Weight gain in children

-possible relation to the development of diabetes

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Linköping 2009

To my family

ABSTRACT

Background: The prevalence of overweight and obesity among children has increased the last decades and is now defined as a global epidemic disease by the World Health Organization. Also the incidence of type 1 diabetes has increased and there are some hypothesises that argue there is a connection between overweight/obesity and type 1 diabetes.

Aim: The general aim of this thesis was to study factors contributing to the development of overweight and obesity among children and to study possible relations to the development of diabetes.

Method: All Babies in Southeast Sweden, ABIS, is a prospective cohort study. The study includes all babies who were born in southeast Sweden between Oct 1st 1997 until Oct1[st] 1999 and the design was to follow them up to school age in ABIS I and to follow them until 14 years in ABIS II, of the eligible 74 % entered the study. The families have answered questionnaires and biological samples were taken mainly from the children at the different time points: birth, 1 year, 2.5 years, 5 years and 8-9 years. In this thesis studies have been made including the whole cohort, but some studies have also been made involving only a part of the children.

Results: The prevalence of overweight and obesity among children in the ABIS study was 12.9% overweight and 2.5 % obese at 5 years of age. One risk factor which appeared to have a great impact on the development of overweight and obesity at 5 years of age was the child's own BMI at an early age and also the heredity for overweight/ obesity and the heredity for type 2 diabetes. If the father had a university degree, the child was less likely to be obese at 5 years of age. Other factors, such as the parents´ age, if the child had any siblings, and if the child lived with a single parent, did not show any significant correlation to the child's BMI at 5 years of age.
Early nutrition has been studied and no correlation could be found between breastfeeding less than 4 months and the development of overweight/obesity at 5 years of age. The parents answered questions about how frequent the child ate different food at 2.5 years and at 5 years. Intake of sweet lemonade was the only single food which was correlated to a higher BMI in 5 years old children. Porridge seemed to be protective against overweight/ obesity. In one of the studies the physical activity was measured by a step counter. The fewer steps the children were taking, the higher BMI and waist circumference they had. Low physical activity was also associated with a higher C-peptide value and decreased insulin sensitivity. Children who spent more time in front of TV/video had a higher fasting blood glucose value.

Conclusions: A strong factor for the development of overweight and obesity among children is the child's own BMI at an early age and also its heredity for overweight/ obesity and the heredity for type 2 diabetes. Early nutrition did not show any obvious correlations with overweight and obesity at 5 year old children. Low physical activity was associated with higher fasting C-peptide value and decreased insulin sensitivity. Low physical activity may cause β-cell stress which might contribute to an autoimmune process in individuals genetically predisposed to autoimmunity and, thereby, to the increasing incidence of Type 1 diabetes in children.

SAMMANFATTNING

Bakgrund: Förekomsten av övervikt och fetma bland barn har ökat under de senaste decennierna och klassas av Världshälsoorganisationen (WHO) som en global epidemi. Antalet barn som insjuknar i typ 1 diabetes har också ökat och det finns en del hypoteser som argumenterar för att det finns en koppling mellan övervikt/fetma och typ 1 diabetes.

Syfte: Den här avhandlingens syfte var att studera faktorer som bidrar till utvecklingen av övervikt och fetma hos barn och att studera om det möjligen finns en relation till utvecklingen av typ 1 diabetes.

Metod: Alla Barn I Sydöstra Sverige, ABIS, är en prospektiv kohort studie. Alla barn, som föddes mellan 1:a oktober 1997 till 1:a oktober 1999 i sydöstra Sverige, erbjöds delta. Barnen följdes sedan upp till skolåldern i ABIS I och till 14 års ålder i ABIS II. Från starten valde 74% av de tillfrågade familjerna att gå med i studien. Familjerna har besvarat frågeformulär, och biologiska prover är tagna huvudsakligen från barnen vid de olika åldrarna: födseln, 1 år, 2.5 år, 5 år och 8-9år. I avhandlingen ingår dels studier med hela ABIS kohorten, men i två av studierna deltar endast en del av barnen.

Resultat: Hos de barn som igår i ABIS studien var 12,9% överviktiga vid 5 års ålder och 2,5% var feta. En faktor som visade sig ha betydelse för utvecklingen av övervikt och fetma hos 5 år gamla barn var barnets eget BMI vid tidig ålder samt hereditet för övervikt/fetma och hereditet för typ 2 diabetes. Om föräldrarna, framförallt pappan, läst på högskola eller universitet var barnen mindre ofta överviktiga/feta. Andra faktorer som föräldrarnas ålder, om barnet hade några syskon och om barnet levde med en ensamstående förälder visade sig inte ha betydelse för utvecklingen av övervikt och fetma hos barnen. Tidig uppfödning har också studerats. Vi fann ingen korrelation mellan kort amning, dvs. mindre än 4 månader, och utvecklingen av övervikt/ fetma hos 5 år gamla barn. Föräldrarna har också fått svara på hur frekvent barnet åt olika livsmedel vid 2.5 år och vid 5 år. Saft var det enda livsmedel som enskilt hade ett samband med utveckling av övervikt och fetma vid 5 år. Gröt föreföll ha en skyddande effekt. I en delstudie har fysisk aktivitet mätts med stegräknare. Ju färre steg ett barn tog, desto större risk förelåg för övervikt och fetma. Låg fysisk aktivitet var också associerad till ett högre C-peptidvärde och minskad insulinkänslighet. Barn som tittar mycket på TV/video hade ett högre fasteblodsocker.

Konklusion: Av betydelse för utveckling av övervikt och fetma hos barn är barnets eget BMI i tidig ålder och dess hereditet för övervikt och fetma samt hereditet för typ 2 diabetes. Tidig nutrition verkar inte ha några uppenbara samband med övervikt och fetma hos 5 år gamla barn. Låg fysisk aktivitet var associerad till högt faste C-peptid och ökad insulinresistens, vilket skulle kunna stressa β-cellerna och därmed, i enlighet med β- cell stress hypotesen, kunna bidra till en ökad förekomst av typ 1 diabetes hos barn.

ORIGINAL PAPERS

This thesis is based on the following papers, conducted within the ABIS-project, which are referred to in the text by Roman numerals given below:

I. Huus K, Ludvigsson J F, Enskär K, Ludvigsson J. Risk factors in childhood obesity - findings from the All Babies In Southeast Sweden (ABIS) cohort.
Acta Paediatrica 2007; 96(9):1321-5.

II. Huus K , Ludvigsson J F, Enskär K, Ludvigsson J. Exclusive breastfeeding of Swedish children and its possible influence on the development of obesity: a prospective cohort study.
BMC Pediatrics 2008; 9;8(1):42.

III. Huus K, Brekke H K, Ludvigsson J F, Ludvigsson J. Relationship of food frequencies as reported by parents to overweight and obesity at 5 years.
Acta Paediatrica 2008 sep 24 (Epub ahead of print)

IV. Ludvigsson J, Huus K, Eklöv K, Klintström R, Lahdenperä A. Fasting plasma glucose levels in healthy preschool children: effects of weight and life style.
Acta Paediatrica 2007; 96(5):706-9.

V. Huus K, Raustorp A, Ludvigsson J. Physical activity, blood glucose and C-peptide in healthy school- children.
Submitted

CONTENTS

ABBREVIATIONS

ABIS	All Babies in Southeast Sweden
AOR	Adjusted Odds Ratio
BMI	Body Mass Index
CHS	Child Health Services
CI	Confidence Interval
DKA	Diabetic Keto Acidosis
FFQ	Food Frequency Questionnaire
GADA	Glutamic Acid Decarboxylase Antibodies
HOMA	Homeostasis Model Assessment
HRQOL	Health-Related Quality Of Life
IAA	Auto-Antibodies to Insulin
ICA	Islet Cell Auto-antibodies
ICC	Intraclass Correlation Coefficient
IOTF	International Obesity Task Force
IR	Insulin Resistance
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
WC	Waist Circumference
WHO	World Health Organization

INTRODUCTION

The prevalence of overweight and obesity among children has increased and the World Health Organization (WHO) describes obesity as a global epidemic disease [1]. Worldwide, more people are overweight than underweight [2]. Also the incidence of type 1 diabetes (T1D) is increasing among children in most regions of the world [3]. There are some hypotheses that argue there is a connection between overweight and T1D [4, 5].

