded minutes, as described in Paper III. Mean Actiheart counts (mAC) in cpm was calculated, for each child, as the sum of the recorded counts (in cpm) divided by the number of recorded minutes. Calculations of mAC and mHR were based on recordings obtained during valid days, i.e. days when wear time plus time spent sleeping were ≥ 19 hours, as described in Paper III.

Actiheart

7.2.8 Activity diary (Paper III)

A modified version of a diary developed for adolescents by Bratteby et al. ⁽⁴¹⁾ was used, as described in Paper III. Parents or other caretakers were asked to enter digits from 1 to 7, representing common activities for children, for all 15-minute intervals throughout the 7-day period. For every 15-minute interval, parents were told to select the dominant activity from one of the following categories: sleeping, lying quietly, passive sitting, active sitting, standing, walking and running. The seven activity categories as well as their assigned MET values as proposed by Ainsworth ⁽⁴²⁾, Torun ⁽⁴⁶⁾ and Adolph ⁽⁴⁷⁾ are shown in **Table 3**. For each child, the number of recorded minutes spent in each activity category during the 7-day period was calculated and multiplied by an appropriate MET value. Thereafter, for each child, the values for all activity categories were summed and then divided by the total number of recorded minutes in order to obtain $PAL_{Ainsworth}$, PAL_{Torun} and PAL_{Adolph} .

Aktivitetsdagbok

Datum för aktiviteterna:
Ifyllt av:

Skriv i rutorna nedan den siffra (se nedre delen av sidan) som bäst beskriver den dominerande aktiviteten under varje 15 minuters period. Vid tveksamhet, kommentera nedan.	Klockslag	0-15	16-30	31-45	46-60
	0	1	1	1	1
	1	1	1	1	1
	2	1	1	1	1
	3	1	1	1	1
	4	1	1	1	1
	5	1	1	1	1
	6	1	1	1	1
	7	2	3	5	6
	8	7	7	5	5
	9	4	4	4	6
	10	3	3	3	4
	11	4	5	5	6

Aktivitetskategorier
 1. Sova
 2. Ligga (stillsamt utan att sova)
 3. Passiva sittande aktiviteter (t ex se på TV, lyssna på saga, åka vagn, åka bil)
 4. Aktiva sittande aktiviteter (t ex leka med klossar, gunga)
 5. Stående aktiviteter (t ex lek stående, hjälpa till med matlagning)
 6. Gående aktiviteter (lek som innebär förflyttning, promenera)
 7. Springa (t ex spela fotboll)

Picture of the activity diary

Table 3. The seven activity categories included in the activity diary as well as the MET values published by Ainsworth et al. ⁽⁴²⁾, Torun ⁽⁴⁶⁾ and Adolph et al. ⁽⁴⁷⁾ used at 1.5 and 3 years of age.

Activity category	Activity (examples)	Ainsworth MET*	Torun MET*	Adolph MET†
1	<i>Sleeping</i>	0.9	0.9	0.9
2	<i>Lying quietly</i>	1	1.1	1.1
3	<i>Passive sitting</i> (watching TV, sitting in a pram or car)	1.3	1.2	1.2
4	<i>Active sitting</i> (eating, drawing, playing with blocks)	1.5	2	1.4
5	<i>Standing</i> (playing standing, participating in cooking)	2	1.4	1.7
6	<i>Walking</i>	2.8	2.2	2.9
7	<i>Running</i> (playing football)	10	5‡	3.8

MET, metabolic equivalent.

* Used at 1.5 and 3 years of age

† Used at 3 years of age.

‡ Obtained as 0.50 times Ainsworth MET value for running in accordance with Torun's method for vigorous activities ⁽⁴⁶⁾.

7.2.9 Tool for Energy Balance in Children (TECH) (Paper IV)

Parents and other caretakers were instructed to take pre- and post-meal photographs of all food items and beverages consumed by their child during one 24-hour period using a mobile phone, as described in Paper IV. Parents were provided with a mobile phone for the study (Nokia 2730c or Sony Ericsson J105i). They also answered six or seven questions at each meal regarding type of foods (i.e. milk, butter/margarine/oil, meat, bread, cereal and sauce) using a JAVA-based questionnaire installed on the mobile phone. The parents were instructed to photograph the meals from three angles, to use table-ware provided for the study and to place a matchbox in each image. Volumes of foods were assessed from images using known sizes of the table-ware and the matchbox by means of the software Paint (Microsoft, version 6.1) and converted into weight by being multiplied by the appropriate weight per volume ⁽⁷⁰⁾. Energy intake was calculated from intakes of foods and drinks through linkage to the Swedish Food Database ⁽⁷¹⁾.

Screenshots showing TECH

7.3 Ethics

The studies in this thesis were conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Research Ethics Committee in Linköping, Sweden (M93-06, T23-08, T55-08, 2011/6832). Written or verbal informed consent was obtained from parents as described previously ^(13; 58; 61; 65).

7.4 Statistics

The complete statistical test procedures have been described in detail in papers I-IV. Values are given as means and standard deviations (SD). Descriptive statistics (line plots) were used to describe the longitudinal development in body composition variables from 1 week to 4.5 years of age and for comparison with reference values ⁽⁶⁸⁾ (Paper II). Significant differences between mean values were identified using t-tests (Papers I and III) or the Wilcoxon matched pairs test (Paper IV) for variables that were not normally distributed. Correlation analyses

were performed using Pearson correlations. For variables that were skewed (Paper IV), Spearman rank order correlations were used. Linear regression was used to analyse associations between PAL_{SMR} (y) and measures of body composition (x) at 1.5 and 3 years of age (Papers I and II). Multiple regression was used to investigate associations between body fatness and PAL_{SMR} that were adjusted for FFMI (Paper II). In Paper III, multiple regression analysis was used to evaluate the fraction of the variation in TEE or AEE that could be explained by mHR and/or mAC, in addition to that explained by TBF and FFM. The Bland-Altman ⁽⁷²⁾ procedure was used to assess agreement between methods in Papers III and IV. In this procedure, the differences (y) between estimated and reference values are plotted against the average of these two estimates (x). To test for bias (the relationship between x and y) in the Bland-Altman plot, linear regression was used. Significance (two-sided) was accepted when $P < 0.05$. Analyses were performed using Statistica Software, version 10 (STAT SOFT, Scandinavia AB, Uppsala, Sweden).

8. RESULTS

8.1 Longitudinal study (Papers I, II)

8.1.1 Longitudinal changes in body composition between 1 week and 4.5 years of age (Paper II)

Our children weighed more and were slightly taller than the reference boy and girl ⁽⁶⁸⁾ at all ages. **Figure 2** shows average and individual values for TBF%, FMI, BMI and FFMI from 1 week to 4.5 years of age for boys (n=16) in the study versus the corresponding reference values ⁽⁶⁸⁾. Measures of TBF% and FMI varied considerably between boys and also between ages for the same individual. As shown in Figure 2, the intra-individual and inter-individual variation in BMI and FFMI were lower than the corresponding values for TBF% and FMI. Average TBF%, BMI and FMI increased considerably from 1 to 12 weeks of age, reached a maximum at 1.5 years, and were thereafter relatively stable. Average FFMI was stable from 1 week until 4.5 years of age. Also, as shown in Figure 1, at all ages the average BMI was comparable to the BMI of the reference boy. However, average TBF% and FMI were considerably higher for boys in the current study from 12 weeks until 4.5 years of age (+10 to +81 %) when compared to the reference boy. Conversely, average FFMI was slightly lower for boys in the present study at all ages (-3 % to -9 %) when compared to the reference boy.

Figure 3 shows average and individual values for TBF%, FMI, BMI and FFMI from 1 week to 4.5 years of age for girls in the study (n=10) compared to the corresponding reference values ⁽⁶⁸⁾. For girls the development of body composition measures, averages as well as variations, was similar to the corresponding development for boys (Figure 2). Thus, average TBF% was higher for the 10 girls at all measurements (+3 to +53 %) and average FMI was higher from 12 weeks until 4.5 years (+11 to +57 %) compared to the reference girl. Average FFMI of the girls was similar to average FFMI of the reference girl at 12 weeks and lower at all other ages (-2 % to -9 %).