Diabetes

The disease diabetes mellitus has been known for more than 3 500 years. The word "diabetes" comes from a Greek doctor, Aretaios from Kappadokien (year 120-180), and it means that a person with diabetes drinks a lot and passes large urine quantities. Mellitus means honey-sweet and refers to the smell of the urine. First in 1921 Banting and Best discovered insulin and were able to state that lack of insulin is the cause of diabetes mellitus [6].

Definition and diagnosis of diabetes

Diabetes mellitus comprises a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Diabetes with chronic hyperglycemia is associated with long-term damage, dysfunction and failure of various organs, especially kidneys, eyes, heart, nerves and blood vessels [7].

Symptoms which are associated with diabetes are caused by hyperglycemia and include polyuria, polydipsia, weight loss and blurring of vision. Children with diabetes often present severe symptoms at onset with very high glucose levels, glucosuria and ketonuria [7].

The diagnosis is based on measurements of plasma/blood glucose in combination with clinical symptoms as mentioned above. The diagnostic criteria are the same for both adults and children, but for children the diagnosis is made by plasma/blood glucose test without an oral glucose tolerance test (OGTT), since the symptoms usually are clear and blood glucose high. If a person does not show any symptoms, the diagnosis should be made only after

repeated plasma/blood glucose tests where the value should be in the diabetic range, or if the OGTT test meets the diagnostic criteria for diabetes [8].

The diagnose of diabetes mellitus can be confirmed by symptoms of diabetes plus casual plasma concentration $\geq$ 11.1 mmol/L (casual is defined as any time of the day without regard to the time when the last meal was taken). Classic symptoms of diabetes include polyuria, polydipsia and unexplained weight loss. Fasting plasma glucose $\geq$ 7.0 mmol/L (fasting is defined as no calorie intake for at least 8 hours), or the 2-h post load glucose $\geq$ 11.1 mmol/L in capillary blood during an OGTT also gives the diagnosis. OGTT should be performed as described by WHO, using a glucose load containing equivalent of 75g anhydrous glucose dissolved in water [7, 9].

Classification of diabetes mellitus

The WHO has divided diabetes into four different groups: T1D, Type 2 diabetes (T2D), gestational diabetes and other specific types, depending on aetiology. Patients with any form of diabetes may need insulin at some stage of their disease but that does not in itself define the aetiological class [8].

T1D

This form of diabetes is a result of an autoimmune mediated destruction of the β-cells in the pancreas which usually leads to absolute insulin deficiency. In some individuals this destruction of β-cells is rapid and in some individuals it is slow. Children often have the rapid form, but also in adulthood the rapid form is seen. The slow form often occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (LADA). The first manifestation of the disease can, especially among children and adolescents, be presented with ketoacidosis. Others have a modest fasting hyperglycaemia and they can, if they are exposed to infection or other stress, rapidly change to severe hyperglycaemia and/or ketoacidosis. Some individuals, especially among adults, can have a residual β-cell function for some years. However, after some eyars of the disease there is usually little or no insulin secretion as manifested by low levels of C-peptide in the plasma. In 85-90% of all individuals with T1D, where fasting diabetic hyperglycaemia is initially detected, there are markers of immune destruction, including auto-antibodies to e.g. insulin and/or to glutamic acid decarboxylase (GAD) [8].

T2D

These patients have relative (rather than absolute) insulin deficiency. The persons are resistant to the action of insulin and at least in the beginning the patients do not need insulin treatment. This type of diabetes can be undiagnosed for many years because it does not give enough noticeable symptoms of hyperglycemia. However, these patients have an increased risk of developing macro vascular and micro vascular complications. The aetiology of this form of diabetes is at least to some extent genetic in combination with increased weight, low physical activity and perhaps stress. The insulin secretion is defective and insufficient to compensate for the insulin resistance. Weight reduction, increased physical activity and/or pharmacological treatment can increase the insulin sensivity or otherwise the own insulin secretion can be stimultated by drugs or insulin can be given [8].

Gestational diabetes

Gestational diabetes is when pregnant women without previously diagnosed diabetes exhibit a high blood glucose value. The women often have very few symptoms and often gestational diabetes is diagnosed through screening. Approximately 7% of all pregnancies are complicated by gestational diabetes. The main problem with gestational diabetes is its negative effects on the foetus and newborn child. Mostly this type of diabetes disappears again after pregnancy, but can sometimes reappear, usually as T2D [10].

Other specific types

This includes less common types of diabetes mellitus where the cause can be identified in a relatively specific manner. One group which includes this form is for example genetic defects on the β-cell function or insulin action. These forms of diabetes are frequently characterized by onset of hyperglycemia at a rather early age (generally before 25 years of age). They are referred to as maturity onset diabetes of the young (MODY) and are characterized by low insulin secretion with minimal or no defects in insulin action. In this type also different rare cases of diabetes are included caused by diseases of exocrine pancreas, drugs or chemicals induced or caused by infections [7].

Aetiology

The aetiology of T1D is in many ways unknown but the development of the disease is believed to be caused by a combination of genetic susceptibility genes, immune dysregulation and environmental factors [11]. The autoimmune process may start years before the clinical onset of T1D and the autoimmune process leading to diminishing insulin production [12]. Different genes for T1D provide susceptibility towards the disease and a number of environmental factors also contribute to the development of T1D (figure 1) [11].

Figure 1. Scheme of T1D development. The interactions between genes and the immune system and with the environmental factors may trigger an autoimmune response leading to β-cell loss and to T1D (adapted from Harrison LC) [13].

Genetic risk of T1D

Several different genes are involved in the pathogenesis for T1D. These genes are not necessary for developing T1D and they are not by themselves sufficient for developing T1D. There are at least four loci with strong evidence of association with T1D. The first is a human

leukocyte antigen (HLA), the most prominent of the loci to increase the risk for T1D [14]. This region is located on chromosome 6p21, including the HLA-DRB1, -DQA1 and –DRQ genes. Other less important loci are: a region 5´ to the insulin gene (INS) on chromosome 11p15, the CTLA-4 gene region on chromosome 2q31 and the protein tyrosine phosphatase-22 (PTPN22) [15].

Auto-antibodies against β-cell antigens

Years before clinical manifestation of T1D, immune and metabolic changes can be detected. The immune changes involve both humoral and cellular responses which persist over a prolonged period until the diagnose of T1D [16].

Islet cell auto-antibodies

Islet cell auto-antibodies (ICA) are not specific for the β-cells because they react also with other cells in the pancreas, but they give valuable information because they could be found in 80-90% of the children who develop T1D (table 1). These auto-antibodies can be found many years before the clinical onset of T1D [17]. However, the ICA are not a truly independent marker, because the ICA signal is proven to be attributable to reactivity against GADA, IA-2 and IAA [18].

Auto-antibodies to insulin

Insulin is the only β-cell specific auto-antigen. Insulin auto-antibodies (IAA) are usually the first auto- antibodies to appear. IAA correlates inversely with age. The older the child is, the lower level of IAA (Table 1) [19].

Glutamic acid decarboxylase

GADA (Glutamic Acid Decarboxylase Antibodies) is an auto-antibody against a peptide, an enzyme, released in the islet of Langerhans in pancreas. The antigen was for the first time detected in plasma from diabetic children in Linköping [20]. GADA is widely used to define if healthy individuals have an increased risk to get T1D, as in ABIS, and is also used to define if diabetes is of an autoimmune type (Table 1) [21]. In a controlled randomized trial in T1D children and adolescents with recent onset T1D it has been shown that GAD-alum treatment preserves residual insulin secretion [22]. Further studies are now ongoing.

Protein tyrosine phosphatase

IA-2A, is an auto-antibody directed against the intracellular part of the IA-2 protein (tyrosine phophotase). This autoantibody is also used for screening of high risk individuals, eg in ABIS (Table 1) [16, 23]

Table 1. Occurrence of auto-antibodies against pancreas in healthy individuals and in individuals with T1D.

Auto-antibodies	Shortening	Healthy individuals	Individuals with type 1 diabetes	References
Islet cell auto-antibodies	*ICA*	*1-3 %*	*85 %*	*18, 21, 24-26*
Auto-antibodies to insulin	*IAA*	*0.7- 3 %*	*40-70 %*	*16, 18, 21, 24, 26-28*
Glutamic acid decarboxylase antibodies	*GADA*	*0-3 %*	*50-80 %*	*16, 18, 21, 24-27, 29*
Protein tyrosine phosphatase	*IA-2A*	*0-2.5 %*	*55-80 %*	*24, 26, 29*

To be able to predict T1D, multiple defined auto-antibodies have been studied. In a cohort of 4 505 school children, 97.5 % had no auto-antibodies, 2.3% had a single auto-antibody and 0.3% of the children had multiple auto-antibodies. Of the children who developed T1D within 8 years, all of them had multiple auto-antibodies [18].

Clinical characteristics at diagnosis of T1D

The most classic symptoms at onset of T1D are **p**olydipsia, polyuria and weight loss. For children developing T1D the symptoms develop quite rapidly, within weeks or months. The mean duration of symptoms before diagnosis is longer the older the child is [30, 31].

When a child develops T1D with clinical symptoms, the destruction of islet β-cells causing insulin deficiency and the glucose concentration in the blood are raised beyond the renal threshold and the reabsorption of glucose in the renal is incomplete. Some glucose remains in the urine, glucosuria. This also results in an increased urine production due to the increased osmotic pressure of the urine and inhibits reabsorption of water. This leads to increased thirst,

lost blood volume will be replaced osmotically by fluid in the body cells which causes dehydration [32].

Some children can also experience blurred vision. It is the prolonged high blood glucose which causes glucose absorption, and this can lead to changes in the shape of the lenses in the eyes. This symptom often disappears when the blood glucose value is normalized [33].

Children developing T1D may initially present diabetic ketoacidosis (DKA). About 29.4% of the children who develop TID gets DKA at onset. This is an extreme state of metabolic dysregulation. The children smell of acetone and their breath is rapid and deep (kussmaul breathing). The children also have polyuria, nausea, are vomiting and have abdominal pain. DKA can lead to coma and death [32, 34].

Epidemiology

The incidence of T1D is increasing among children in most regions of the world from 0.1/100 000 children below the age of 15 in low incidence countries as China and Venezuela to 32-40/100 000 in high incidence countries as Sweden, Finland, and Sardinia in Italy [35, 36]. The incidence rate has increased dramatically over the last decades in the western countries and in Sweden the incidence has almost doubled during the last 20 years [37, 38]. Boys and girls are equally affected in most populations and the increase seems to be highest in the youngest age group [3]. However, according to a study of Pundziute-Lycka et al (2002) the increasing incidence of T1D in Swedish children may to some extent be a shift so the children are younger when they get the diagnosis [39].

Living with T1D

The families´ lived experience, when a child develops T1D, is described like an ongoing process including learning about the inevitable and about the extent. The families have to learn new things depending on what has happened and they therefore need individualized treatment in the beginning [40]. After one year the families describe that they have had an ordinary, yet different, year, but it is important that health professionals make use of the families´ experience to be able to support them [41]. Studies asking the child or its family about living with T1D show that the children experience the restrictions in their social life, the need to live a regular life and a feeling of being different as aspects they find difficult living with [42-44].

Factors related to development of T1D

Both genetic and environmental risk factors contribute to the risk of T1D in children. Different environmental factors have been suggested as trigger mechanisms of this autoimmune β-cell destruction.

Infections, such as viruses, are one of the most probable risk factors, but this has been very difficult to prove because of the time between their possible trigger effect and the manifestation of T1D. New evidence strongly suggests that enterovirus infections can be one major trigger of T1D [45]. Enteroviruses, and in particular the Coxsackie virus, have been proposed as triggers of β-cell autoimmunity and T1D [46]. An additional hint was also that it was observed that T1D was usually diagnosed in late summer and fall, this matching the seasonal pattern of enterovirus in northern Europe [47]. Another study has shown that countries with high incidences of T1D (Finland and Sweden) also have a lower frequency of enterovirus infections compared to countries where T1D is less common. This finding is congruent with the polio hypothesis, which is based on experience from another enterovirus disease, poliomyelitis, where the risk virus-induced motor neuron damage increases when the frequency of polivirus infections in the population decreases [48, 49].

Another possibility is that the enterovirus infection occurs in utero or neonatally in countries with low frequency of enterovirus infections. As maternal enterovirus antibody levels are low in these countries, the child gets no protection [50].

Even if the association between enterovirus infections and T1D has been studied in many epidemiological studies, there are still conflicting findings and the causality has not been proved. It is still possible that this association is caused by some unidentified confounding factor [46, 50].

Hygiene, Kolb and Elliot (1994) described the hygiene hypothesis. It was from the beginning introduced in the field of allergy research but could also be linked to T1D. The hygiene hypothesis suggests that due to better hygiene resulting in reduced exposure to microbial antigens early in life and by that a reduced need for a strong immune defense [51] which may lead to imbalance of the immune system with for instance autoimmune diseases or allergies as a consequence. Infections, especially in childhood, may in fact prevent or delay diseases [52].

Early infant feeding has also been suggested as a trigger for β -cell destruction. Short duration of breastfeeding (≤ 2months), an early introduction of cow's milk, and late introduction of gluten as well as a high consumption of milk (at the age of 1 year) have been found to be risk factors for the introduction of β-cell auto-antibodies in 2.5-years-old children [53]. Breast milk protects the child against infections during the first months by maternally transmitted immunity and this could also increase the child's resistance to other possible triggers of diabetes associated autoimmunity [54]. It is the cow´s milk protein which has been proposed as a trigger of the activation of the immune system in the destructive process leading to T1D [55].

Another study on pre-school children confirmed the results that long duration of breastfeeding, late introduction of bottle feeding and current consumption of cow´s milk seem to reduce the risk of developing T1D [56].

In a review of Virtanen and Knip 2003 they discuss the role of nutritional factors as potential risk factors in the development of T1D. Many different factors, such as infant feeding, cow´s milk, different vitamins (C, D and E), have been reported as possibly protective against T1D. But the conclusions are that more longitudinal studies are needed from pregnancy until the children develop T1D [57].

Maternal food consumption during pregnancy has been suggested as a trigger of β -cell destruction. Different food groups, such as potatoes, other root-vegetables, gluten-containing foods, non-gluten cereal grains, cow´s milk products, fruits, vegetables, meat and fish, have been studied. It was found that an increased consumption of potatoes during the last three months of pregnancy was associated with a delayed time in the development of islet autoimmunity (IA) [58]. It is not the potato in itself but the toxic contamination of a class of streptomyces toxins that can accelerate the onset of autoimmune diabetes [59]. Also the intake of vitamin D has been studied. Mothers who continuously used cod liver oil during pregnancy, had lower risk of T1D in the offspring [60]. Measures of the intake of vitamin D in the food during pregnancy shows that it may have a protective effect on the appearance of IA in the offspring [61]. However, if the mother used multivitamin supplements during pregnancy, this did not give the same results [60, 61].

Psychological stress has also been suggested as a risk factor. It is known that psychological stress decreases the insulin sensitivity and increases the insulin resistance and may hence be important in the development of T1D [62, 63]. Also, the mothers´ experience of serious life events is associated with diabetes-related autoimmunity in children [64].

The link between child overweight/obesity and the development of T1D

Wilkin TJ (2001) proposed the accelerator hypothesis which could be the link between child overweight/obesity and the development of T1D. This hypothesis suggests that T1D and T2D have the same origin. The increased insulin resistance is associated with the epidemic of (childhood) overweight and obesity and creates greater insulin secretory demand on the islets, leading to acceleration of β-cell destruction [4]. The different genes affect the tempo of the β-cell loss and thus determine the age of the child when getting diabetes [65].

An extension of the acceleration hypothesis is the β-cell stress hypothesis suggesting that any phenomenon that induces insulin resistance and/or increased insulin demand and thereby adds extra pressure on the β-cells should be regarded as a risk factor for T1D [5, 66]. Psychological stress, puberty and rapid growth could be such phenomena [64, 66].

Overweight/ obesity

Nowadays humans are among the fattest of all mammals. For humans, fat is an energy reserve. For other mammals fat mostly appears to serve as insulation from the cold. Obesity has never been a common health problem in the human history, nor has it been realistically possible for most of the people to be obese because of frequent food shortages [66]. However, in our days the prevalence of overweight and obesity among children has increased rapidly during the last 20 years. The WHO describes obesity as a global epidemic disease [1]. Also in Sweden the prevalence is increasing [67]. For the first time in 200 years life expectancy for young people may be decreased because of the prevalence of obesity [68].