Figure 2. Boys.

Legend to figure 2. Measures of body composition versus age for boys ($n=16$) in the study. Each line represents one boy. Red line: mean values; blue line: reference data by Fomon et al. ⁽⁶⁸⁾.

A) TBF, total body fat, %

B) BMI, body mass index (kg/m^2)

C) FMI, fat mass index (kg/m^2)

D) FFMI, fat-free mass index (kg/m^2)

Figure 3. Girls.

Legend to figure 3. Measures of body composition versus age for girls ($n=10$) in the study. Each line represents one girl. Red line: mean values; blue line: reference data by Fomon et al. ⁽⁶⁸⁾.

A) TBF, total body fat, %

B) BMI, body mass index (kg/m^2)

C) FMI, fat mass index (kg/m^2)

D) FFMI, fat-free mass index (kg/m^2)

8.1.2 Body composition in relation to PAL at 1.5 and 3 years of age (Papers I, II)

Table 4 shows TEE, SMR, PAL_{SMR} , AEE and body composition variables for children in the study at 1.5 and 3 years of age. PAL_{SMR} at 1.5 years was not correlated to PAL_{SMR} at 3 years of age ($r=0.04$, $P=0.86$). **Figures 4** and **5** show correlations between body composition variables and PAL_{SMR} at 1.5 and 3 years of age, respectively. TBF% was negatively correlated with PAL_{SMR} both at 1.5 ($r=-0.40$, $P=0.008$) and at 3 years of age ($r=-0.48$, $P=0.004$), while BMI and FMI were not significantly associated with PAL_{SMR} at any of these ages. FFMI was strongly and positively correlated with PAL_{SMR} at 3 years of age ($r=0.74$, $P<0.001$) while this correlation was weaker at 1.5 years ($r=0.28$, $P=0.062$).

Table 5 shows results of regression analyses at 1.5 and 3 years of age with PAL_{SMR} as the dependent variable and TBF% or/and FFMI as independent variables. At 1.5 years of age, TBF% explained 14% of the variation in PAL_{SMR} . When fitting TBF% and FFMI in the same model, slightly more (19%) of the variation in PAL_{SMR} was explained but only TBF% was significant ($P=0.0090$). At 3 years, TBF% explained 21% of the variation in PAL_{SMR} while the corresponding figure for FFMI was 54%. When fitting both TBF% and FFMI as independent variables in the same model, the amount of the variation in PAL_{SMR} explained was similar (adjusted R^2 : 53%) and only FFMI was significant ($P<0.001$).

Table 4. Energy metabolism, PAL_{SMR}, AEE and different measures of body composition for the children in the study at 1.5 and 3 years of age*

		TEE (kJ/24h)		SMR (kJ/24h)		PAL _{SMR}		AEE (kJ/24h)		TBF %		FMI (kg/m ²)		FFMI (kg/m ²)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.5 years	Boys (n=23)	4060	410	3020	320	1.35	0.16	1060	400	28.4	3.4	5.01	0.94	12.6	1.0
	Girls (n=21)	3930	420	2740	250	1.44	0.17	1170	410	27.3	3.3	4.65	0.75	12.3	0.7
	All (n=44)	4000	420	2890	320	1.39	0.17	1110	410	27.9	3.3	4.84	0.86	12.4	0.9
3 years	Boys (n=22)	5190	650	3270	220	1.59	0.14	1950	520	26.8	5.7	4.48	1.21	12.1	0.83
	Girls (n=11)	4950	590	3100	300	1.60	0.18	1810	480	26.4	6.8	4.41	1.22	12.2	1.18
	All (n=33)	5110	630	3210	260	1.59	0.15	1900	500	26.6	6.0	4.46	1.20	12.1	0.94

TEE, total energy expenditure measured using the doubly labelled water method; SMR, sleeping metabolic ratio measured by indirect calorimetry; PAL_{SMR}, physical activity level calculated as TEE divided by SMR; TBF%, total body fat %; BMI, body mass index; FMI, fat mass index; FFMI, fat-free mass index.

* Energy metabolism from 30 children and energy metabolism and physical activity from 31 of the 33 children at 3 years of age have been published in Papers IV and III, respectively.

Figure 4. 1.5 years of age.

Legend to figure 4. PAL_{SMR} at 1.5 years of age (y) regressed on different measures of body composition at the age of 1.5 years (x), $n = 44$. Correlation and regression analysis showed significant relationships in figure 1A ($r = -0.40$, $P = 0.0076$; $y = 1.94 - 0.0197 x$). Non-significant relations in figure 1B ($r = 0.015$, $P = 0.93$, $y = 1.42 - 0.0018 x$), 1C ($r = -0.29$, $P = 0.062$, $y = 1.66 - 0.055 x$) and 1D ($r = 0.28$, $P = 0.062$, $y = 0.65 + 0.060 x$).

PAL_{SMR} , physical activity level (calculated as TEE divided by SMR); TBF, total body fat; BMI, body mass index; FMI, fat mass index; FFMI, fat-free mass index.

Figure 5. 3 years of age.

A

B

C

D

Legend to figure 5. PAL_{SMR} at 3 years of age (y) regressed on different measures of body composition at the age of 3 years (x), $n = 33$. Correlation and regression analysis showed significant relationships in figure 1A ($r = -0.48$, $P = 0.0044$, $y = 1.92 - 0.012 x$) and in figure 1D ($r = 0.74$, $P = 0.000001$, $y = 0.13 + 0.12 x$). Non-significant relations in figure 1B ($r = 0.24$, $P = 0.17$, $y = 1.11 + 0.029 x$) and 1C ($r = -0.32$, $P = 0.071$, $y = 1.77 - 0.041 x$).

PAL_{SMR} , physical activity level (calculated as TEE divided by SMR); TBF, total body fat; BMI, body mass index; FMI, fat mass index; FFMI, fat-free mass index.

Table 5. Multiple regression results showing PAL_{SMR} (y) regressed on TBF% (x₁) and FFMI (x₂) at 1.5 and 3 years of age

	Model	Independent variables	Intercept	Slope	P	R ² model	P model
1.5 years (n=44)	A	TBF%	1.94	-0.020	0.0076	0.14	
	B	FFMI	0.65	0.060	0.062	0.058	
	C	TBF%	1.24	-0.19	0.0090	0.19	0.0057
		FFMI		0.055	0.068		
3 years (n=33)	D	TBF%	1.92	-0.012	0.0044	0.21	
	E	FFMI	0.13	0.12	<0.001	0.54	
	F	TBF%	0.37	-0.0036	0.33	0.53	<0.0010
		FFMI		0.11	<0.0010		

PAL_{SMR}, physical activity level calculated as total energy expenditure divided by sleeping metabolic rate ; DLW, doubly labelled water; TBF, total body fat; FFMI, fat-free mass index.

8.2 Evaluation of Actiheart and the activity diary (Paper III)

8.2.1 Actiheart

At 1.5 years of age, mHR was correlated with AEE ($r=0.33$; $P=0.029$) and TEE ($r=0.41$; $P=0.006$), as described in Paper III. The corresponding correlations at 3 years of age were $r=0.33$; $P=0.07$ (AEE) and $r=0.37$; $P=0.042$ (TEE). mAC was not correlated with AEE or TEE at 1.5 or 3 years of age ($r=0.17$ - 0.21).

Table 6 shows results obtained when TBF, FFM, mHR and mAC were independent variables in multiple regression models with TEE as the dependent variable at 1.5 and 3 years of age.