Definition of overweight/ obesity

Obesity is the result of excess body fat. Body Mass Index (BMI) is used to classify overweight and obesity in adults. BMI is the weight in kilograms divided by the square of the height in meters (kg/m^2) [1]. It is the same for both genders and for all ages of adults. WHO defines overweight in adults as BMI equal to or more than 25 and obesity as BMI equal to or more than 30. Overweight and obesity among children are defined according to International Obesity Task Force (IOTF) based on a study of Cole et al. This cut-off is equivalent to BMI in adults (overweight BMI >25 and obese BMI >30) (Figure 2). The definition is based on an international survey of six large nationally representative cross sectional growth studies [69]. To assist international comparisons in epidemiological studies, the Cole et al reference values have several strengths, they are based on large data set from different countries, the BMI cut-offs are linked to adult cut-offs , and they are simple to use. They are also consistent for children and adolescents. But there are also some concerns regarding the definition. Almost all data derive from western populations. In the population, around which the cut-offs are built, there is a variation in the prevalence of overweight/obesity, and the characteristics of the population, like the anthropometry, are unknown [70].

Figure 2. The lines represent the BMI cut-offs within the different ages (Source: Nowicka P, Flodmark CE. Barnövervikt i praktiken- evidensbaserad familjeviktskola. P:16. Printed with permission from Studentlitteratur, Sweden)

Another way to measure overweight and obesity is Waist Circumference (WC). In adults it is well known that a more central fat distribution is associated with an increased risk of ill health [66]. However, also the prediction of health risks associated to overweight/obesity in children is improved by additional inclusion of WC [71].

Aetiology

Overweight and obesity have been seen as something which could disappear if a person changed life style [72]. However, research has shown that it is not that simple. There are many different factors which interfere with each other in the development of overweight and obesity, such as genes, social factors, behavior, and cultural factors [72-74].

Childhood obesity leads to health consequences both in childhood and in adulthood. Obesity in childhood might lead to psychological consequences. Obese children are more likely to have low self-esteem and behavioral problems than non–obese children, and that risk seems to be even greater in girls than in boys. Obesity in childhood also promotes cardiovascular risks, such as dyslipidaemia and hypertension. Another clinical consequence is an increased risk of developing asthma symptoms. Also a higher risk of developing T1D has been reported [75, 76].

Long term consequences of obesity in childhood have an adverse effect on the social and economic outcomes. There is some evidence that this effect might be more marked in women. Cardiovascular risk factors are higher among persons who were obese as children [76]. Obesity in childhood leads to adult obesity. Four out of five obese teenagers remain obese in adulthood [77].

Epidemiology

During the past two decades, the prevalence of obesity has increased worldwide [1]. In a study from the US it was found that between 1999-2004 there was an increase in overweight among children, adolescents and men, while it might have stabilized among women [78]. In Europe, especially in the southern and western parts, the prevalence of overweight is higher among children [79]. Compared to other countries, Sweden has a low incidence of overweight and obesity among children, but the development during the last decades is alarming. In a study from the county of Östergötland it has been found that the prevalence of overweight among 10- year old children was 22% in both boys and girls, of which 4% and 5% respectively were obese [80]. In another Swedish study published in 2007 it was found that the obesity epidemic in children 10-11 years of age may be decreasing in urban Sweden and among girls it may possibly be reversed. From 2000/2001 until 2004/2005 the prevalence among overweight girls decreased from 19.6% to 15.9% and among obese girls from 3.0% to 2.5% [81].

Obese children, both girls and boys, are at risk of becoming obese adults. A study following children from 3 to 35 years of age showed that the higher the children´s or adolescents´ BMI is, the greater the risk becomes of being overweight at 35 years [82].

Living with overweight/ obesity

Children with overweight/obesity have a lower health-related quality of life (HRQOL) compared to normal weight children [83]. Especially obese children have a lower health quality of life in physical, social and school domains compared with normal weight children. The moderately obese children had similar emotional and school HRQOL, but they had lower HRQOL in the physical domain. Parents to obese children perceived their children´s quality of life events lower than the children did themselves [84].

Factors related to development of overweight/ obesity

By some estimates **Genetic variation** stands for 40-70% of the within-population variation in obesity. It is very complex to find out which genes that are involved in causing obesity in humans. Mostly there is an interaction of multiple genes, environmental factors and behaviour. There are at least 21 different genes or markers which can be linked to obesity [73].

Breastfeeding. Some studies report that breastfeeding protects against obesity [85-88], while other studies show that breastfeeding does not protect against obesity [89, 90]. Several possible biological mechanisms for a protective effect against obesity have been discussed. Rolland-Cachera et al (1995) observed that a higher intake of protein early in life, regardless of type of feeding, was related to an increased risk of obesity [91]. Infants who are breastfed have a lower intake of protein and reduced energy metabolism [92]. Another possible biological mechanism could be that breastfed and formula-fed infants have different hormonal response to feeding. Formula feeding leads to a greater insulin response resulting in fat deposition and that the adipocytes increase in number [93]. Other studies suggest that infants who have been breastfed more readily adapt to new foods, such as vegetables, thus reducing the caloric density of their subsequent diets [94].

Decreased **physical activity** has been proposed to be one of the greatest reasons for obesity [95]. TV-viewing, using computers and other sedentary occupations are also proposed as causes of obesity. However, the results are pointing in different directions [96]. Reduced television viewing and computer use may lead to a decrease in BMI, but this result might be due to the change in **energy intake** more than to the change in physical activity [97].

There are studies which show an association between **dietary factors** and BMI [98-105]. Especially dietary fat intake [98-102, 104, 105] but also the protein intake [105, 106] have been related to BMI. However, other studies have not confirmed these results [103, 107-109].

Modern Life style

The life style for children has changed during the last decades. Their life style has changed to include less physical activity and a change of the food consumption. Food has become more affordable to a larger number of people over the last decades and also the price of food has decreased substantially in relation to the income. The means of nourishment have also changed to be a marker of life style and a source of pleasure [110]. Inactivity, such as television viewing and computer games, is associated with increased prevalence of obesity [111]. In the last decades inactivity among children has increased and it has been found that parents prefer letting their children watch television at home rather than playing outside because then the parents can keep up with what they are doing and still be able to have an eye on the children. Children are also more often driven by their parents to school and they participate less frequently in sports and physical education [110, 112].

AIM OF THE THESIS

The general aim of this thesis was to study factors contributing to the development of overweight and obesity among children and to study possible relations to the development of diabetes

Specific aims:

- To identify risk factors for overweight/obesity among children.

- To investigate if breastfeeding and early nutrition are related to overweight/obesity in 5 year -old children.

- To examine whether a modern life style with high-energy intake and low levels of physical activity influences the blood glucose value and insulin sensitivity in healthy children.

METHOD

All papers in the current thesis are based on data from the All Babies In Southeast Sweden (ABIS) project or from parts of the ABIS project. ABIS is a prospective, longitudinal cohort study aiming at studies of environmental factors affecting development of immune-mediated diseases in children. The study was initiated by Professor Johnny Ludvigsson at the Department of Molecular and Clinical Medicine. Division of Pediatrics, Faculty of Health Sciences, Linköping, Sweden.

The ABIS study is divided into two parts, ABIS I and ABIS II.
The study was designed to include all babies who were born in southeast Sweden between Oct 1st 1997 until Oct 1st 1999 and to follow them up to school age in ABIS I and to follow them until 14 years in ABIS II. In ABIS I data were collected at 4 different time points, at birth, at 1 year, at 2. 5 years and at 5 years. In ABIS II data were collected at 8-9 years and are planned to be collected at 11 years and 14 years (Figure 3).

Figure 3: The time table of the ABIS projects

At each time point questionnaire data and biological samples, mainly from the children, were collected. The data in ABIS I were collected by nurses at the Child Health Services (CHS).

Data were collected in connection with the four childhood check-ups at the CHS, i.e. at 1 week, 1 year, 2-3 years and 5-6 years of age. In the 8-9 year follow-up of ABIS II the questionnaire and equipment for biological samples were sent home to the families. The parents and their children went to the nearest welfare center to take blood samples and they sent in the other biological samples by themselves together with the questionnaires.

Each questionnaire contained approx 150 questions. The questions asked in the different studies concerned BMI of the children and their parents and other questions regarded siblings and heredity for T1D and T2D among relatives. Several questions regarding food frequency, including breastfeeding, activity and inactivity. Regarded the parents, questions concerning their age, education, if they were a single parent, if they were born abroad and their smoking habits were analyzed.