At 1.5 years of age, TBF and FFM together explained 48 % of the variation in TEE ($P<0.001$) (Model 1A). Adding mHR as another independent variable explained 8 % more of this variation ($P=0.006$) (Model 1B), while adding mAC instead explained no additional variation in TEE (Model 1C). Furthermore, using both mHR and mAC as additional independent variables (model 1D) explained 55 % the variation in TEE, which was similar to that obtained for mHR alone, 56% (model 1B). At the age of 3 years, TBF and FFM together explained 68 % of the variation in TEE ($P<0.001$) (Model 2A). When adding mHR or mAC an additional 8 % of this variation could be explained ($P=0.004$) (Models 2B and 2C). Together mHR, mAC, TBF and FFM explained 78 % of the variation in TEE (model 2D), a figure similar to that (76 %) obtained when only mHR or only mAC was added (models 2B and 2C).

Corresponding results for regression models with AEE as the dependent variable are described in Paper III ⁽⁵⁸⁾. Briefly, at 1.5 years of age, TBF and FFM explained only 23 % of the variation in AEE, and mHR or mAC did not explain any additional variation ($P>0.05$). At 3 years of age, TBF and FFM explained 59 % of the variation in AEE, and adding mHR or mAC explained another 6 % ($P=0.03$).

Table 6. Multiple regression results obtained at 1.5 and 3 years of age when total energy expenditure (kJ/24h) was regressed on total body fat, fat-free mass and Actiheart variables (mean heart rate, mHR, and mean activity counts, mAC).

Age	Model	Independent variables	Intercept	Slope	P	R ²	SEE (kJ/24h)	P model
1.5 years (n=44)	1A	TBF (kg)	926.9	-207.3	0.016	0.48	301	<0.001
		FFM (kg)		438.9	<0.001			
	1B	mHR (bpm)	-1554.0	20.3	0.006	0.56	278	<0.001
		TBF (kg)		-163.4	0.041			
		FFM (kg)		403.2	<0.001			
	1C	mAC (cpm)	931.1	-0.14	0.94	0.46	305	<0.001
		TBF (kg)		-208.6	0.018			
		FFM (kg)		440.4	<0.001			
	1D	mHR (bpm)	-1640.8	21.3	0.005	0.55	279	<0.001
		mAC (cpm)		-1.09	0.50			
		TBF (kg)		-171.3	0.036			
		FFM (kg)		413.8	<0.001			
3 years (n=30)*	2A	TBF (kg)	715.0	-14.4	0.81	0.68	339	<0.001
		FFM (kg)		396.4	<0.001			
	2B	mHR (bpm)	-1863.4	22.7	0.004	0.76	295	<0.001
		TBF (kg)		-14.0	0.79			
		FFM (kg)		381.8	<0.001			
	2C	mAC (cpm)	-268.7	5.5	0.004	0.76	295	<0.001
		TBF (kg)		29.1	0.60			
		FFM (kg)		415.4	<0.001			
	2D	mHR (bpm)	-1746.1	15.7	0.052	0.78	278	<0.001
		mAC (cpm)		3.80	0.051			
		TBF (kg)		15.95	0.76			
		FFM (kg)		399.4	<0.001			

TBF, total body fat; FFM, fat-free mass; bpm, beats per minute; cpm, counts per minute; R², adjusted coefficient of determination for the model; SEE, standard error of estimation of the model; P model, P-value of the model.

*n=30 due to invalid Actiheart recordings for all four days for one child at 3 years of age.

8.2.2 Activity diary

Table 7 shows $PAL_{Ainsworth}$, PAL_{Torun} , PAL_{Adolph} and PAL_{SMR} for children in the study at 1.5 and 3 years of age. At these ages, average $PAL_{Ainsworth}$ was 1.44 and 1.59, respectively, and not significantly different from PAL_{SMR} (1.39 and 1.61, respectively). At 1.5 years of age average PAL_{Torun} was 1.33 and significantly ($P=0.014$) lower than PAL_{SMR} . At 3 years of age, average PAL_{Torun} and PAL_{Adolph} were 1.43 and 1.42, respectively. Both these values were significantly ($P<0.001$) lower than PAL_{SMR} (1.61).

The Bland-Altman plots for $PAL_{Ainsworth}$ and PAL_{Torun} versus PAL_{SMR} at 1.5 years of age are shown in **Figure 6 A and B**. The limits of agreement were wide in both plots. Furthermore, the activity diary overestimated low and underestimated high PAL values for both these two sets of MET values. The Bland-Altman plots for $PAL_{Ainsworth}$, PAL_{Torun} and PAL_{Adolph} versus PAL_{SMR} at 3 years of age are shown in **Figure 7 A, B and C**. The limits of agreement were wide in all three plots. The activity diary underestimated low and overestimated high PAL values when using Ainsworths' MET values. Figure 7 B and C show that PAL_{Torun} and PAL_{Adolph} were lower than PAL_{SMR} in 28 and 27 of 31 children, respectively. Furthermore, the underestimation by PAL_{Adolph} increased with increasing PAL (Figure 7C) and a trend ($P=0.086$) for a negative relationship was also found in the corresponding plot for PAL_{Torun} (Figure 7B).

Table 7. PAL assessed by means of an activity diary using different MET values in comparison to PAL_{SMR} at 1.5 and 3 years of age

	1.5 years (n=44)				3 years (n=31)			
	Mean	SD	Range		Mean	SD	Range	
PAL _{SMR}	1.39	0.17	1.09-1.78		1.61	0.14	1.36-1.90	
PAL _{Ainsworth}	1.44	0.11	1.27-1.74		1.59	0.25	1.35-2.66	
PAL _{Torun}	1.33*	0.06	1.22-1.44		1.43†	0.11	1.28-1.85	
PAL _{Adolph}	-	-	-		1.42†	0.10	1.23-1.75	

MET, metabolic equivalent; PAL_{SMR}, physical activity level calculated as TEE divided by SMR; PAL_{Ainsworth}, PAL calculated using MET values for adults published by Ainsworth et al. ⁽⁴²⁾; PAL_{Torun}, PAL calculated using MET values published by Torun ⁽⁴⁶⁾; PAL_{Adolph}, PAL calculated using MET values published by Adolph et al. ⁽⁴⁷⁾.

* Significantly different from the corresponding PAL_{SMR} (P=0.014).

† Significantly different from the corresponding PAL_{SMR} (P<0.001).

Figure 6.

A Bland-Altman plot comparing the physical activity level (PAL), assessed by means of a 7-day activity diary using different sets of metabolic equivalent (MET) values with PAL_{SMR} calculated as TEE divided by SMR at 1.5 years of age.

(A) PAL calculated using MET values published by Ainsworth *et al.* ⁽⁴²⁾ (PAL_{Ainsworth}). PAL_{Ainsworth}-PAL_{SMR} was 0.05 (2SD was 0.35). The regression equation was $y = -0.75x - 1.03$ ($r = -0.38$, $P = 0.01$).

(B) PAL calculated using MET values published by Torun ⁽⁴⁶⁾ (PAL_{Torun}). PAL_{Torun}-PAL_{SMR} was -0.06 (2SD was 0.32). The regression equation was $y = -1.35x + 1.78$ ($r = -0.80$, $P < 0.001$).

Figure 7

A Bland-Altman plot comparing the physical activity level (PAL), assessed by means of a 7-day activity diary using different sets of metabolic equivalent (MET) values with PAL_{SMR} calculated as TEE divided by SMR at 3 years of age.

(A) PAL calculated using MET values published by Ainsworth ⁽⁴²⁾ ($PAL_{Ainsworth}$). $PAL_{Ainsworth}-PAL_{SMR}$ was -0.02 (2SD was 0.53). The regression equation was $y=0.86x-1.40$ ($r=0.49$, $P=0.005$).

(B) PAL calculated using MET values published by Torun ⁽⁴⁶⁾ (PAL_{Torun}). $PAL_{Torun}-PAL_{SMR}$ was -0.18 (2SD was 0.32). The regression equation was $y=-0.52x+0.61$ ($r=-0.32$, $P=0.086$).