Participants

All 21 700 parents-to-be in Southeast Sweden during Oct 1st 1997 and Oct 1st 1999 were invited to participate in the ABIS study. The questionnaire response rate at infant birth was 74% yielding a sample of 16 058 subjects (51.8% boys and 48.2% girls). The 1-year questionnaire was completed by 10 836 families, and 7 356 completed the 5-year questionnaire. In ABIS II the questionnaire was sent by regular post to all who participated from birth and answered that questionnaire. Parents of 3 952 children and 3 837 children themselves answered the 8-9 year questionnaire. No reminders have been sent out (September 2008, the collection is ongoing) (Figure 2).

Figure 2. Number of participating families in the different years when the data were collected (collection of data of the 8 year follow up is still ongoing, September 2008).

The representation of participants in the ABIS study is shown in table 2. Characteristics for children and parents at baseline (child birth) for all participants and for those who completed the 5 year questionnaire are presented. In addition, the table shows data from answers of the 5 year questionnaires. The numbers are based on those from whom we have data on the specific parameters.

Table 2. Characteristics of the children and the parents at baseline (in all participants) and at baseline and 5 years in those who continued to participate until 5 years of age.

	Baseline (n ≥14 244)	Baseline (n ≥ 5 999)	5 years (n ≥ 5 999)
Boys/girls, %	48.2/51.8	48.1/51.9	48.1/51.9
BMI girls*	-	-	16.01 (1.77)
BMI boys*	-	-	16.09 (1.59)
BMI boys and girls*	-	-	16.05 (1.68)
Maternal age, years	29.6	29.9	34.9
Paternal age , years	32.1	32.3	37.3
BMI mother*	23.8 (3.9)#	23.7 (3.9)#	24.1 (4.0)
BMI father*	25.0 (3.0)#	25.0 (2.9)#	25.6 (3.1)
Mother born abroad, %	6.6	5.3	5.3
Father born abroad, %	7.2	5.2	5.2
University degree, mother %	31.7	34.5	39.4
University degree, father %	24.6	25.3	26.6
Single parent , %	2.1	1.2	6.6
Siblings*	1.4(0.5)	1.4(0.5)	-
Smoking, mother %	11.1	7.8	10.7
Smoking, father %	-	-	9.3

BMI = Body Mass Index

* Data are presented as mean values (standard deviation)

Information collected at 1-year-examination.

The baseline values for parental BMI, parental age, level of education, if the parents were born abroad, maternal smoking habits and parental civil status differ very little between the initial cohort and between those who still participated at 5 years of age. Parents in the families who dropped out of the study, tended to be slightly younger and to have a lower level of education. These parents were often smokers, were often born in a foreign country and were often a single parent at the time of birth.

Samples

Three of the papers are based on data collected from the questionnaire sent out in the ABIS study (papers I. II. III) and two of the papers (papers IV, V) are based on subsamples of the ABIS study.

Papers I, II and ***III*** were based on the whole ABIS cohort and questions from the birth, 1 year, 2.5 year and 5 year questionnaires. In the different studies all available data are used even if it meant that the number of participants differs in the analyses.

Paper IV was based on a subsample of the ABIS study, 127 parents and their children from 6 different pre-school units in the Linköping area participated. The children were between 5 and 7.5 years old and the mean age was 6.6 years. There were 56% girls and 44% boys. Fasting plasma glucose samples were provided from 106 children.

Paper V was based on a subsample of the ABIS study. Totally 199 children participated and questions in the 8 year questionnaire have been answered by 154 parents. Number of pedometer steps was collected from 192 children, 53.2% girls and 51.8% boys.

Procedure

In the ABIS study the at-birth questionnaire was given to the mothers when they left the hospital after the delivery. Biological samples, such as blood, urine, stool, hair from the mother and breast milk, were taken. The questionnaire was filled in and returned either immediately or to the Child Health Services at the first check up.

The questionnaires at 1 year, 2,5 years and 5 years were handed out to the parents at the Child Health Service. They were returned immediately or mailed back when completed. Biological samples, such as blood, urine and stool, were taken. Some Child Health Services reminded (by phone) those parents who had not returned the questionnaires. Also at 1 year the parents were contacted if they had not completed the full questionnaire. This was not done in the 2.5 year and 5 year questionnaires.

In study IV, information letters and questionnaires were sent out to the children and their parents by the pre-school teacher who also collected them after they had been filled in. The

fasting plasma glucose measurement was made one morning before breakfast. Also WC, weight and height were measured.

The 8 years questionnaire was sent home to the parents and their children by ordinary mail. The participants were also given the opportunity to answer the questionnaire on the home page on internet. In paper V, which is a subsample of the whole cohort, the school nurse measured height and weight. Also fasting blood samples were gathered.

Physical activity was measured by a step counter, cable tie sealed Yamax pedometers (SW-200 Tokyo, Japan) during four consecutive weekdays. The number of daily mean steps was measured over four consecutive days as recommended in order to assure reliable results and avoid reactivity [113, 114]. The school nurses and physical educators carried out the data collections. The pedometers were collected each day (every 24 hours) by a research assistant, who unsealed them, documented the number of daily mean steps, reset and resealed the devices and returned them to the children. The children were also asked to complete a brief survey to verify that the pedometers were worn according to the instructions during the entire time on the previous day.

Data collection

The ABIS questionnaires cover a large number of different subjects, such as medical issues, environmental factors, nutrition, psychological variables (117-196 questions). Relevant to the current thesis are questions about weight, height, WC, nutrition including breastfeeding, physical activity and some socioeconomic factors.

Weight and height

All the papers include variables with weight and height. Overweight and obesity were defined according to Cole et al [69], age and gender adjusted. Weight and height data were received from the parents in papers I, II and III. In papers IV and V, weight and height were measured at the same time as other data were collected.

Paper I

BMI was related to different variables which could be associated with overweight/obesity, family situation (single vs. cohabitant), maternal age ($\leq$ 34 years at child birth vs. $\geq$ 35 years), paternal age ($\leq$ 36 years at child birth vs.$\geq$37 years), number of siblings (0 vs. $\geq$1), infant birth weight ($\leq$94[th] percentile vs.$\geq$ 95th percentile), maternal BMI ($\leq$ 24.99 vs $\geq$ 25), paternal BMI ($\leq$ 24.99 vs $\geq$ 25), maternal and paternal education (university degree: no vs. yes). Heredity for obesity or T1D was also used.

Paper II

The questionnaire at 1 year contained questions about exclusive breastfeeding, introduction of infant formula or gruel and the introduction of cow´s milk and the response alternatives were: < 1 month to >8 months. Regarding any breastfeeding there was a question in the 2.5 year questionnaire where the parents stated how many months after birth the mother stopped breastfeeding the child. Short-term exclusive breastfeeding was defined as < 4 months of exclusive breastfeeding.

Breastfeeding and the children's BMI were related to different variables such as family situation (single vs. cohabitant), maternal age ($\leq$34 years at child birth vs. $\geq$35 years), paternal age ($\leq$36 years at child birth vs.$\geq$37 years), number of siblings (0 vs. $\geq$1), maternal BMI ($\leq$ 24.99 vs $\geq$25), paternal BMI ($\leq$ 24.99 vs $\geq$25), maternal and paternal education (university degree: no vs. yes).

Paper III

The questionnaire at 2.5 years contained a 30 items Food Frequency Questionnaire (FFQ) and the questionnaire at 5 years contained a 34 items FFQ regarding the children's diet. The frequency categories used were: A. daily, B. 3-5 times/week, C. 1-2 times/week, D. less than once a week and in the 5 year questionnaire, E. never. Fat content of dairy foods was not specified in the questionnaire. Foods which were expected to be associated with overweight or obesity due to high energy content (foods high in fat and/or sugar like snack foods/ candy, cheese, sausage) and foods with low energy content (vegetables, fruits, porridge) as well as foods associated to BMI in previous studies (milk, sugar containing beverages) were included in the analyses.

Paper IV

The questionnaire which was used in this paper was answered by the parents and a part of the questions was a FFQ containing 16 items regarding the children's diet. The frequency categories which were used were: A. more than 1/day, B. every day, C. 3-6 times/week, D.1-2 times/week, E. 1-3 times/month, F. more seldom. There were also questions about physical activity/inactivity, heredity for T1D and heredity for overweight/obesity. Weight, length, WC were measured and fasting plasma glucose levels were registered.