(C) PAL calculated using MET values published by Adolph ⁽⁴⁷⁾ (PAL_{Adolph}). $PAL_{Adolph}-PAL_{SMR}$ was -0.19 (2SD was 0.30). The regression equation was $y=-0.65x+0.80$ ($r=-0.40$, $P=0.025$).

8.4 Evaluation of TECH (Paper IV)

The average energy intake assessed using TECH was 5400 ± 1500 kJ/24h and not significantly different ($P = 0.23$) from TEE (5070 ± 600 kJ/24h). Figure 1 shows a Bland-Altman plot comparison ⁽⁷²⁾ for energy intake compared to TEE. The limits of agreement were wide and there was a significant trend in the Bland-Altman plot indicating that TECH overestimated high and underestimated low energy intakes.

Figure 8.
A Bland-Altman plot comparing energy intake estimated using Tool for Energy Balance in Children (TECH) with total energy expenditure (TEE) measured using the doubly labelled water method in 30 healthy children at 3 years of age.

Energy intake estimated using TECH - TEE was 330 kJ/24h (2SD was 2990 kJ/24h). The regression equation was $y=1.27 x- 6312.67$ ($r=0.73$, $P< 0.001$).

9. GENERAL DISCUSSION

9.1 Comments on the population and methods

9.1.1 Study population

The sample sizes of the studies in this thesis were small, which may limit generalisability. Furthermore, the proportion of parents with university degrees was considerably higher in the studies than in Swedish national data (58 versus 36 % ⁽⁷⁴⁾ had a university degree). The proportions of overweight and obese parents were in accordance with the Swedish population of the appropriate age and sex ⁽⁷⁵⁾. Also, the body size of the participating children was comparable to Swedish reference data for contemporary Swedish children ⁽⁶⁰⁾ and their TEE values and body composition were similar to comparable estimates in Western children ^(66; 76). The proportions of overweight and obese children at 3 and 4.5 years of age were similar to reported data from Swedish children at 4 years of age ⁽⁷⁾. Thus, although the children in this thesis were not randomly selected, they were comparable to contemporary Western children regarding body size and composition.

9.1.2 Methods to assess body composition and energy metabolism

Major strengths in these studies include the use of accurate methods to measure body composition (the DLW method and ADP) and the DLW method or/and indirect calorimetry to measure energy metabolism (i.e. TEE, SMR, PAL and AEE).

At 1.5 and 3 years of age, isotope dilution was used to assess body composition, while ADP was used at 1 and 12 weeks as well as at 4.5 years of age. The isotope dilution assessments and ADP measurements at 1 and 12 weeks were based on data for FFM hydration and density provided by Fomon et al. ⁽⁶⁸⁾ while ADP measurements at 4.5 years were based on FFM density as reported by Lohman ⁽⁶⁹⁾. Both these methods provide valid measures of body composition in infants and children ^(16; 17; 59). It is noteworthy that values for hydration as well as for density of FFM are very similar when data by Fomon et al. ⁽⁶⁸⁾ and Lohman ⁽⁶⁹⁾ are compared. Therefore it is unlikely that using two different body composition methods has influenced the results or conclusions of this thesis in any important way.

Measures of TEE and possibly SMR in young children will include some energy expenditure in response to growth. However, for children below 3 years of age such energy expenditure is equivalent to only about 2 % of TEE on average ^(68; 77).

It is common to correct AEE for the so-called dietary-induced thermogenesis, DIT (5-15 % of TEE). AEE and PAL are generally calculated using BMR, measured after 12 hours of fasting while awake but at complete rest. Because these requirements cannot be fulfilled in young children, SMR was measured in a non-fasting state and consequently included some DIT. Therefore, calculations of AEE were not adjusted for DIT.

9.1.3 Relationship between PAL and body composition

The presented associations between PAL_{SMR} and body composition are observational and cross-sectional and it cannot be definitely stated whether PAL_{SMR} influences body composition or vice versa. In this thesis, body composition is presented as the independent variable and PAL_{SMR} as the dependent variable. The reason for choosing this approach is that Tennefors et al. ⁽⁷⁸⁾ found an association between PAL and TBF% and suggested the presence of a vicious cycle, where high body fatness counteracts physical activity, leading to a positive energy balance with subsequent accumulation of body fat. In this thesis it was of interest to investigate whether this mechanism may also be present at 1.5 and 3 years. Obviously, it is also conceivable that PAL_{SMR} is the independent variable. However, it seems likely that PAL needs to be changed for a long period of time in order to influence the body composition while, conversely, body composition may influence activity patterns instantaneously.

9.1.4 Choice of reference values

Body composition variables were compared to reference values published by Fomon et al. in 1982 ⁽⁶⁸⁾. These data are based on weight, height and body composition studies of American infants and children collected around 1970. Even though the authors stressed that these reference data must be considered preliminary and crude ⁽⁶⁸⁾, they have been used frequently. These reference values were chosen in the present thesis since they are the only available longitudinal description of body composition development of reference children that cover the period from birth to 4.5 years of age ⁽⁶⁸⁾. When these reference data were collected, overweight and obesity in childhood were much less common than today, and malnutrition was also uncommon. Therefore these reference data may be regarded as a biological standard

describing the normal development of healthy children. It should be noted that developments that differ from these reference data are not necessarily less healthy.

9.2 Main findings: interpretation and implications

9.2.1 Development of body composition during infancy and early childhood

One main finding in this thesis is that average TBF% and FMI in the children in the study from 12 weeks up to 4.5 years of age were considerably higher than the reference values by Fomon et al. ⁽⁶⁸⁾. Also, from 1.5 years and onwards almost all individual children had TBF% and FMI above the corresponding reference value ⁽⁶⁸⁾. In contrast, the average FFMI of the children was lower at all ages (except for girls at 12 weeks when values were similar) compared to the corresponding reference values ⁽⁶⁸⁾. The children's body weight and height were slightly higher than reference values ⁽⁶⁸⁾, but BMI was quite similar. Interestingly, even if BMI was similar, the children's body composition was characterized by a higher body fatness and slightly lower FFMI when compared to the reference child ⁽⁶⁸⁾. This finding may demonstrate a secular trend in body composition development characterized by a high body fatness during infancy and childhood until 4.5 years of age. This suggestion can be reconciled with previous research in older children ^(79; 80; 81) which has identified secular trends with higher body fatness in contemporary children. In addition, higher body fatness when compared to Fomon et al. has also been observed in an American population from birth up to two years of age ⁽⁸²⁾. The results in this thesis indicate that a secular trend may already be established during infancy and early childhood. However, the explanation for this trend is unknown and possible explanations are only a matter of speculation. An obvious possibility is that changes in feeding practices of infants and young children may have taken place resulting in a higher intake of dietary energy. It is also conceivable that contemporary infants and young children are less physically active ⁽⁸³⁾ when compared to children used to develop the reference values. Furthermore, as discussed in Papers I and II, the relationship between PAL_{SMR} and TBF% at 1.5 years of age may represent a considerable potential for a positive energy balance and consequently fat retention at least at a young age. Other as yet unknown explanations may also contribute. Finally, it should be pointed out that there is no way to definitely confirm that the data by Fomon et al. really represent an appropriate body composition reference.

9.2.2 Associations between PAL and body composition at 1.5 and 3 years of age

In this thesis FFMI and TBF% were positively and negatively, respectively, correlated with PAL_{SMR} at 3 years of age. These findings can be reconciled with studies conducted in older children. In such studies negative associations between physical activity and measures of body fatness ^{e.g. (23; 84)} as well as a positive relationship between physical activity and lean mass ⁽⁸⁴⁾ have been reported. At 3 years of age, the correlation between FFMI and PAL_{SMR} in this thesis is very strong, and the multiple regression analyses in Table 5 showed that the variation in PAL_{SMR} could be explained by the variation in FFMI rather than by the variation in TBF%. Conversely, at 1.5 years of age variations in TBF% but not variations in FFMI could explain the variations in PAL_{SMR} . It is conceivable that FFMI reflects the capacity of the muscular system to perform physical activity, since this measure reflects the weight of the FFM in relation to height. Thus a possible interpretation of the different results at 1.5 and 3 years of age is that a high body fatness inhibits PAL_{SMR} when the capacity to perform physical activity is limited, as it is at 1.5 years of age, while this is not the case at 3 years of age when this capacity is better developed.