Paper V

In this paper the 8-years questionnaire from ABIS was used. Five different questions about physical activity were analysed: Number of hours spent in front of TV/video, hours spent in front of computer, hours spent in physical activity (playing, running around), hours spent reading /homework plus one further question concerning hours spent in car transportation. The scales used for these questions were 1= 0-15 minutes, 2= 1/2 hour, 3= 1 hour, 4= 2 hours, 5=3 hours, 6=4 hours, 7= 5 hours, 8= 6 hours, 9= 7 hours or more. The questions were asked with regard to schooldays and weekend days respectively. Physical activity was measured by a step counter, height, weight and WC were also measured and blood samples, such as fasting blood glucose, HbA1c and C-peptide, were taken.

Analyses and statistics

SPSS version 11.5- 14 for windows was used [115].

Descriptive statistics with means or medians were calculated in all the papers. In paper IV scatter plots were used to illustrate the results.

All participating children with data on each question were used in the different analyses. This explains why the number of participants differs between the analyses.

Correlations were assessed using Pearson's correlation for parametric variables and Spearman´s correlation for non-parametric variables (Papers IV and V).

In paper I we used the Chi-square test. This test compares the observed numbers in each of the four categories in the contingency table with the numbers to be expected [116]. The Chi-square test was used for analyzing heredity for overweight/obesity in paper I.

Student's t-test (independent) was used to compare mean values in the different groups. The t-test was used in paper V when we compared boys and girls and when comparing normal weight children with overweight/obese children.

Simple and/or multiple logistic regressions were used in the papers I, II and III. Multiple logistic regressions were used to investigate how a dichotomous outcome variable (dependent variable) is related to more than one exposure variable (independent variable). The logistic regression analysis was used to explore predictors and to calculate Odds Ratio (OR) with 95% confidence intervals (CI). In paper I logistic regressions were used to investigate the relationship between a number of risk factors and the risk of overweight/obese. In paper III different foods were related to normal weight and overweight/ obese children. In paper II different risk factors were related to both short and long breastfeeding and to normal weight or overweight/obese children.

Intraclass correlation coefficient (ICC) was used to be able to demonstrate the similarity between two different test methods that measure a continuous variable. In the validation of the children's height and weight intraclass correlation is used. The reason for using ICC instead of another correlation like the Pearson's was that Pearson's correlation is between two different groups, for instance between men and women. However, in the intraclass correlation the trait´s mean and variance are derived from pooled estimates across all members of all groups. The intraclass correlation gives the between group variance divided by the total variance [116].

P-values (significance level) are used to assess the strength of evidence against the null hypothesis. This means that there is no true association in the population from which the sample was drawn. The smaller the P-value is, the stronger the evidence is against the null hypothesis [117]. In papers I, II, IV and V the P-value < 0.05 was considered statistically significant. In paper III, due to multiple comparisons and to the risk of false-positive statistical significance, only the P-value < 0.01 was regarded as statistically significant.

Homeostatic model assessment (HOMA) are used to assessing β-cell function and insulin resistance (IR) from fasting glucose and insulin or C-peptide concentrations [118]. In paper V, HOMA IR and HOMA β-cell were calculated.

Validity

The validity term means that the measurement shows what it claims to measure [119]. All data in the ABIS project are given by the parents. In all papers data on weight and height are used. Therefore a validation of these data has been done. Eight different schools in the ABIS area where they had the children's records from the CHS were included in this validation. Data on 145 children at 1 year and 5 years of age were registered from the child health records and compared with the data from the parents. This validation showed a high correlation between weight and height having an intraclass correlation coefficient of $P<0.001$. A validation of height and weight has also been done at 8 years of age (intraclass correlation coefficient of $P<0.001$).

A validation has also been done in paper V where the parents answered questions about the children's physical activity and inactivity. Physical activity was measured also by a step counter/ pedometer.

Reliability

This is the precision of the measuring. Reliability measures the degree at which it is consistent and stable. A test which is stable and repeatable has reliability [119]. P-values (significance level) are used to assess the strength of evidence against the null hypothesis. This means that there is no true association in the population from which the sample was drawn. The smaller the P-value is, the stronger the evidence is against the null hypothesis [117]. In papers I, II, IV and V the P-value < 0.05 was considered statistically significant. In paper III, due to multiple comparisons and to the risk of false-positive statistical significance, only the P-value < 0.01 was regarded as statistically significant.

Ethical considerations

When conducting research involving children, the research should be performed according to accepted ethical guidelines and rules. The ABIS study was formally approved by the Research Ethics Committee of the Faculty of Health Science at the University of Linköping, Sweden. The ABIS study was also approved by the Research Ethic Committee of the Medical Faculty of Lund University.

Performing research including children and their families in a large scale study as the ABIS can cause ethical conflicts. Ethical questions and principles were considered in the following way with respect to the four ethical principles, respect for autonomy, beneficence, nonmaleficence and justice [120].

Respect for autonomy

Autonomy is the capacity for self-determination. To respect an autonomous individual is to acknowledge that person´s right to make choices out of his or her own values and beliefs [121]. In the ABIS study the parents received oral information and they were also invited to see a video film about the ABIS project before their child was born. On the first page of the questionnaire information was given that participation is voluntary. Return of the completed at-birth questionnaire and/or biological samples were considered as an informed consent to participate. In study IV blood-samples and some other measurements were taken from pre-school children and they were informed that the participation was voluntary. Also in the last study V blood-samples were taken from 8 years old children. The children carried a pedometer for 4 days and some other measurements were taken. They were also informed about the voluntariness and some chose to participate only in some parts.

Beneficence

The principle is that we should do good to others [121]. There are no sharp breaks between nonmaleficence and beneficence, but the beneficence principle potentially demands more than nonmaleficence because it requires positive steps to help others [120]. The main aim of the ABIS study is to study the aetiology of diabetes and other immune-mediated diseases, and, if answers can be found and thus also a cure or a way of making life easier for children with diabetes or other immune mediated diseases, we have done something good. However, children who have participated in the ABIS study can, if the research finds a way to prevent

and treat T1D and if the research shows how to identify risk individuals depending on how specific the prevention must be, get direct benefits from the study.

Nonmaleficence

This principle implies not to harm intentionally or willingly [121]. In the ABIS study the parents and children answered questionnaires, and biological samples, such as blood-samples, were also taken. It could always be discussed if the child is harmed or not. When the child are small, the parents make the decision, but the older the children are, the more they can decide for themselves. The parents and the children have got information about that their participation is voluntary, and sometimes, when a child has not consented to take some biological samples, the parents have nevertheless answered the questionnaire.

Justice

In general, justice means fairness, for example ensuring equality of opportunity for all individuals and equality of the outcome for different groups in society [121]. In ABIS the opportunity to participate in the study was the same for all mothers in the southeast of Sweden who visited the Maternal Health Services. All participating families have answered the same questionnaires and the same biological samples have been asked for. However, papers IV and V are based on subsamples of the ABIS where the opportunity to participate has been given to a small group in a pre-school class or in an ordinary school class.

The ABIS study is a large prospective cohort study and in studies like this there are often ethical concerns about the risk of creating distress and anxiety. Therefore, in the ABIS study there have been several papers on ethical concerns. The first study about ethical concerns was made in 2002 by Gustafsson-Stolt et al. and thereafter a number of studies have been made. All these studies, either based on interviews or questionnaires, show that a majority believed that a study like the ABIS was good and they wanted to participate because they felt they could do something for the research. The mothers who did not want to participate were concerned about putting the child through frequent blood testing and some felt that the project involved some kind of "experimentation" [122]. Gustafsson-Stolt et al (2003) found that the parents´ attitudes towards the project were prominently positive. The parents were positive to research screening and felt that this kind of research is important [123].

RESULTS

All papers include BMI of the children and in table 3 the BMI of the whole cohort is presented. In papers IV and V, subsamples of the whole cohort are used.