9.2.3 Assessment of physical activity and energy intake in young children

A key issue when measuring physical activity and dietary intake is whether the methodology that is used provides accurate estimates. One finding in this thesis is that Actiheart recordings explained only a small fraction (6-8 %) of free-living energy expenditure above that explained by body composition variables. No comparable data exist for this age group; however, the results are in good agreement with those obtained in a study in 4-10-year-old children ⁽⁸⁵⁾. Explaining only an additional 6-8 % of the variation in AEE or TEE might seem low, but how much a monitor should be able to explain in order to be useful for practical application is a matter of discussion. For adults, Masse et al. considered the ability of accelerometer counts to explain 5 % of the variation in TEE above that explained by body size as “meaningful” ⁽⁸⁶⁾. Furthermore, mHR and mAC were registered by means of Actiheart using one-minute intervals. The technology of activity monitors is constantly improving which includes for instance larger memory capacity of the devices used as well as more advanced software processing of the data collected. The increased memory capacity allows data collection over shorter time intervals (seconds instead of minutes) and longer total time periods (several weeks). The use of shorter time intervals (10 or 15-second intervals) may capture more variation in physical activity. Furthermore, recently the possibility to process

raw acceleration data (instead of summary estimates such as counts) has attracted interest. This might be an advantage since it has been suggested that physical activity outcome is best evaluated when accelerometer signals are processed as raw data ⁽⁸⁷⁾.

The activity diary produced mean PAL values in agreement with reference values on the group level, but the limits of agreement in the Bland-Altman plots were wide, implying low accuracy in individuals. This is in agreement with results from previous studies in adults ⁽⁴⁰⁾ and children ⁽⁸⁸⁾ showing that self-reporting is not valid on an individual level. Future studies may investigate whether the accuracy of the diary can be improved for individuals by adding more alternatives to the included activity categories. Also, using 5-minute intervals might improve the accuracy, since it may be difficult to categorize a full 15-minute period.

This pilot validation study of TECH shows that one day of recordings using TECH provided average values for energy intake that were not significantly different from average TEE. However, the Bland-Altman plot showed wide limits of agreement indicating low accuracy in individuals. Furthermore, there was a bias where low energy intakes were under-reported while high intakes were over-reported. The results are comparable to previous similar validation studies (for established dietary methods) in children aged 3-6 years, both in terms of mean difference and limits of agreement, as shown in **Table 10**. Those results were obtained despite the fact that TECH was applied for only one day. Thus further validation studies of TECH should evaluate if more assessment days can improve the accuracy in individuals. Furthermore, underlying reasons for the observed bias may be differences in parental factors such as socioeconomic status, BMI or gender. This pilot study is too small to evaluate such factors; however, the identification of these factors may be the topic for future studies.

Table 10. A comparison of results using TECH with previous validation studies of established dietary assessment methods in pre-school children (3-6 years) when using the doubly labelled water method as reference.

Author, year	Country	Age (yrs)	N	Dietary method	Energy intake (kJ/24h)	TEE (kJ/24h)	Mean difference energy intake-TEE (kJ/24h)	Limits of agreement (kJ/24h)
This study	Sweden	3	30	TECH	5400	5070	+330 (+7 %)	2990 (59 % of TEE)
Börnhorst et al., 2014 ⁽⁸⁹⁾	Spain, Belgium	4-6	14	Repeated 24 h recalls	5326	5799	-472 (-8 %)	3849 (66 % of TEE)
Collins et al., 2013 ⁽⁹⁰⁾	Australia	3.2	12	FFQ	4950	5234	-284 (-5 %)	2326 (44 % of TEE)
Collins et al., 2013 ⁽⁹⁰⁾	Australia	3.2	12	WFR 4 days	4932	5234	-301 (-6 %)	1782 (34 % of TEE)
Reilly et al., 2001 ⁽⁹¹⁾	Scotland	3-4	41	Repeated 24 h recalls	6500	5800	+660 (+11 %)	3018 (52 % of TEE)
Livingstone et al., 1992 ⁽⁹²⁾	UK	3	8	Diet history interview	5260	5910	-650 (-13.9 %)	+/-36%*
Kaskoun et al., 1994 ⁽⁹³⁾	USA	4-6	45	FFQ	9120	5740	+3390 (+59 %)	4900 (85 % of TEE)
Davies et al., 1994 ⁽⁶⁶⁾	UK	2.5-3.5	31	WFR 4 days	4635	4874	-238(-5%)	1933 (40 % of TEE)

TECH, Tool for energy balance; FFQ, Food frequency questionnaire; WFR, weighed food record; TEE, total energy expenditure measured using the doubly labelled water method; yrs, years; SD, standard deviation. Limits of agreement: ± 2 SD for the mean difference between energy intake and TEE.

* Absolute values not provided.

9.2.4 Assessment of energy metabolism and physical activity during growth

The studies in this thesis provide some unique and interesting observations regarding movement patterns, physical activity and body composition in young children. Interestingly, some of these observations indicate possible differences between children at 1.5 and 3 years of age. For example, TBF (kg) was correlated with TEE at 1.5 years but not at 3 years of age (Table 6). Also, counts using Actiheart could explain a significant fraction of the variation in TEE at 3 years but not at 1.5 years. Furthermore, there was no correlation between PAL_{SMR} values at 1.5 and at 3 years of age. Finally, the relationship between TBF% and PAL_{SMR} was explained by a relationship between PAL_{SMR} and FFMI at 3 years of age, but not at 1.5 years (Table 5). All these observations indicate that movement patterns and exercise physiology such as relations between body composition, physical activity and partition of energy expenditure may differ between young children and older children or adults. These differences might be important to consider in future research when studying relationships between physical activity and body composition in young children.

10. CONCLUSIONS

- From 1 week until 4.5 years of age TBF% and FMI of healthy children were higher than corresponding reference values, indicating a secular trend in body composition development of Swedish children characterized by a high body fatness.
- A relationship between TBF% and PAL was found both at 1.5 and 3 years of age. At 3 years, but not at 1.5 years, this could be explained by a relationship between PAL and FFMI. These findings suggest that body fatness counteracts physical activity at 1.5 years of age when the capacity to perform physical activity is limited, but not at 3 years of age when such a capacity is better developed.
- Actiheart recordings explained a significant but quite small fraction of the variation in free-living TEE at 1.5 and 3 years of age, and in AEE at 3 years of age, above that explained by body composition variables.
- The activity diary produced mean PAL values in agreement with reference PAL when using MET values published by Ainsworth et al., but it underestimated low and overestimated high PAL-values. Furthermore, the accuracy for individual children was low for the activity diary using MET values proposed by Torun, Adolph or Ainsworth.
- The evaluation of the Tool for Energy Balance in Children (TECH) showed that one day of recordings by means of this tool provided average values for energy intake at 3 years of age that were not significantly different from average TEE measured using the DLW method. However, high energy intakes were overestimated while low energy intakes were underestimated and the accuracy in individual children was low.

11. ACKNOWLEDGEMENTS

My warmest thanks to all those who made this thesis possible.

Firstly, I wish to express my sincere gratitude to the families who participated in these studies. Many thanks for all the time and effort you contributed to my project. Without your participation there would be no thesis!

Marie Löf, my supervisor, for sharing your excellence in scientific thinking and writing with me. For guiding me through my PhD period with great patience, for all your help and support, for always being available and for being so encouraging and considerate. Thank you for believing in me!

Elisabet Forsum, my co-supervisor, for sharing your expertise in this research area with me, for your invaluable comments and sharp eye for details and for always being so generous with your time.

Britt Eriksson, for good collaboration, for recruiting and measuring children at 1 and 12 weeks of age and for being a good friend.