Table 3. BMI distribution in the ABIS study (the whole cohort)

	Overweight	*Obesity*
Children at 2. 5 years	*12.9%*	*2.5%*
Children at 5 years	*12.9%*	*4.3%*
Children at 8-9 years	*12.0%*	*2.3%*
*Mothers	*23%*	*7%*
*Fathers	*41%*	*6%*
■ Mothers	*23%*	*8.5%*
■ Fathers	*46%*	*7.5%*

*1 year after the child´s birth
■ 5 years after the child´s birth

Paper I

In paper I a multiple logistic regression with the children's BMI > 25 and > 30, age and gender adjusted according to Cole et al [69] as our dependent variable and different risk factors as independent variables was made. The independent variables include the parents´ age, parents´ education, if the child had any siblings, and if the child lived with a single parent. The analysis shows that children whose fathers had a university degree were less likely to be obese at 5 years of age (adjusted odds ratio (AOR) = 0.74; 95% CI= 0.60-0.91; p= 0.005) and the result was similar when BMI >30 (age and gender adjusted) was used as an dependent variable. A borderline association was also found for maternal education and children´s BMI>25 (AOR= 0.84; 95% CI= 0.70-1.00; p=0.054). The other variables in the analysis were not significantly associated.

In another multiple regression analysis of the children´s BMI as a dependent variable the children's birth BMI > 95th percentile and the parents´ BMI >25 were used as independent variables. A high birth BMI was positively associated with BMI at 5 years of age (AOR= 2.25; 95% CI= 1.68-3.02; p= < 0.001) and also the parental BMI was positively associated with the children's BMI at 5 years of age (high BMI in mothers: AOR = 1.97; 95% CI = 1.67-2.32; p< 0.001; and high BMI in fathers: AOR= 1.96; 95% CI= 1.64-2.33; p< 0.001). Similar results were found when the parents BMI < 30 was used as an independent variable.

Heredity: It was found that children, who had heredity for T2D, had a higher BMI (p<0.001; N= 6 213) at 5 years of age and children, who had heredity for overweight, also had a higher BMI (p< 0.001; N= 6 213) at 5 years of age.

Paper II

In paper II it was found to be a weak association between short-term exclusive breastfeeding and obesity in 5 year old children (simple logistic regression: OR=1.44; 95% CI=1.00-2.07; P=0.050). However, when adjusting for other variables, such as parental age, single parent, parent born abroad, parents´ smoking habits and the parents´ education, the association between short-term exclusive breastfeeding and obesity in 5 year old children did not exist any longer. The only association found in the multivariate analysis was maternal smoking and obesity at 5 years of age (AOR= 1.72; 95% CI= 1.00-2.93; P=0.048).

At the age of three months 78.4% of the children were exclusively breastfed and the median age for introduction of infant formula or gruel was 5 months. The median duration of any breastfeeding was 8 months. Any breastfeeding was also associated with the parents´ age. Older mothers breastfed for a longer time than younger mothers (R2= 0.028, P<0.001).

Short-tem exclusive breastfeeding (less than 4 months) was in a multiple logistic regression associated with maternal BMI> 30 (AOR=1.07; 95% CI=1.05-1.09; P<0.001) and maternal smoking (AOR=1.43; 95% CI= 1.05-1.95; P= 0.023). It was also more common with short exclusive breastfeeding among single mothers (AOR= 2.10; 95% CI= 1.43-3.09; P<0 .001). It was less common with short exclusive breastfeeding among parents with a university degree (mother: AOR=0.74; 95% CI= 0.61-0.90; P=0.003, father: AOR=0.73; 95% CI= 0.58-0.92; P=0.008).

Paper III

In an analysis where overweight/obese children at 5 years of age and food frequencies at 2.5 years were studied, the only food that was positively associated with (contributed to) overweight/obesity was the consumption of cheese (AOR=0.070; 95% CI= 0.55-0.90; P= 0.005). Negatively associated with (protect against) overweight/obesity was porridge (AOR= 0.57; 95% CI= 0.39-0.84; P= 0.004), fried potatoes /pommes frites (AOR=0.77; 95% CI= 0.54-0.92; P= 0.005) and cream /crème fraiche (AOR=1.19; 95% CI= 1.00-1.41; P= 0.046).

Based on previous results from this population [124], analyses were performed adjusted for parental BMI, parental education and heredity for T1D and T2D. When adjusting for these risk factors, the frequency in intake of fried potatoes/pommes frites (AOR=0.75; 95% CI=0.62-0.92; P= 0.006) and porridge (AOR=0.55; 95% CI= 0.36-0.85; P=0.007) remained significantly negatively associated with overweight/obesity.

In another analysis where overweight/obesity at 5 years of age and food frequencies at 5 years were studied. The intake of chocolate (AOR=0.76; 95% CI=0.65-0.90; P= 0.001) and lemonade (AOR=1.38; 95% CI=1.12-1.70; P= 0.003) were positively associated, whereas cream/crème fraiche (AOR=1.23; 95% CI=1.06-1.43; P= 0.007), pastries (AOR=0.43; 95% CI=0.24-0.79; P=0.007), chocolate (AOR=0.76; 95% CI=0.65-0.90; P= 0.001) and candy (non-chocolate) (AOR=1.46; 95% CI=1.14-1.87; P= 0.003) were negatively associated with overweight/obesity at 5 years of age. When adjusting for known risk factors only the frequency in intake of candy (non-chocolate) AOR=1.60; 95% CI=1.22-2.12; P=0.001) remained negatively associated with overweight/obesity.

Paper IV

In paper IV, playing outdoors was associated with a higher degree of physical exercise ($p<0.05$, R^2=0.082) and it was also correlated to a higher BMI ($p<0.001$, R^2=0.228). Children who watched TV often had a higher BMI ($p< 0.01$, R^2=0.228) and the WC was correlated with how many hours per week the children played outdoors ($p<0.01$, R^2=0.151). BMI was also correlated to the consumption of sweet lemonade ($p< 0.05$, R^2= 0.041). In paper IV no correlations were found between fasting plasma glucose levels and physical activity, eating habits or the children's BMI.

Paper V

When the children carried a pedometer for 4 days it was found that boys took more steps than girls, even though boys were said to spend more time in front of the computer (paper IV). The fewer steps the children were taking, the higher BMI (p=0.019) and WC (p= 0.018) they had. C-peptide (P=0.044), HOMA IR (P= 0.046) and HOMA β-cell (P=0.041) were also significantly correlated to how many daily mean steps the children were taking. A higher HOMA IR was also correlated to a larger WC (P=0.041) .

How many hours per day the child spent in front of TV / video were correlated to larger WC (school days: P= 0.033 weekend days: P= 0.016). Children who spent more hours per day in front of TV / video were also correlated to higher fasting blood glucose values (school days P=0.016). Children who spent long time sitting in a car had higher fasting blood glucose values (P= 0.037). The more time the children spent in front of TV/DVD on schooldays, the less time they spent on physical activity in the weekends (P= 0.045), Table 4.

Table 4. Correlations within physical variables and questionnaire data

		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
1.	BMI	-															
2.	Waist circum ference	.797**	-														
3.	Fasting blood glucose	.079	.079	-													
4.	Fasting C-peptide	.149	.175	.028	-												
5.	HbA1C	-.062	-.126	-.028	.016	-											
6.	HOMA IR	.172	.221*	.082	.991*	.009	-										
7.	HOMAβ-cell	.040	.072	.468**	.831**	.030	.805**	-									
8.	Daily mean steps	-.185*	-.212*	.095	-.205*	-.062	-.210*	.239*	-								
9.	Hours spent in front of TV video/DVD (S)	.136	.198*	.233*	.185	.072	,174	-.077	.051	-							
10.	Hours spent in front of TV video/DVD (W)	.133	.206*	.063	.064	.084	.041	-.075	-.070	.623**	-						
11.	Hours spent in front of computer (S)	-.027	.055	-.077	.073	-.153	.037	.087	.117	.163*	.232*	-					
12.	Hours spent in front of computer (W)	-.020	.032	-.002	.016	-.167	-.006	.019	.109	.109	.193*	.749**	-				
13.	Hours spent in physical activity (playing, running around) (S)	-.059	-.135	.015	-.004	.009	-.017	-.008	.085	-.033	-.004	-.097	-.083	-			
14.	Hours spent in physical activity (playing, running around) (W)	-.124	-.102	-.059	.029	.610	.009	.030	-.030	-.164*	-.076	-.127	-.128	.705*	-		
15.	Hours spent reading/ homework (S)	-.009	.119	-.009	.042	-.218*	.046	.035	-.046	.091	.071	.154	.072	-.018	.009	-	
16.	Hours spent reading/ homework (W)	-.031	.089	.043	.100	-.194*	.131	.094	-.121	-.042	-.007	.073	.021	-.026	.063	.663**	-
17.	Hours spent in the car (per week)	-.031	.050	.202*	.037	.047	.009	-.134	-.075	-.127	-.040	-.206*	-.157	.116	.145	-.028	.112