Eva Flinke Carlsson, for good collaboration in collecting data and for brightening my coffee breaks.

Ulf Hannestad, for skilfully running the massspectrometer.

Katrin Bergström, for analysis of food images.

Christine Delisle, for help with analysis of food images and for linguistic assistance.

Elisabeth Olhager, for medical coverage.

Stephanie Bonn, Anna Bergström, Katarina Bälter and Olle Bälter, for good collaboration in paper IV.

Eva Hagberg, director at Cityhälsan Norr, for giving me the opportunity to complete this thesis.

Monika Hardmark, Anette Wiklund, Ann-Christine Gilmore-Ellis, Chatarina Malm, Gunilla Linghammar, Viveca Axén and Annelie Munther, for excellent help with administrative matters.

Jane Wigertz, for linguistic assistance.

All my dietician colleagues, for your support and interest in the progress of my research!

All my friends, especially **Veronica Carlberg, Eva Rosendahl and Helena Granberg**, for distracting me from work-related thoughts, brightening my life and for all the laughs we have shared.

My family, my mother and father **Ingrid and Jan Olsson**, for all your love and support. You have always been there for me, feeding me with all your love and delicious food ☺. My sister, **Agnes Olsson**, for always being kind, loving and thoughtful. I am so grateful for having a person in my life who shares all good and bad times with me. My brother, **Olof Olsson**, for always making me laugh and for all the good times we have shared.

And most of all....

My children, **Elias and Siri**, for giving me so much love, joy and happiness and for teaching me the art of living in the present.

Pontus, my loving husband, for making me so happy, for your endless love and support, for your kind heart and for your invaluable input throughout the study period. Without you there would have been no thesis.

12. SAMMANFATTNING PÅ SVENSKA

Barnfetma är enligt världshälsoorganisationen en av 2000-talets största utmaningar för folkhälsan. Prevalensen av barnfetma är hög, både globalt och i Sverige. Detta är ett stort bekymmer eftersom fetma i barndomen tenderar att bestå in i vuxen ålder. Övervikt och fetma hos små barn kan bero på faktorer tidigt i livet, men dessa är till stor del okända. En bidragande orsak till detta är att det saknas enkla och tillförlitliga metoder för att mäta kroppssammansättning, fysisk aktivitet och kostintag hos barn. Syftet med denna avhandling var: 1) att beskriva den longitudinella utvecklingen av kroppssammansättning från 1 vecka till 4,5 års ålder; 2) att studera samband mellan fysisk aktivitetsnivå ("physical activity level", PAL) och olika mått på kroppssammansättning vid 1,5 och 3 års ålder; 3) att utvärdera om rörelse- och pulsregistrering via Actiheart kan förklara variation i total energiförbrukning och energiomsättning orsakad av fysisk aktivitet ("total energy expenditure", TEE och "activity energy expenditure", AEE) vid 1,5 och 3 år; 4) att utvärdera potentialen hos en aktivitetsdagbok att mäta PAL vid 1,5 och 3 år; 5) att utvärdera ett nytt verktyg (TECH) för att mäta energiintag via mobiltelefon vid 3 års ålder.

Friska barn studerades vid 1 och 12 veckor (n=44), samt vid 1,5 (n=44), 3 (n=33) och 4,5 (n=26) års ålder. Kroppssammansättning mättes med hjälp av s.k. helkroppsspletysmografi vid 1 och 12 veckor samt 4,5 års ålder. Vid 1,5 och 3 år mättes kroppssammansättning, TEE, PAL och AEE med hjälp av dubbelmärkta vattenmetoden och indirekt kalorimetri. Puls och rörelser registrerades med Actiheart (4 dagar) och barnens aktiviteter noterades i en dagbok (7 dagar). Energiintaget mättes med hjälp av TECH under en dag.

I genomsnitt var andelen kroppsfett ("total body fat", TBF% och "fat mass index", FMI) högre (3-81%), medan andelen fettfri massa i kroppen ("fat-free mass index", FFMI) var något lägre (-2 till -9 %), för barnen i studien jämfört med referensvärden (från 12 veckor till 4,5 års ålder). Ett negativt samband mellan TBF% och PAL hittades vid både 1,5 och 3 års ålder. Vid 3 år, men inte vid 1,5 år, kunde detta samband förklaras av ett motsvarande positivt samband mellan PAL och FFMI. Rörelse- och pulsregistrering via Actiheart förklarade en liten del (8%) av variationen i TEE vid 1,5 och 3 år, och AEE (6%) vid 3 års ålder, utöver det som förklarades av barnens kroppssammansättning. PAL via aktivitetsdagboken var i genomsnitt inte signifikant skilt från referens PAL vid 1,5 och 3 år, men tillförlitligheten för

individer var låg. Genomsnittligt energiintag via TECH var inte signifikant skiljt från TEE, men tillförlitligheten på individnivå var låg.

De resultat som presenteras i denna avhandling visar att:

- Barnen i avhandlingen hade högre kroppsfetthalt jämfört med referensbarnen. Detta visar på en möjlig identifiering av en sekulär trend i utveckling av kroppssammansättning som kännetecknas av en hög kroppsfetthalt.
- En hög kroppsfetthalt kan hämma fysisk aktivitet vid 1,5 års ålder då kapaciteten för fysisk aktivitet är begränsad men inte vid 3 års ålder, när denna har utvecklats.
- Actiheart registreringar förklarade en statistiskt signifikant, men liten, del av variationen i TEE vid 1,5 och 3 år, och i AEE vid 3 års ålder, utöver det som kunde förklaras av barnens kroppssammansättning.
- Aktivitetsdagboken och TECH gav medelvärden som överensstämde med respektive referens (PAL och TEE), men tillförlitligheten för enskilda barn var låg.

Sammanfattningsvis tyder resultaten från denna avhandling på en sekulär trend i utveckling av kroppssammansättning hos friska svenska barn som kännetecknas av hög kroppsfetthalt under de första 4,5 levnadsåren. Utvärderingarna av metoder för att bedöma småbarns fysiska aktivitet och energiintag gav tillfredställande resultat på gruppnivå, men det krävs mer forskning för att öka tillförlitligheten av dessa metoder för individuella barn.

13. REFERENCES

1. Perlhagen (2007) Fetma hos barn -prevention enda realistiska lösningen på problemet. *Läkartidningen* **104** (3) 138-141.
2. Stockholm County Council (2003), Stockholm County Council website <http://dok.slso.sll.se/CES/FHG/Folkhalsoarbete/Halsa%20Stockholm/folkhalsorapport.2003.pdf> (accessed April 2015).
3. Singh AS, Mulder C, Twisk JW *et al.* (2008) Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obes Rev* **9**, 474-488.
4. World Health Organization (2015), World Health Organization website <http://www.who.int/dietphysicalactivity/childhood/en/> (accessed April 2015).
5. Ng M, Fleming T, Robinson M *et al.* (2014) Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **384**, 766-781.
6. Ahrens W, Pigeot I, Pohlabeln H *et al.* (2014) Prevalence of overweight and obesity in European children below the age of 10. *Int J Obes* **38** Suppl 2, 99-107.
7. Bergstrom E, Blomquist HK (2009) Is the prevalence of overweight and obesity declining among 4-year-old Swedish children? *Acta Paediatr* **98**, 1956-1958.
8. Moraeus L, Lissner L, Sjöberg A (2014) Stable prevalence of obesity in Swedish schoolchildren from 2008 to 2013 but widening socio-economic gap in girls. *Acta Paediatr* **103**, 1277-1284.
9. Swedish National Institute of Public Health (2015), the Swedish National Institute of Public Health website <http://www.folkhalsomyndigheten.se/pagefiles/17825/Folkhalsan-i-Sverige-arsrapport-2014.pdf> (accessed April 2015).
10. Bammann K, Gwozdz W, Lanfer A *et al.* (2013) Socioeconomic factors and childhood overweight in Europe: results from the multi-centre IDEFICS study. *Pediatr Obes* **8**, 1-12.
11. Cole TJ, Bellizzi MC, Flegal KM *et al.* (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. *Bmj* **320**, 1240-1243.
12. Wells JC (2014) Toward body composition reference data for infants, children, and adolescents. *Adv Nutr* **5**, 320S-329S.
13. Forsum E, Flinck Carlsson E, Henriksson H *et al.* (2013) Total body fat content versus BMI in 4-year-old healthy Swedish children. *J Obes* **2013**, 206715.
14. Flegal KM, Shepherd JA, Looker AC *et al.* (2009) Comparisons of percentage body fat, body mass index, waist circumference, and waist-stature ratio in adults. *Am J Clin Nutr* **89**, 500-508.