Pearsons correlation
Spearman correlation
*significant level 0.05
** significant level 0.01
(S) = school days
(W) weekend days

DISCUSSION

Main methodological issues

All 21 700 parents-to-be in Southeast Sweden during Oct 1st 1997 until Oct 1st 1999 were invited to participate in the ABIS study. The questionnaire response rate at birth was 16 058 subjects. No selection was made by the researchers. At 1 year 10 836 families answered the questionnaire, at 5 years 7 356 families answered the questionnaire and at 8 years 3 952 parents and 3 837 children answered the questionnaire. One weakness of the ABIS study is the drop-out rate. It has been discussed how much loss that can be acceptable in prospective cohort studies, and some researchers have tried to put a number on it. Kristman et al propose that acceptable follow-up rates are 60%-70% [125], others suggest 80% [126]. However, in a new study of Fewtrell et al [127] they suggested that the most important is to give unambiguous information about the cohort throughout the study, to discuss potential bias, if the drop-out has affected the generalization of the findings and that appropriate sensitivity analyses are used [127]. In the papers in this thesis, the initial cohort is compared with the cohort which is still participating in the 5 year follow-ups in those variables that were used. There is no selection bias with regard to the parents´ BMI and almost none with regard to the parents´ age. However, there is a slight overrepresentation of parents with a high education, fewer parents born abroad, fewer single parents and fewer smoking parents at the 5 year follow-up.

All data in the ABIS study are self reported. Therefore, there is a possibility that some variables, like the food frequency consumption, to some extent can be underreported. The main objective of the ABIS study is to examine risk factors for T1D, and by tradition there is a belief among people that T1D is caused by or related to sugar consumption. Therefore, it can be an underreporting in these variables. There has also been an increased awareness of obesity among children and, therefore, some parents might have underreported the intake of fat [128]. However, the weight and height data were validated against those recorded at the Child Health Service. Eight different schools in the ABIS area where they had the children's records from the Child Health Service were included in this validation. The weight and height data on 145 children at 1 year and 5 years were registered from the child health records and compared with data from the parents. This validation showed a high correlation between

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weight and height, having an intraclass correlation coefficient of P<0.001. Another study also shown that parents are good at estimating their children's weight [129].

Main results

The incidence of T1D is increasing [3], and also the incidence of overweight and obesity among children is increasing [1, 67]. The reason for the increase in T1D is unknown, but one hypothesis discussed in recent years is the β-Cell Stress Hypothesis. This means that any phenomena which induce insulin resistance and/ or increased insulin demand and thereby add extra pressure on the β-cells should be regarded as a risk factor for T1D [5, 130]. These phenomena could be psychological stress, puberty and rapid growth [64, 130]. In this thesis phenomena which could contribute to the development of overweight/obesity in children and thereby to the development of diabetes have been studied (Figure 3).

Figure 3. Scheme of phenomena which could contribute to the β-cell stress and maybe to T1D.

Heredity

The parents´ BMI is one factor associated with the children's BMI. There is an interaction of multiple genes which could be linked to obesity [73], and in paper I it was found that children with heredity for overweight and T2D had a higher BMI at 5 years of age. Also the heredity for T2D are associated to a higher BMI at 5 years of age as shown in paper I.

Socioeconomic factors

Parents´ education

The parents´ education was the only socioeconomic factor in this study which was found to influence the children's weight (paper I). This is also confirmed in other studies [131, 132] where it also has been found that socioeconomic factors, such as smoking, education, and family income, influence the weight development in children.

Manner of living

Early nutrition

One factor which has been studied is breastfeeding. There are theories that breastfeeding protects against obesity. One theory is that children who are breastfed have a lower intake of protein and by that less risk for developing obesity [91]. The mothers in the ABIS breastfed for a long period, 78.4% breastfed exclusively for at least three months. In paper II no association was found between exclusive breastfeeding (< 4 month) and the children's BMI at 5 years when adjusting for other factors.

In paper III food frequencies are used to find associations between early nutrition and the child's BMI at 5 years of age. The food frequency questionnaire was one way to measure how often the child consumed a certain type of food. It gives a rather rough estimation and says nothing about portion size. However, the advantage with the food frequency questionnaire in the ABIS study is that it reflects the dietary habits for a long time even if it is influenced by attitudes and knowledge of the parents. Food frequencies do not give any simple explanation for overweight and obesity at 5 years. Porridge at 2.5 years may be

protecting against overweight/obesity and lemonade may contribute to overweight/obesity at 5 years. These results are confirmed by a study from the US where it was found that consumption of sugar-sweetened drinks is associated with obesity in children. The risk for overweight/ obesity increased by each additional glass of sugar-sweetened drink the child consumed every day [133].

Physical activity/ inactivity

Physical activity is measured by an objective method; step counters (paper V) and by questionnaires to the parents (paper IV and V). This is associated not only to the child´s BMI, but also to their WC and to blood glucose control and insulin sensitivity. When measuring physical activity, it was found that lower physical activity is associated with higher BMI and larger WC. Another finding was that children who were more physically inactive, spent more time in front of TV/video, and had more time spent in the car have higher fasting blood glucose. Those with low physical activity measured by step counters also have higher fasting C-peptide and increased HOMA IR index which indicate insulin resistance.

A life style with high energy intake and low levels of physical activity may in fact already at these low ages increase the β-cell stress and could, according to the β-cell stress hypothesis [5], contribute to an increasing incidence of T1D in children.

Individual factors of the child

BMI in early age

One of the purposes of this thesis was to study factors contributing to the development of overweight and obesity among children. One of the most important factors is the children´s own BMI. Early overweight/obesity has to be taken seriously. Overweight and obesity at 1 and 2.5 years of age increase the risk of being overweight at 5 years (paper I) and obesity in childhood leads to obesity later in adult life [77].

Overweight and obesity among children are increasing when comparing the children in the ABIS study with children born a few years earlier in the same area [134]. There are few effective treatments for childhood obesity [135, 136] and, therefore, the need of identification of risk factors is important to be able to prevent childhood obesity.

CONCLUSIONS

This thesis has shown:

- A strong factor for the development of childhood overweight and obesity is the child´s own BMI at a very early age, the parents´ BMI and the child's heredity for T2D and the child´s heredity for overweight.

- If the parents have a low education (no university degree) this is a risk factor for childhood overweight and obesity.

- Physical activity is associated with the children's BMI.

- Low physical activity is associated with higher fasting C-peptide and decreased insulin sensitivity, which may stress the β-cells and could, according to the β-cell stress hypothesis, contribute to an increasing incidence of T1D in children.

- Early nutrition did not show any obvious relation to overweight/ obesity at 5 years of age. The foods that showed a correlation in the early nutrition of the child were sweet lemonade which could cause overweight/obesity and porridge which was found to protect against overweight/obesity.

Clinical implications

These factors, the child's BMI, the parents´ BMI, heredity for T2D and obesity together with the parents´ education, can be identified at the CHS and, maybe, it could be possible to start prevention early. Prevention could be advice about the importance of physical activity as a prevention of later overweight/ obesity and also advice about the importance of early nutrition, such as the intake of sweet lemonade. The advice should be about how important it is with physical activity and also how important it is to avoid physical inactivity. At the CHS, advice could be given on limiting the children's screen time. If the parents could do that, the children spontaneously would be more physically active and as a result overweight and obesity would be prevented. Also, when the children get a little older, it is important to promote physical activity in school from the beginning and to give advice about nutrition in school, not only regarding the school meals but also other intakes at school.

Anna-Greta Huus, mother in law, for language checking, support and encouragement.

All doctoral students and colleges at the department of pediatrics, Linköping University, for support, helpful criticism during seminaries, and stimulating discussions during this period.

The colleagues and friends at the University College of Health Sciences, Jönköping, who inspired me to begin and encouraged me to carry on. Thanks for all support.

The Child group at Jönköping University for helpful criticism during seminaries, for support and friendship.

Fredrik, my husband for being understanding and encouraging.

Hampus, Linus and Kasper our wonderful children who bring us happiness.

At last, but not at least my family, my father and the rest of my family, for support and for believing in me. All my friends outside the scientific world who are very important to me and who all have supported and encouraged me during these years.

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