15. Urlando A, Dempster P, Aitkens S (2003) A new air displacement pletysmograph for the measurements of body composition in infants. *Pediatr Res* **53**, 486-492.
16. Ellis K, Yao M, Shypailo R *et al.* (2007) Body-composition assessment in infancy: air-displacement plethysmography compared with a reference 4-compartment model. *Am J Clin Nutr* **85**(1), 90-95.
17. Fields DA, Allison DB (2012) Air-displacement plethysmography pediatric option in 2-6 years old using the four-compartment model as a criterion method. *Obesity (Silver Spring)* **20**, 1732-1737.
18. Fields DA, Goran MI, McCrory MA (2002) Body-composition assessment via air-displacement plethysmography in adults and children: a review. *Am J Clin Nutr* **75**, 453-467.
19. Heymsfield SB, Lohman TG, Wang Z *et al.* (2005) *Human Body Composition*. Champaign, US: Human Kinetics.
20. Reilly JJ (2008) Physical activity, sedentary behaviour and energy balance in the preschool child: opportunities for early obesity prevention. *Proc Nutr Soc* **67**, 317-325.
21. Hebestreit A, Bornhorst C, Barba G *et al.* (2014) Associations between energy intake, daily food intake and energy density of foods and BMI z-score in 2-9-year-old European children. *Eur J Nutr* **53**, 673-681.
22. Berkey CS, Rockett HR, Field AE *et al.* (2000) Activity, dietary intake, and weight changes in a longitudinal study of preadolescent and adolescent boys and girls. *Pediatrics* **105**, E56.
23. Prentice-Dunn H, Prentice-Dunn S (2012) Physical activity, sedentary behavior, and childhood obesity: a review of cross-sectional studies. *Psychol Health Med* **17**, 255-273.
24. Timmons BW, Naylor PJ, Pfeiffer KA (2007) Physical activity for preschool children--how much and how? *Can J Public Health* **98 Suppl 2**, S122-134.
25. Tennefors C (2003) Total energy expenditure and body composition in healthy Swedish children 9 and 14 months of age. *Eur J Clin Nutr* **57**, 647-653.
26. Weir J (1949) New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* **109**, 1-9.
27. Black A, Prentice A, Coward W (1986) Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. *Hum Nutr Clin Nutr* **40**, 381-391.
28. Speakman JR (1997) *Doubly labelled water. Theory and practice*. London: Chapman & Hall.
29. Henry CJ (2005) Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutr* **8**, 1133-1152.

30. Burrows TL, Martin RJ, Collins CE (2010) A systematic review of the validity of dietary assessment methods in children when compared with the method of doubly labeled water. *Journal of the American Dietetic Association* **110**, 1501-1510.
31. Montgomery C, Reilly JJ, Jackson DM *et al.* (2004) Relation between physical activity and energy expenditure in a representative sample of young children. *Am J Clin Nutr* **80**, 591-596.
32. Reilly JJ, Kelly LA, Montgomery C *et al.* (2006) Validation of Actigraph accelerometer estimates of total energy expenditure in young children. *Int J Pediatr Obes* **1**, 161-167.
33. Sijtsma A, Schierbeek H, Goris A *et al.* (2013) Validation of the TracmorD tri axial accelerometer to assess physical activity in preschool children. *Obesity* **21** (9):1877-83.
34. Lopez-Alarcon M, Merrifield J, Fields DA *et al.* (2004) Ability of the actiwatch accelerometer to predict free-living energy expenditure in young children. *Obes Res* **12**, 1859-1865.
35. Butte NF, Wong WW, Lee JS *et al.* (2013) Prediction of Energy Expenditure and Physical Activity in Preschoolers. *Med Sci Sports Exerc* **46** (6):1216-26
36. Brage S, Brage N, Franks PW *et al.* (2005) Reliability and validity of the combined heart rate and movement sensor Actiheart. *Eur J Clin Nutr* **59**, 561-570.
37. Assah FK, Ekelund U, Brage S *et al.* (2011) Accuracy and validity of a combined heart rate and motion sensor for the measurement of free-living physical activity energy expenditure in adults in Cameroon. *Int J Epidemiol* **40**, 112-120.
38. Villars C, Bergouignan A, Dugas J *et al.* (2012) Validity of combining heart rate and uniaxial acceleration to measure free-living physical activity energy expenditure in young men. *J Appl Physiol*.
39. Butte NF, Wong WW, Adolph AL *et al.* (2010) Validation of cross-sectional time series and multivariate adaptive regression splines models for the prediction of energy expenditure in children and adolescents using doubly labeled water. *J Nutr* **140**, 1516-1523.
40. Neilson HK, Robson PJ, Friedenreich CM *et al.* (2008) Estimating activity energy expenditure: how valid are physical activity questionnaires? *Am J Clin Nutr* **87**, 279-291.
41. Bratteby LE, Sandhagen B, Fan H *et al.* (1997) A 7-day activity diary for assessment of daily energy expenditure validated by the doubly labelled water method in adolescents. *Eur J Clin Nutr* **51**, 585-591.
42. Ainsworth BE, Haskell WL, Herrmann SD *et al.* (2011) 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc* **43**, 1575-1581.
43. Ainsworth BE, Haskell WL, Leon AS *et al.* (1993) Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc* **25**, 71-80.

44. Ainsworth BE, Haskell WL, Whitt MC *et al.* (2000) Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* **32**, S498-504.
45. Ridley K, Ainsworth BE, Olds TS (2008) Development of a compendium of energy expenditures for youth. *Int J Behav Nutr Phys Act* **5**, 45.
46. Torun B (1990) *Activity, Energy Expenditure and Energy Requirements of Infants and Children*. Proceedings of an IDECG workshop held in Cambridge, Massachusetts, USA, November 14-17, 1989. Lausanne (Switzerland): International Dietary Energy Consulting Group.
47. Adolph AL, Puyau MR, Vohra FA *et al.* (2011) Validation of Uniaxial and Triaxial Accelerometers for the Assessment of Physical Activity in Preschool Children. *J Phys Act Health*.
48. Hill RJ, Davies PS (2001) The validity of self-reported energy intake as determined using the doubly labelled water technique. *Br J Nutr* **85**, 415-430.
49. Finndahl O (2015) *Svenskarna och internet*. URL:<http://www.internetstatistik.se>
50. Zhu F, Bosch M, Woo I *et al.* (2010) The Use of Mobile Devices in Aiding Dietary Assessment and Evaluation. *IEEE J Sel Top Signal Process* **4**, 756-766.
51. Martin CK, Nicklas T, Gunturk B *et al.* (2014) Measuring food intake with digital photography. *J Hum Nutr Diet* **27 Suppl 1**, 72-81.
52. Wang DH, Kogashiwa M, Kira S (2006) Development of a new instrument for evaluating individuals' dietary intakes. *J Am Diet Assoc* **106**, 1588-1593.
53. Sharp DB, Allman-Farinelli M (2014) Feasibility and validity of mobile phones to assess dietary intake. *Nutrition* **30**, 1257-1266.
54. Gemming L, Utter J, Ni Mhurchu C (2015) Image-assisted dietary assessment: a systematic review of the evidence. *J Acad Nutr Diet* **115**, 64-77.
55. Nicklas TA, O'Neil CE, Stuff J *et al.* (2012) Validity and feasibility of a digital diet estimation method for use with preschool children: a pilot study. *J Nutr Educ Behav* **44**, 618-623.
56. Small L, Sidora-Arcoleo K, Vaughan L *et al.* (2009) Validity and Reliability of Photographic Diet Diaries for Assessing Dietary Intake Among Young Children. *ICAN: Infant, Child, & Adolescent Nutrition* **1:27**.
57. Mitchell SA, Miles CL, Brennan L *et al.* (2010) Reliability of the School Food Checklist for in-school audits and photograph analysis of children's packed lunches. *J Hum Nutr Diet* **23**, 48-53.
58. Henriksson H, Forsum E, Lof M (2014) Evaluation of Actiheart and a 7 d activity diary for estimating free-living total and activity energy expenditure using criterion methods in 1.5- and 3-year-old children. *Br J Nutr* **111**, 1830-1840.

59. Ma G, Yao M, Liu Y *et al.* (2004) Validation of a new pediatric air-displacement plethysmograph for assessing body composition in infants. *Am J Clin Nutr* **79**, 653-660.
60. Albertsson-Wikland KA, Luo ZC, Niklasson A *et al.* (2002) Swedish population-based longitudinal reference values from birth to 18 years of age for height, weight and head circumference. *Acta Paediatr* **91**, 739-754.
61. Eriksson B, Lof M, Forsum E (2010) Body composition in full-term healthy infants measured with air displacement plethysmography at 1 and 12 weeks of age. *Acta Paediatr* **99**, 563-568.
62. Henriksson H, Bonn S, Bergström A *et al.* (2015) A New Mobile Phone-Based Tool for Assessing Energy and Certain Food Intakes in Young Children: A Validation Study. *JMIR mHEALTH AND uHEALTH*. doi:10.2196/mhealth.3670.
63. Ma G YM, Liu Y, Lin A, Zou H, Urlando A, Wong WW, Nommsen-Rivers L, Dewey KG (2004) Validation of a new pediatric air-displacement plethysmograph for assessing body composition in infants. *Am J Clin Nutr* **79**(4), 653-660.
64. Thielecke F, Noack R (1997) Evaluation of an automated equilibration technique for deuterium/hydrogen isotope ratio measurements with respect to assessing total energy expenditure by the doubly labelled water method. *J Mass Spectrom* **32**, 323-327.
65. Eriksson B, Henriksson H, Löf M *et al.* (2012) Body composition development during early childhood and energy expenditure in response to physical activity in 1.5-year-old children. *Am J Clin Nutr*.
66. Davies PS, Coward WA, Gregory J *et al.* (1994) Total energy expenditure and energy intake in the pre-school child: a comparison. *Br J Nutr* **72**, 13-20.
67. International Atomic Energy Agency (2009) *Assessment of Body Composition and Total Energy Expenditure in Human Using Stable Isotope Techniques*. Human Health Series no 3. Vienna: International Atomic Energy Agency.
68. Fomon SJ, Haschke F, Ziegler EE *et al.* (1982) Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* **35**, 1169-1175.
69. Lohman TG (1989) *Assessment of Body Composition in Children*. *Peadiatr Exerc Sci* **1**.
70. National Food Administration (1992). *Weight for foods and dishes*. Uppsala, Sweden: National Food Administration.
71. National food administration, 2015. Swedish Food Database. URL:<http://www.slv.se/en-gb/> (accessed April 2015).
72. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1**, 307-310.

73. Schofield WN (1985) Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr* **39 Suppl 1**, 5-41.
74. Swedish National Institute of Public Health (2015). The Swedish National Institute of Public Health website <http://www.folkhalsomyndigheten.se/amnesomraden/livsvillkor-och-levnadsvanor/folkhalsans-utveckling-malomraden/ekonomiska-och-sociala-forutsattningar/utbildningsniva/> (accessed April 2015).
75. Swedish National Institute of Public Health (2015). The Swedish National Institute of Public Health website <http://www.folkhalsomyndigheten.se/amnesomraden/statistik-och-undersokningar/enkater-och-undersokningar/nationellafolkhalsoenkaten/levnadsvanor/overvikt-och-fetma/> (accessed April 2015).
76. Butte NF, Wong WW, Wilson TA *et al.* (2014) Revision of Dietary Reference Intakes for energy in preschool-age children. *Am J Clin Nutr* **100**, 161-167.
77. Roberts SB, Young VR (1988) Energy costs of fat and protein deposition in the human infant. *Am J Clin Nutr* **48**, 951-955.
78. Tennefors C, Coward WA, Hernell O *et al.* (2003) Total energy expenditure and physical activity level in healthy young Swedish children 9 or 14 months of age. *Eur J Clin Nutr* **57**, 647-653.
79. Ruxton CH, Reilly JJ, Kirk TR (1999) Body composition of healthy 7-and 8-year-old children and a comparison with the 'reference child'. *Int J Obes Relat Metab Disord* **23**, 1276-1281.
80. Sun SS, Deng X, Sabo R *et al.* (2012) Secular trends in body composition for children and young adults: the Fels Longitudinal Study. *Am J Hum Biol* **24**, 506-514.
81. Olds TS (2009) One million skinfolds: secular trends in the fatness of young people 1951-2004. *Eur J Clin Nutr* **63**, 934-946.
82. Butte N, Hopkinson J, Wong W *et al.* (2000) Body composition during the first 2 years of life: an updated reference. *Pediatr Res* **47**, 578-585.
83. Knuth AG, Hallal PC (2009) Temporal trends in physical activity: a systematic review. *J Phys Act Health* **6**, 548-559.
84. Ness AR, Leary SD, Mattocks C *et al.* (2007) Objectively measured physical activity and fat mass in a large cohort of children. *PLoS medicine* **4**, e97.
85. Ojiambo R, Konstabel K, Veidebaum T *et al.* (2012) Validity of hip-mounted uniaxial accelerometry with heart-rate monitoring vs. triaxial accelerometry in the assessment of free-living energy expenditure in young children: the IDEFICS Validation Study. *J Appl Physiol* **113**, 1530-1536.
86. Masse LC, Fulton JE, Watson KL *et al.* (2004) Influence of body composition on physical activity validation studies using doubly labeled water. *J Appl Physiol* **96**, 1357-1364.

- 87.** Plasqui G, Bonomi AG, Westerterp KR (2013) Daily physical activity assessment with accelerometers: new insights and validation studies. *Obes Rev* **14**, 451-462.
- 88.** Corder K, van Sluijs EM, Wright A *et al.* (2009) Is it possible to assess free-living physical activity and energy expenditure in young people by self-report? *Am J Clin Nutr* **89**, 862-870.
- 89.** Bornhorst C, Bel-Serrat S, Pigeot I *et al.* (2014) Validity of 24-h recalls in (pre-)school aged children: comparison of proxy-reported energy intakes with measured energy expenditure. *Clin Nutr* **33**, 79-84.
- 90.** Collins CE, Burrows TL, Truby H *et al.* (2013) Comparison of energy intake in toddlers assessed by food frequency questionnaire and total energy expenditure measured by the doubly labeled water method. *J Acad Nutr Diet* **113**, 459-463.
- 91.** Reilly JJ, Montgomery C, Jackson D *et al.* (2001) Energy intake by multiple pass 24 h recall and total energy expenditure: a comparison in a representative sample of 3-4-year-olds. *Br J Nutr* **86**, 601-605.
- 92.** Livingstone MB, Prentice AM, Coward WA *et al.* (1992) Validation of estimates of energy intake by weighed dietary record and diet history in children and adolescents. *Am J Clin Nutr* **56**, 29-35.
- 93.** Kaskoun MC, Johnson RK, Goran MI (1994) Comparison of energy intake by semiquantitative food-frequency questionnaire with total energy expenditure by the doubly labeled water method in young children. *Am J Clin Nutr* **60**, 43-47.

Papers

The articles associated with this thesis have been removed for copyright reasons. For more details about these see:

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-117420>